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SHORT COMMUNICATION

GENETIC DIVERGENCE ANALYSIS ON PEANUT BY RAPDs

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ABSTRACT

This study demonstrated the utility of random amplified polymorphic DNAs (RAPDs) to analyse genetic divergence of peanut genotypes in the South Vietnam. Nucleic acid extracts from 29 Arachis hypogea L. cultivars were amplified with five random decamers by PCR. Markers were used as RAPD 2, RAPD 3, RAPD 5, RAPD 6, OPC 11. The distinctive RAPD patterns generated from these cultivars could be used as genomic fingerprint to establish the identity of a given genotype. 29 peanuts were clearly separated in distinct subclusters in a phyllogram obtained by unweighted pair group method analysis (UPGMA) of genetic distances.

Key words: RAPD, Genetic distance, PCR, Peanut

INTRODUCTION

Peanut (*Arachis hypogea* L) is an important food and oil crop in Vietnam. A cross breeding program is needed to improve peanut's economic value. Since peanut is a self- pollinating plant, a cross breeding procedure is useful for producing new varieties of this crop. However, it takes a long time to breed this plant by crossing and crossed products occur at a low rate. Tallury *et al.* 1995). Breeders need to know the genetic similarity between potential parents. Hence, it is necessary to develop a system of genomic fingerpringting so that the identity of specific cultivars can be rapidly determined. Such a system can be used by geneticist to study the evolution of this genus and it may enable breeders to formulate reasonable breeding plans. A genetic fingerprinting system will also be useful in protecting the rights of the plant patent holders. In recent years, random amplified polymorphic DNA (RAPD) analysis through the polymerase chain reaction (PCR) has become widely use to characterize and trace the phylogeny of diverse plant and animal species. The main advantages of RAPD analysis over other methods are its low sample DNA requirement and the high frequency of polymorphic bands detechted (Williiams *et al.* 1900).

This reported with the establishment of genomic fingerprinting system for peanut, staring with the rapid micro extraction of nucleic acid from 28 cultivars, The generation of RAPD bands by 10 random decamers through PCR estimation of the genetic distances and phylogenetic analysis.

MATERIALS AND METHODS

Data pertaining to the cultivars included in this study were obtained from CLRRI (Cuu Long Delta Rice research Institute) and AGI (Agricultural Genetics Research Institute) (Table 1)

DNA isolation

A crude DNA preparation suitable for PCR analysis was prepared using a simplified miniscale procedure (Lang 2002). A single piece of healthy young leaf was harvested and placed in a labelled 1.5 ml centrifuge tube in ice. The leaf sample was macerated using thick glass rod after adding 400 μl of extraction buffer (50 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 300 mM NaCl and 1% SDS). The leaf was grounded until the buffer turned into green colour. After grinding, another 400 μl of extraction buffer was added and mixed by pipetting. The contents were centrifuged at 12,000 g in micro centrifuge for 10 min.

Nearly 400 µl of lysate was extracted with 400 µl of chloroform. The top aqueous supernatant was transferred to another 1.5ml tube and DNA was precipitated with absolute ethanol. The contents were centrifuged for 3 min at full speed and the supernatants were discarded. The pellet was washed with 70% ethanol. The DNA was air dried and re-suspended in 50 µl of TE buffer (10mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0). One ml of aliquot was used for PCR analysis and the remaining solution was stored at –20^{0}C for any further use.

RAPD methodology

The PCR amplification (Lang 2002) was performed in a volume of 20 µl containing 50 mM KCl, 10 mM Tris–HCl (pH = 9.0), 2.5 mM $MgCl_2$, 0.2 mM dNTPs each, 0.4 µM 10-mer primer (Sangon Company), 20 ng of genomic DNA, and 1 unit of *Taq* DNA polymerase. PCR was performed with an MJ Research PTC-100 thermalcycler (MJ Research Inc.) using the following cycling: 94^{0}C/4 min; three cycles at 94^{0}C/15 s, 35^{0}C/15 s, 72^{0}C/75 s; 35 cycles at 94^{0}C/15 s, 40^{0}C/15 s, 72^{0}C/75 s; one cycle at 72^{0}C/7 min; 4^{0}C soak. A total of nine random decamer primers were used to identify primers with both a relatively high level of polymorphism and banding pattern reproducibility. After initial screening, five primers were selected to detect polymorphisms in 28 accessions (Table 1). The products were separated on 1.2% agarose gels in one. TAE buffer (40 mM Tris–acetate, 1 mM ethylenediaminetetra acetic acid, pH 8.0) under 95 V for 1.5 h. The gels were stained with ethidium bromide and immediately photographed with a camera under UV light.

Data analysis

Data (*i.e.* SSR and RAPD bands) were scored as discrete variables, using 1 to indicate presence and 0 to indicate absence of a band. A pairwise similarity matrix was generated with SPSS 10.0 software (SPSS Inc., Chicago, IL, USA) using Jaccard's coefficient values as follows: Jij = a/(a+b), where a is the number of bands common to both accessions and b is the number of missing bands in one accession, but present in the other (Lang 2002). The GD between two samples was calculated as D = 1) J_{ij}. The degree of concordance between SSR and RAPD markers was determined by visual appraisal of graphic depictions generated from the analysis of GD values and correlation analysis using Microsoft Excel 2000. Cluster analysis was performed based on SSR and RAPD marker data using the PHYLIP version 3.5c software package (Scientific American, Inc., Seattle, Washington, USA) with an unweighted pair-group method using an arithmetic averaging (UPGMA) algorithm.

RESULTS AND DISCUSSION

The analysis of variance revealed highly significant difference among lines and characteristics studied (Table 1).

Table 1. Lines, origin, physiological characters of peanut cultivars used in the study

No.	Lines	Origin	One seed / pod	Two seeds / pod	Three seeds/ pod	Number seed/plant
1	OMĐP1	CLRRI	5.00 a	36.00 a	0.33 d	302.67 ab
2	OMĐP2	CLRRI	3.33 abc	18.33 defg	2.67 b	212.00 cdefghi
3	OMĐP3	CLRRI	4.67 ab	36.33 a	1.00 c	223.33 bcdefghi
4	OMĐP4	CLRRI	2.67 abc	16.67 efgh	0.00 d	269.33 abcde
5	OMĐP5	CLRRI	2.00 c	6.67 h	4.00 a	281.33 abcd
6	OMĐP6	CLRRI	3.67 abc	34.67 ab	0.00 d	250.00 cdefg
7	OMĐP7	CLRRI	4.00 abc	27.33 bcdef	0.00 d	288.00 abc
8	OMĐP8	CLRRI	4.00 abc	30.33 abcd	0.00 d	228.67 bcdefgh
9	OMĐP9	CLRRI	3.67 abc	32.33 abc	0.00 d	316.67 a
10	OMĐP10	CLRRI	4.67 ab	30.33 abcd	0.00 d	289.33 abc
11	OMĐP11	CLRRI	2.67 abc	16.33 fgh	0.00 d	170.67 hi

No.	Lines	Origin	One seed / pod	Two seeds / pod	Three seeds/ pod	Number seed/plant
12	OMĐP12	CLRRI	2.33 bc	12.00 gh	0.33 d	218.00 cdefghi
13	OMĐP13	CLRRI	3.00 abc	27.67 bcdef	2.67 b	292.00 abc
14	OMĐP14	CLRRI	5.00 a	21.67 cdefg	0.00 d	180.67 fghi
15	OMĐP15	CLRRI	3.67 abc	27.00 abcdef	0.00 d	173.67 ghi
16	OMĐP16	CLRRI	4.00 abc	28.67 abcde	0.00 d	194.00 efghi
17	OMĐP17	CLRRI	3.67 abc	24.33 abcdef	0.00 d	257.33 bcdef
18	OMĐP18	CLRRI	3.33 abc	24.33 abcdef	0.33	168.67 hi
19	OMĐP19	CLRRI	2.67 abc	29.00 abcd	0.00 d	275.33 abcd
20	VD1	AGI	4.67 ab	25.67 abcdef	0.33 d	144.67 i
21	VD2	AGI	2.33 bc	22.67 bcdefg	0.00 d	202.67 efghi
22	VD3	AGI	3.67 abc	24.67 abcdef	0.00 d	154.67 hi
23	VD4	AGI	2.67 abc	16.33 fgh	0.00 d	170.67 hi
24	VD5	AGI	2.67 abc	16.33 fgh	0.00 d	170.67 hi
25	VD99-4	AGI	2.33 bc	12.00 gh	0.33 d	218.00 cdefghi
26	VD99-5	AGI	3.00 abc	27.67 bcdef	2.67 b	292.00 abc
27	VD99-6	AGI	5.00 a	21.67 cdefg	0.00 d	180.67 fghi
28	TXAG-4	An Giang	3.67 abc	27.00 abcdef	0.00 d	173.67 ghi
29	VA861102	Australia	2.33 bc	12.00 gh	0.00 d	228.67 bcdefgh

The six random primers generated a total of 15 RAPD bands: RAPD 2, RAPD 3, RAPD 5, RAPD 6, OPC 11 produced 1, 4, 3, 2 and 4 polymorphic bands, respectively. Figure 1 shows the RAPD patterns generated by RAP 5, RAPD 3 and OPC 11.

Table 2. Numbers of observed and polymorphic bands of 29 varieties .

No.	Primer	Sequencing 5' ---------- 3'	allele	Size markers(kb)
1	RAPD2	GTTTCGCTCC	2	0.7 – 1.6
2	RAPD3	GTAGACCCGT	4	0.2 – 3.0
3	RAPD5	AACgCgCAAC	3	1.1 – 2.1
4	RAPD6	CCCGTCAGCA	2	1.1 – 2.5
5	OPC11	AAAGCTGCGG	4	0.5 – 2.8

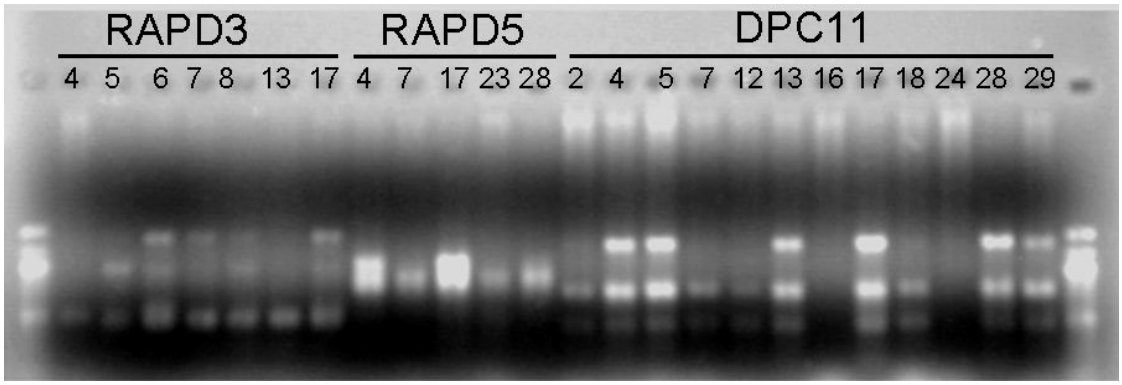

Figure 1. RAPD band generated by RAPD 3, RAPD 5 and OPC 11.Lane 2: OMDP 2, lane 4: OMDN P, lane 5: OMDP 5, lane 7: OMĐP7, lane 8: OMDP 8, lane 13: OMĐP 13, lane 12: OMĐP12, lane 13: OMDP 13, lane 16: OMĐP 16, lane 18: OMĐP18, lane 23: VD 4, lane 24: VD 5, lane 28: TXAG 4, lane 29: VA 861102

RAPD-based GD estimates

Five RAPD primers produced distinct, clearly visible polymorphisms (*i.e*. bright reproducible bands), and were thus used for the genetic relationship analysis. In a total of 14 assessments of genetic relationships among groundnut, 14 allele sites were scored with a high degree of confidence. The polymorphisms identified were then used to generate the GD matrix (above the diagonal of Table 1). The molecular weights of bands analyzed ranged between 200 and 2,800 bp, of which 96% were polymorphic.

The genetic distance value provides a useful estimate of relationship between a specific pair and a small number of genotypes. Phylogenetic analysis, however, is more appropriate for the interpretation of all possible relationships among a large group of genotypes. The genes can be divided into two clusters based on the 5 primers. Pairwise comparison and average similarity coefficients among all accessions were calculated based on the presence or absence of polymorphic bands on non-denaturing accessions ranged from 0.00 to 0.74 (average of 0.56). The largest cluster I, consisted mainly of the 26 lines. Cluster II consisted mainly of OMDP 17, OMDP 7, and OMDP. The various relationships among these cultivars are shown in the phyllogram (fig. 2), generated by UPGM analysis of the genetic distances. The OMDP 17, OMDP 7, and OMDP 4 were closely related. This is also indicated by the great similarity in the flower structure and color patterns of these three cultivars. The close similarity between OMDP 6 and OMDP 8 may be because they have the same male parent.

The distribution of the hybrid type into different subclusters may be an indication of the diversity of the parental species used to generate this population. The VD 1 and OMDP 9 were the most closely related among OMDP 3, OMDP 9.

The phyllogram can be used in the formulation of breeding plans. For example, if crosses involving the most distantly related cultivars are desired then crosses between the OMDP 17 and OMDP 1 type can be recommended. Crosses between closely related genotypes like OMDP 1 and OMDP 3 are less likely to produce heterosis.

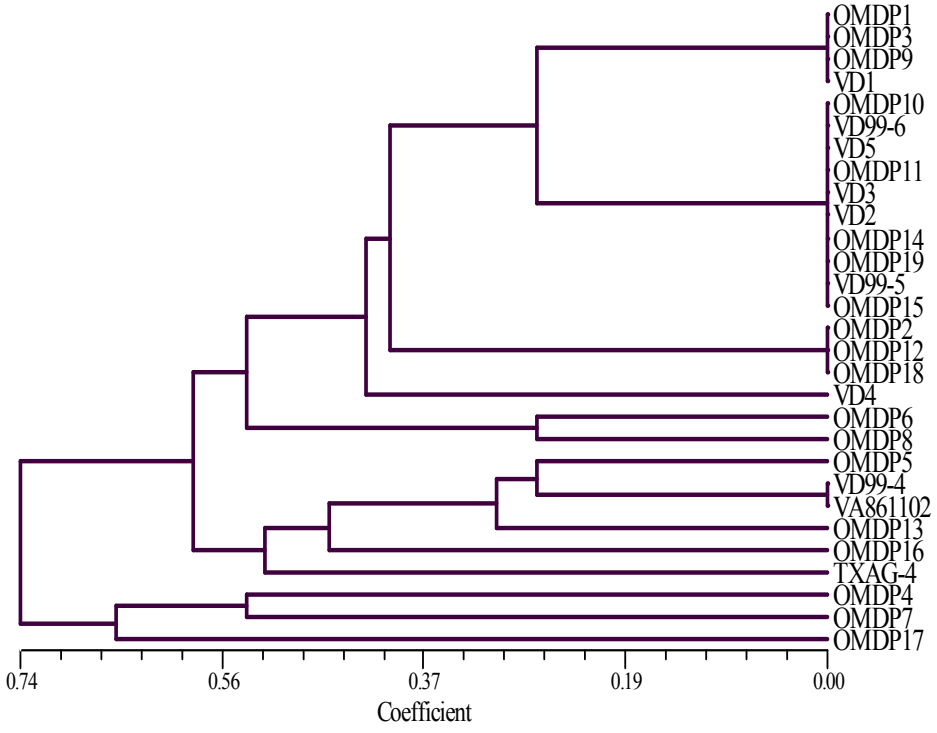

Figure 2. Phyllogram obtained by unweighted pair group method analysis of 15 RAPD markers generated by five random primers of 29 peanut varieties.

CONCLUSION

The rapid nucleic acid extraction procedure and the corresponding PCR protocol for the generation of RAPD marker have proved useful in the fingerprinting and classification of groundnut. A phyllogram generated by unweighted based on the available pedigree data. Hence, RAPD analysis should be a primary tool in the future research into the evolution of this genus.

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Phân tích đa dạng di truyền giữa các giống đậu phộng bằng phương pháp RAPD

Nghiên cứu này được thực hiện nhằm chứng minh lợi ích của RAPD marker trong sự kiện tách các tần suất allele khác nhau. Nucleic acid ly trích từ 29 giống đậu phộng được khuếch đại với 5 oligomer ngẫu nhiên bằng phương pháp PCR : RAPD 2, RAPD 3, RAPD 5, RAPD 6, OPC 11. Các RAPD chuyên biệt được tạo ra từ các giống này có thể được sử dụng như là cách đánh dấu genome để thiết lập tính đồng nhất của một kiểu gen được đưa vào. 29 giống đậu phộng được tách biệt rõ ràng thành các nhóm phụ riêng biệt trong sơ đồ phân nhóm bằng phương pháp UPGMA dựa vào các khoảng cách di truyền. Qua phân tích ghi nhận các giống với mức độ tương đồng từ 0.00 đến 0.74 (trung bình 0,56). Với mức độ này, chúng ta có thể chia ra hai nhóm. Nhóm 1 gồm 26 giống, nhóm hai 3 giống. Từ số liệu trên cung cấp nhiều thông tin phục vụ cho chọn vật liệu ban đều để lai tạo giống đậu phộng.