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GENETIC DIVERGENCE ANALYSIS ON *Cucumis* spp. BY RAPD MARKER

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ABSTRACT

The study of genetic diversity is necessary for efficient utilization, conservation and management of germplasm collections in cucumber. In this paper, the number of accessions, the variation of traits, the genetic diversity indexes and the coefficient of variation were employed to study geographical distribution of accessions, genetic divergence of major characters of cucumber by statistical analysis of CLRRI database. In this study, RAPD markers were used to investigate relationships among 14 cucumber cultivars. A total of 6 RAPD primed sites were used to calculate Jaccard's distance coefficients for cluster analysis using a unweighted pair-group method using an arithmetic averaging (UPGMA) algorithm. The genetic relationships identified using RAPD markers were highly concordant, such that the correlation between SSR and RAPD genetic distance (GD) estimates was r = 0.73 through RAPD analysis of 14 accessions allowed for their grouping into two distinct groups designated as CS and CM.

Keywords: Cucumber, Genetic diversity, Germplasm, RAPD

INTRODUCTION

The genus Cucumis of the *Cucurbitaceae* can be divided into an Asian group (x = 7) possessing only one species *C. sativus* and an African group (x = 12) comprising 30 species. The latter group can be further subdivided into six series: Humifructuosi, Melo, Hirsuti, Metuliferus, Angurioidei and Myriocarpi. *Cucumis hystrix* Chakr is a wild *Cucumis* spp. uniquely indigenous to South China. For *Cucumis hystrix* is the Asian *Cucumis* spp. described with its chromosome number 2n = 24 (Chen *et al.* 1997a). Although *C. hystrix* and *C. melo* L. possess the same number of chromosomes, isozyme patterns suggest that the phylogenetic relationship between *C. hystrix* and *C. sativus* (cucumber) is closer than that between *C. hystrix* and *C. melo* (melon) (Chen *et al.* 1995, 1997). Molecular markers have proved usefully as tools for assessing phylogenetic relationships in plant species (Burstin *et al.* 2001, Rossetto *et al.* 2002, Pham *et al.* 2003, Phan 2003, Buu and Lang 2004). Random amplified polymorphic DNA (RAPD) markers have been used for intra-genera analysis among *Cucumis* spp. Because its inherent technology is easy to perform, comparatively inexpensive The RAPD polymorphism level in cucumber and melon is 3–8 and 12–15%, respectively. *Cucumis melo* exhibits higher level of polymorphism in simple sequence repeat (SSR) (71%; Katzir *et al.* 1996) and restriction fragment length polymorphism (33%; Neuhausen 1992).

This reported with the establishment of genomic fingerprinting system for cucumber, starting with the rapid micro extraction of nucleic acid from 14 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 were summarized in 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 labeled 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◦C for any further use.

RAPD methodology

The PCR amplication (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 mM 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 thermal cycler (MJ Research Inc.) using the following cycling: 94◦C/4 min; three cycles at 94◦C/15s, 35◦C/15 s, 72◦C/75s; 35 cycles at 94◦C/15s, 40◦C/15s, 72◦C/75s; one cycle at 72◦C/7 min; 4◦C soak. A total of 9 random decamer primers were used to identify primers with both a relatively high level of polymorphism and banding pattern reproducibility. After initial screening, 5 primers were selected to detect polymorphisms in 28 accessions (Table 1). The products were separated on 1.2% agarose gels in 1M TAE bufer (40mM Tris–acetate, 1mM ethylene diaminetetraacetic 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 (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: $J_{ij} = a/(a+b)$, 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. 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 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

In order to understand overall genetic diversity, distribution of genetic diversity and formation modes of genetic diversity we analyzed the data from 14 accessions using several parameters. The hypotheses proposed here will supply a reference point for other studies on genetic diversity of local cucumber from An Giang and Ben Tre as well as for a better utilization, conservation and management of cucumber. This distribution was also affected by landscape, soil and climate conditions. A line could be roughly drawn from the Bay Nui (mountainous region) in the An Giang and Ba Tri, Ben Tre (coastal areas) based on the number of accessions and the locations where collections came from. The genetic diversity of cucumber genetic diversity was detected by analyzing variation in eight qualitative traits and four quantitative traits. The results were shown in Table 1. Generally, qualitative traits revealed less genetic diversity than quantitative traits.

The genetic diversity of different traits and the geographical distribution of genetic diversity were various. Genetic diversity varying from large to little was found for 12 traits with fruit weight, days to maturity,

leaf shape, number of flower, length of leaf.... The geographical distributions of accessions, the genetic diversity indexes of 12 traits were measured and observed.

Table 1. Characteristics of cucumber cultivars (14 varieties)

	Designation	Height after 25 days (cm)	Height after 47 days (cm)	Number of leaves	Length of leaf (cm)	Width of leaf (cm)	Number of flowers
1	PhungTuong	70.92b	173.19b	23.78a	15.10ab	19.57ab	4.54b
2	BinhPhu	85.91ab	221.04a	26.86a	14.78bc	18.80c	9.43ab
3	OMdua1	90.87ab	200.40ab	24.55a	14.36cd	17.33efg	8.76ab
4	OMdua2	92.24ab	188.47b	23.89a	13.95de	17.58def	6.76ab
5	OMdua3	86.89ab	194.76ab	24.85a	13.91de	17.90de	7.51ab
6	GiongTrom	89.58ab	184.75b	23.48a	15.06ab	19.78ab	5.02ab
7	DuaBaTri	78.66ab	198.68ab	22.86a	13.77ef	17.03fg	10.05a
8	OMdua4	83.56ab	196.85ab	23.33a	12.73g	15.90h	7.84ab
9	OMdua5	99.91a	198.81ab	24.67a	13.76ef	17.06fg	8.92ab
10	OMdua6	87.90ab	203.41ab	25.39a	14.90b	19.20bc	5.32ab
11	MaLai597	71.30b	190.71ab	22.53a	13.40f	17.60def	6.12ab
12	TriTon	70.43b	172.15b	22.29a	13.43f	16.85g	8.31ab
13	OMdua7	77.66ab	194.35ab	23.96a	14.10de	18.10d	5.95ab
14	DaXanh	86.51ab	192.25ab	24.53a	15.40a	20.11a	6.38ab
	CV (%)	16.01	8.25	13.03	1.79	2.05	36.05

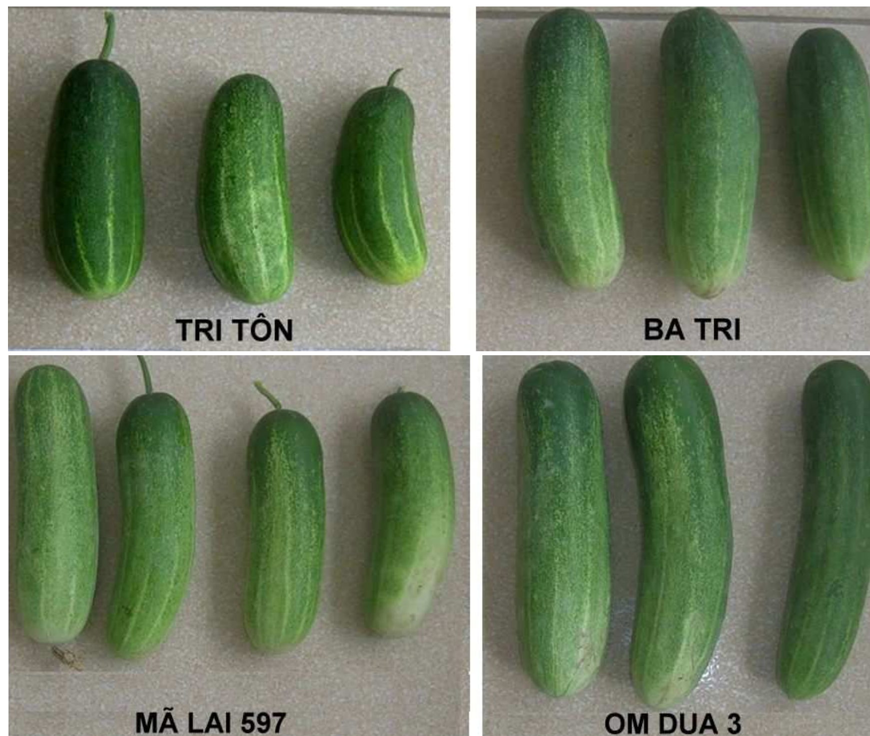

Figure 1. Evaluated phenotype of Cucumbers from different genotypes

Table 2. Yield and yield components

No	Lines	Weight of fruits (g)	Length of fruits (cm)	Width of fruits (cm)	Number of fruits/plant	Yield/ plant (kg)	Yield (t/ha)
1	PhungTuong	121.67c	14.23d	12.23cd	6.25ab	0.79bcd	16.47bcd
2	BinhPhu	172.78a	17.90a	13.47ab	8.70a	1.51a	31.53a
3	OMdua1	163.33ab	16.10bc	12.56bcd	8.05ab	1.35ab	28.23ab
4	OMdua2	134.22bc	15.82c	11.41d	5.21ab	0.70cd	14.66cd
5	OMdua3	166.39ab	17.44ab	12.09cd	4.60b	0.77bcd	15.97bcd
6	GiongTrom	120.00c	14.17d	11.97cd	4.39b	0.53d	11.13d
7	DuaBaTri	168.89a	16.33bc	13.92a	7.24ab	1.19abc	24.90abc
8	OMdua4	135.28bc	14.46d	12.79abc	7.62ab	1.02abcd	21.39abcd
9	OMdua5	148.33abc	16.96abc	11.88cd	5.81ab	0.86bcd	17.86bcd
10	OMdua6	135.00bc	14.40d	13.07abc	6.54ab	0.86bcd	17.94bcd
11	MaLai597	132.56bc	13.28de	12.49bcd	7.90ab	1.05abcd	22.04abcd
12	TriTon	115.56c	12.24e	12.79abc	6.80ab	0.78bcd	16.33bcd
13	OMdua7	120.00c	13.50de	11.87cd	5.50ab	0.70cd	14.52cd
14	DaXanh	115.00c	13.07de	12.06cd	5.70ab	0.64cd	13.38cd
	CV (%)	12.89	5.31	4.96	29.10	34.15	34.20

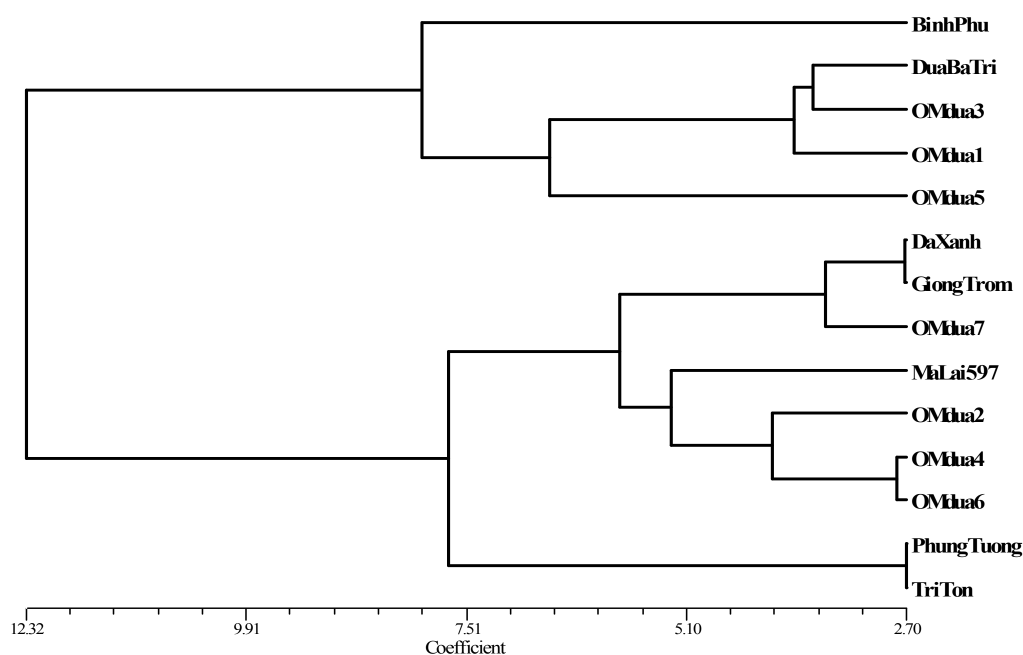

Figure 2. Dendrogram obtained by unweighted pair group method through phenotyping analysis from 14 cucumbers.

Table 3. The results of phenotyping analysis of 14 cucumbers

Group	Designation	Number of lines
A	Binh Phu, OMdua1, OMdua3, OMdua5, Dua Ba Tri	5
B	Phung Tuong, OMdua7, Tri tôn, Ma Lai 597, OMdua2, OMdua6, OMdua4, Giong Trom, Da Xanh	9

Genotyping by RAPD

PCR-based marker analysis used in the study revealed significant genetic divergence among genotypes These results were consistent with morphological and ecological divergence. All loci tested in this study were significant between variations of genotype components.

Table 4. List of RAPDs used in genetic divergence analysis

No.	RAPD	Sequence 5' ---------------- 3'	Number of alleles	Number of bands
1	AK 12	AGTGTAGCCC	5	21
2	OPA 10	GTGATCGCAG	6	47
3	OPC 11	AAAGCTGCGG	5	32
4	OPD 13	GGGGTGACGA	5	41
5	RAPD 2	GTTTCGCTCC	5	36
6	RAPD 5	AACGCGCAAC	2	5

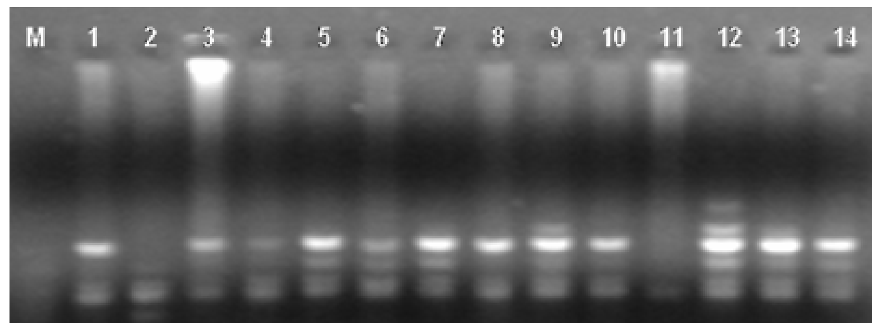

Figure 3. RAPD bands generated by RAPD at locus OPA10

1: Phung Tuong, 2: Binh Phu, 3: OMdua1, 4: OMdua2, 5: OMdua3, 6: Giong Trôm, 7: Dua Ba Tri, 8: OMdua4, 9: OMdua5, 10: OMdua6, 11: Ma Lai 597, 12: Tri Tôn, 13: OMdua7, 14: Da Xanh

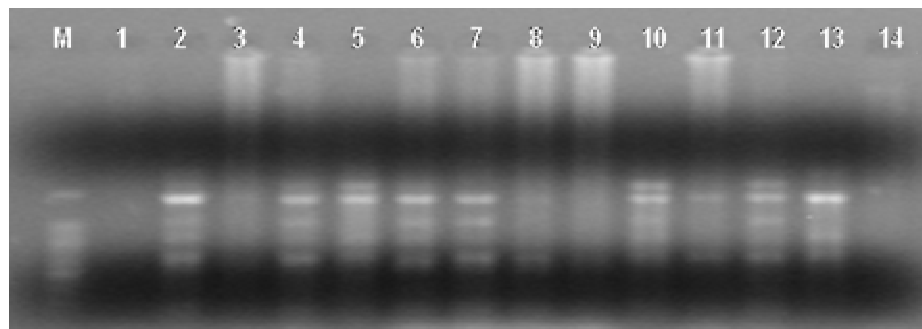

Figure 4. RAPD bands generated by RAPD at locus OPC11

1: Phung Tuong, 2: Binh Phu, 3: OMdua1, 4: OMdua2, 5: OMdua3, 6: Giong Trôm, 7: Dua Ba Tri, 8: OMdua4, 9: OMdua5, 10: OMdua6, 11: Ma Lai 597, 12: Tri Tôn, 13: OMdua7, 14: Da Xanh

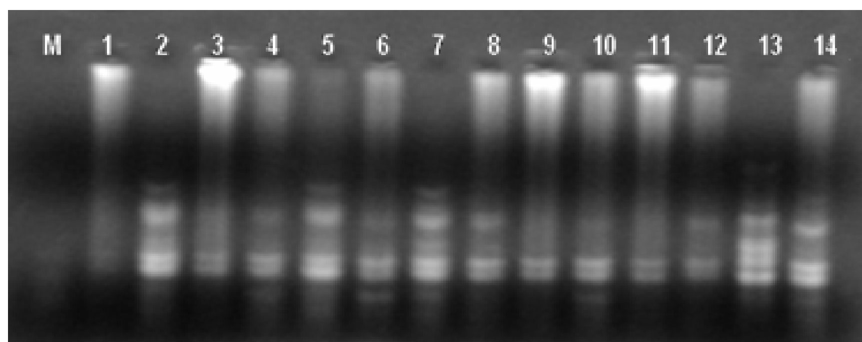

Figure 5. RAPD bands generated by RAPD at locus OPD13

1: Phung Tuong	4: OMdua2	7: Dua Ba Tri	10: OMdua6	13: OMdua7
2: Binh Phu	5: OMdua3	8: OMdua4	11: Ma Lai 597	14: Da Xanh
3: OMdua1	6: Giong Trôm	9: OMdua5	12: Tri Tôn	

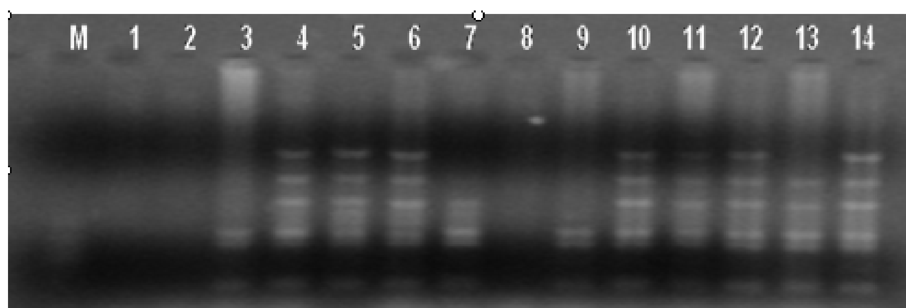

Figure 6. RAPD bands generated by RAPD at locus RAPD2

1: Phung Tuong	4: OMdua2	7: Dua Ba Tri	10: OMdua6	13: OMdua7
2: Binh Phu	5: OMdua3	8: OMdua4	11: Ma Lai 597	14: Da Xanh
3: OMdua1	6: Giong Trôm	9: OMdua5	12: Tri Tôn	

RAPD-based GD estimates

Six RAPD primers produced distinct, clearly visible polymorphisms (*i.e*. bright reproducible bands), and were thus used for the genetic relationship analysis. With a total of 14 cucumber accessions, 28 allele sites were scored with high degree of confidence. The polymorphisms identified were then used to generate the GD matrix (above the diagonal of Table 2). The molecular weights of bands analyzed ranged between 200 and 2,800 bp, of which 96% were polymorphic.

Genetic similarities among all individuals ranged from 0.33 to 0.66 with mean similarity of 0.30. For RAPD dendrogram, cophenetic correlation was estimated at r= 0. 73, corresponding to a very good fit. 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 genotypes could be divided into four groups based on the 6 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 0.33 to 0.66 (average of 0.56). The cluster A composed of only one variety: Dua Ba Tri. The largest cluster B included mainly 7 lines: OM dua leo 2, Dua Giong Trom, Dua Tri Ton, OM dua leo 6, Da Xanh, OMdua leo 3 and OM dua leo 7. Cluster C consisted 2 lines MaLai and Binh Phu. Cluster D included 4 lines: OM dua leo 4, OM dua leo 5, OM dua leo 1 and Phung Tuong. The various

relationships among these cultivars were shown in the phyllogram (Fig.2), generated by UPGM analysis of the genetic distances. The OM dua leo 1 and Dua Phung Tuong were closely related. This is also indicated by the great similarity in the flower structure and color patterns of these two cultivars. The close similarity between OM dua leo 1 and Dua Phung Tuong was observed because they had the same male parent.

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 OM dua leo 1 and Dua Ba Tri type can be recommended. Crosses between closely related genotypes like OM dua leo 1 and Dua Ba Tri are less likely to produce heterosis.

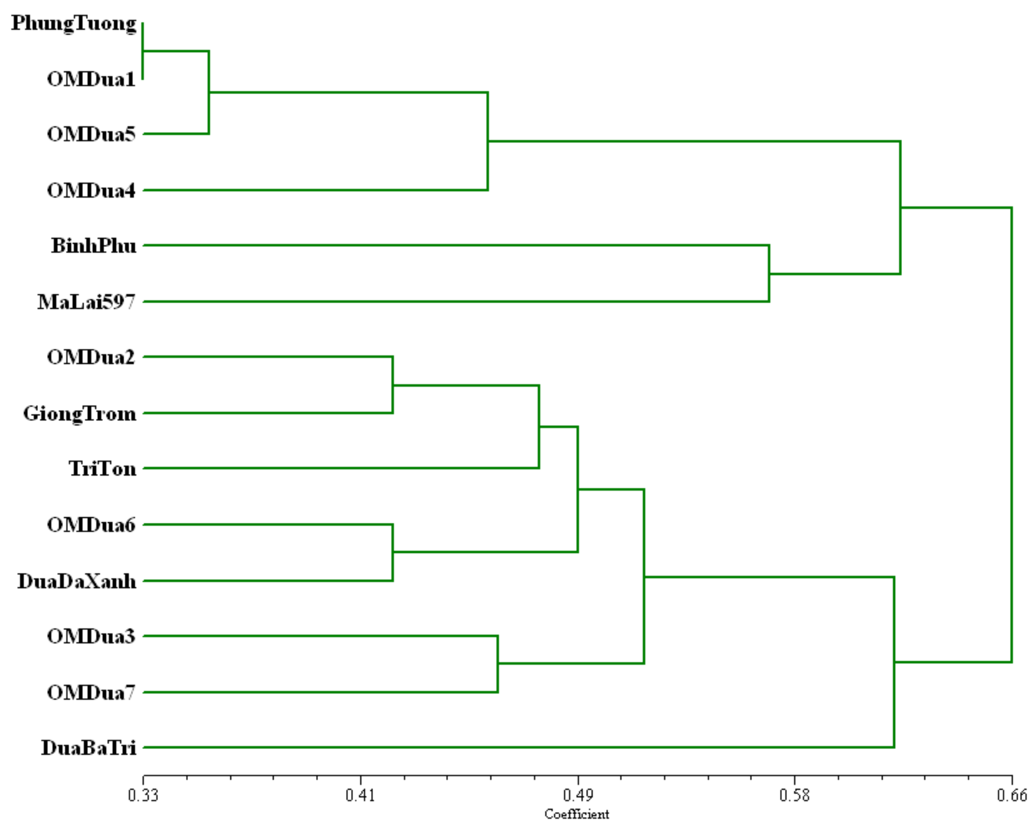

Figure 7. Dendrogram obtained by unweighted pair group method through genotyping analysis from 14 cucumbers.

Table 5. Genetic clustering of 14 cucumber accessions through genotyping analysis

Number	Designation	Cluster	Number	Designation	Cluster
1	Ba Tri	A	8	Ma Lai	C
2	OM dua leo 2	B	9	Binh Phu	C
3	Giong Trôm	B	10	OM dua leo 7	B
4	Tri Ton	B	11	OM dua leo 4	D
5	OM dua leo 6	B	12	OM dua leo 5	D
6	Da xanh	B	13	OM dua leo 1	D
7	OM dua leo 3	B	14	Phung Tuong	D

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Phân tích đa dạng di truyền của một số giống dưa leo

Nghiên cứu đa dạng di truyền vô cùng cần thiết cho nhà chọn giống trước khi lập kế hoạch chương trình cải tiến giống dưa leo. Chúng tôi đã thực hiện cả hai phân tích đánh giá kiểu hình và đánh giá kiểu gen nhờ RAPD marker để phân tích nhóm di truyền của 14 mẫu giống dưa leo được sưu tập ở ĐBSCL.

Phân tích trên 12 tính trạng nông học cần thiết, chúng tôi ghi nhận có 2 nhóm di truyền chính.

Phân tích kiểu gen nhờ RAPD tại 6 loci, chúng tôi ghi nhận có 4 nhóm di truyền chính với giá trị khoảng cách di truyền làm dữ liệu cho nhà chọn giống tham khảo trước khi quyết định sử dụng vật liệu lai.