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SHORT COMMUNICATION

SOYBEAN GENETIC DIVERSITY ANALYSIS

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ABSTRACT

PCR amplification of total genomic DNA using 20 random primers yielded scorable amplification products. The amplification product obtained with each of these primers was resolved on 1.2 % agarose gel. The size of amplification producted on agarose ranged between 0.3 and 3.0 kb. Seventeen primers detected polymorphism of all soybean accessions analyzed in the study.

The reliability of RAPD data for the classification of soybean was tested by subjecting the data to unweighted pair group method analysis of arithmetic means (UPGMA) in order to explore the possibility of classifying the cultivars using RAPD analysis. The phenotypic analysis of primers revealed the presence and extent of genetic similarities among the cultivars. The cluster analysis of RAPD data separated out the cultivars into five distinct clusters. However RADP data separated out OMDN varieties into three clusters. Cluster 2 included OMDN43 and OMDN29, cluster 3: OMDN34 and OMDN33, cluster 4: OMDN32, OMDN30, OMDN31. One genotype HL2 was separated farthest apart in the phenogram. This study confirm a close relationship between cultivars and evaluated genetic variation in 50 germplasm accessions of soybean, provides information about amount and distribution of genetic diversity within and among germplasm.

Key words: genetic cluster, polymorphism, RAPD, soybean germplasm

INTRODUCTION

Traditionally, the study of genetic diversity has fallen within population genetics which has focussed on measuring its extent in natural populations, in comparing levels of genetic divesity within and among populations and in making references on the nature and intensity of evolutionary processese from the observed patterns of genetic diversity. Hence, there is a long tradition as well as a wealth of conceptual tools in population genetics for analyzing, measuring and partitioning genetic diversity.

Furthermore, analysis of gene marker data enables esimation of the mating system and monitoring of genetic changes caused by factors affecting the reproductive biogogy of a species.

Soybean is considered as an important crop in rice-based cropping system in the Mekong Delta. It has obtained the highest average yield in the country as compared to other soybean cultivation areas. Its commercial value now becomes better due to the development of aquaproducts such as shrimp, fish for export in which they need a rich protein and high energy food from soybean, rice bran, and maize. All efforts by plant breeders have been focussed to introduction of soybean genotypes, pure line selection of traditional varieties and soybean hybridization to improve grain yield, short duration, resistance to major pests and diseases, and high grain quality. Genetic diversity analysis provides the understanding how to select appropriate parents from given clusters for succesful breeding program.

MATERIALS & METHODS

Fifty accessions of soybean were collected including inbred lines (varieties), landraces, introduction lines (varieties) (table 1). DNA extraction from these genotypes was done at CLRRI lab.

Table 1. List of soybean genotypes

No.	Name	0rigin	No.	Name	Origin
1	MTD664	CTU	26	OMDN 29	CLRRI
2	MTD455-3	CTU	27	OM DN 30	CLRRI
3	MTD652	CTU	28	OMDN 21	CLRRI
4	Valiant		29	OMDN 32	CLRRI
5	MTD 176	CTU	30	OMDN 33	CLRRI
6	G 25-2	CLRRI	31	OM DN 34	CLRRI
7	MTD 574	CTU	32	GC 9004-8-86	VASI
8	TL57	OCRI	33	AK 05	IAS
9	AK 05	IAS	34	OHFG1	Philippine
10	GC 90012-18-25-3		35	OHFG 3	Philippine
11	HSP3	Philippine	36	ATF15	IAS
12	Kehnam	Philippine	37	OMDN 43	CLRRI
13	OHFG 3	Philippine	38	VD N 2	OCRI
14	CLS 2111	OCRI	39	V 48	VASI
15	AGS 376	VASI	40	GC 90013-21-15-10	VASI
16	MSBR 22	OCRI	41	GC 90013-21-23-4	VASI
17	GC9001-1-41-9	VASI	42	A 20	VASI
18	VDN 1	OCRI	43	GC 9004-8-9-6	VASI
19	MTD 483-4	CTU	44	GC 90012-18-13-11	VASI
20	Meltrose	IAS	45	GC 90013-21-23-4	VASI
21	AGS 374	VASI	46	GC 90013-21-15-6	VASI
22	AGS 366	VASI	47	GC 9004-8-9-6	VASI
23	L3 75	IAS	48	GC 9001-41-5	VASI
24	DT 84	AGI	49	GC 9001-1-41-9	VASI
25	DT 93	AGI	50	GC 9001-8-86	VASI

AGI: Agricultural Genetics Institute, CLRRI: Cuu Long Delta Rice Research Institute, VASI: Vietnam Agricultural Science Institute, OCRI: Oil Crop Research Institute, IAS: Institute of Agricutural Science for South Vietnam, CTU: CanTho University.

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°C for 5 minutes followed by 35 cycles of PCR amplification using the following parameters: 1minute denaturation of 93°C, 1minute primer annealing at 37°C and 2 min primer extension at 72°C. Completion of

primer extension was alllowed by a final 8 min incubation at 72°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

Genetic distances were computed according to Nei (1975). Computation were facilitated by the PC- based program NTSYS (Rohlf 1992).

RESULTS & DISCUSSION

Primer screening

To identify primers that detect polymorphism, 20 primers from the OPA kit were screened on the total DNA obtained from the leaves of 50 varieties. The amplified products obtained with each of these primers were resolved on 1.2% agarose gels and scutinized for the polymorphism and consistency of amplifications and the 12 discriminatory primers

Of these 20 primers, three failed to yield amplification products. The remaining (17) yielded reproducible fragments and at least 61 loci were scorable. The size of the fragments ranged from 300 to 3500 bp. Figure 1, 2, and 3 show the fragments in amplified DNAs obtained with OPA primers.

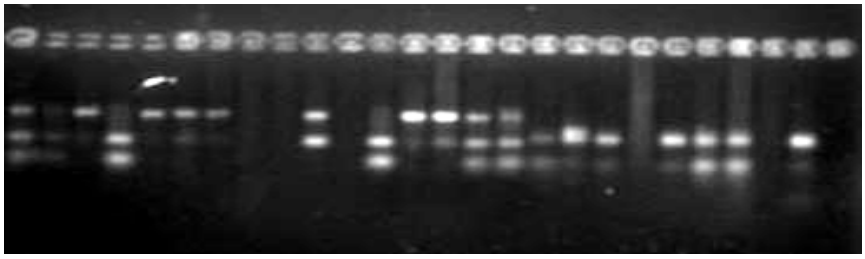

Figure 1: RAPD fragments amplified by OPA primers in 25 soybean genotypes .

These are probably sufficient for identifying the distinct cultivars of soybean, as 50 accessions included in this study represent the whole range of variation occuring naturally.

PCR amplification of total genomic DNA using 20 random 10-mer primers yielded scorable amplification products. The size of amplification produced scored in 1.2 % agarose gels ranged between 0.2- 3.0 kb.

Table 2. List of RAPD primers

Primer	Sequence	No. of bands detected	Size of products (kb)
OPA-03	GCTCAGCCAC	2	0.3-0.2
OPA-04	AATCGGGCTG	2	0.4-3.0
OPA-10	GTGATCGCAG	5	0.3-2.5
OPA-13	CAGCACCCAC	6	0.2-3.0
OPC-06	GAACGGACTC	2	0.5-2.5
OPC-11	AAAGCTGCGG	4	0.3-1.7
OPC-15	GACGGATCAG	3	0.3-3.0
OPD02	GGACCCAACC	5	0.3-2.5
OPD -03	GTCGCCGTCA	4	0.3-3.0
OPD 07	TTGGCACGGG	4	0.2-3.0
OPD-08	GTGTGCCCCA	3	0.3-2.5
OPD-13	GGGGTGACGA	4	0.2-2.0

The usefulness of a technique for germplasm characterization depends on its ability to sample any portion of the genome, study markers on all the linkage groups, detect genetic differences among distinct genotypes, classify the accessions into specific groups which should be comparable to the accepted classifications and screen large number of samples as required in a gene bank.

The reliability of RAPD data for clasification of soybean was tested by subjecting the data to unweighted pair group method analysis of arithmetic means (UPGMA) in order to explore the possibility of classifying the cultivars using RAPD analysis. The phenotypic analysis primers revealed the presence and extent of genetic similarities among the cultivar (GC) (figure 3). However RADP data separated out OMDN varieties into three clusters. Cluster 2 included OMDN43 and OMDN29, cluster 3: OMDN 34 and OMDN 33, cluster 4: OMDN32, OMDN30, OMDN31. One genotype HL 2 was separated farthest apart in the phenogram.

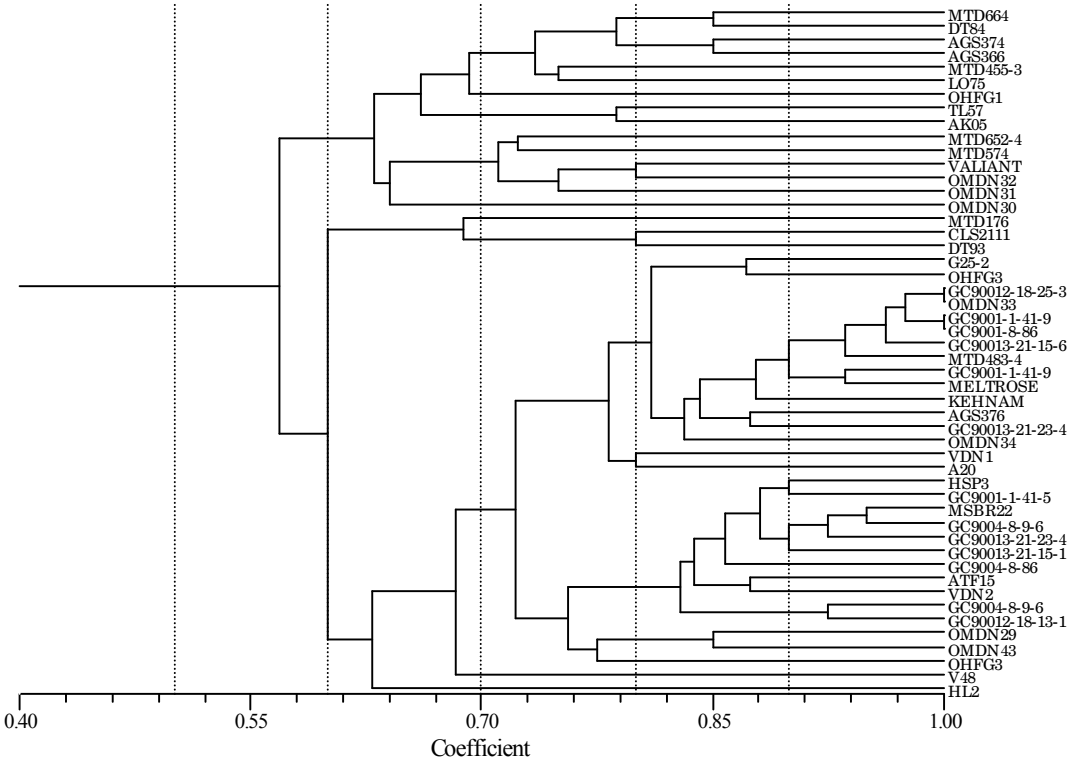

Figure 2: Phenogram resulting from RADP analysis of relationships among 50 accessions

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SUMMARY IN VIETNAMESE

Phân tích đa dạng di truyền bộ giống đậu nành ở ĐBSCL

Đậu nành là cây trồng đáp ứng yêu cầu chuyển đổi cơ cấu cây trồng phục vụ sản xuất nông nghiệp ở Đồng Bằng Sông Cửu Long. Nghiên cứu bước đầu điều tra và sưu tập nguồn gen cây đậu nành, đồng thời phân nhóm di truyền với 50 giống du nhập từ các nguồn khác nhau, thông qua áp dụng kỹ thuật chỉ thị phân tử RAPD.

PCR khuếch đại phân tử DNA với 20 primers ngẫu nhiên, có mã trình tự là 10 mer. Sản phẩm khuếch đại phân tích điện di trên agarose gel 1,2%. Kích thước trọng lượng phân tử biến thiên từ 0,2 đến 3,0 kb. Với kỹ thuật RAPD, thông qua 17 chỉ thị, biểu hiện đa hình trên tất cả các giống phân tích đã được ghi nhận. Phân tích dựa trên UPGMA cho thấy có 5 nhóm chính và nhiều nhóm phụ. Các giống thuộc chương trình lai tại tại VLĐBSCL ký hiệu là OMDN được tách ra thành ba nhóm. Nhóm 2: OMDN43 và OMDN29, nhóm 3: OMDN 34 và OMDN33, nhóm 4: OMDN32, OMDN30, OMDN31. Giống HL 2 nằm trong một nhóm riêng biệt có khoảng cách rất xa với nhóm khác.