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Research Article
Evaluation of Genetic Diversity of Black Soybean [*Glycine max* (L.) Merr] by Using RAPD and ISSR Markers

[1]Thanh Tuan Nguyen, [1]Van Loc Nguyen, [2]Quoc Khanh Nguyen, [2]Truong Son Dinh, [3]Thi Huong Giang Doan and [3]Thi Lieu Le

[1]Department of Genetics and Plant Breeding, Faculty of Agronomy, Vietnam National University of Agriculture, Hanoi, Vietnam
[2]Department of Plant Biotechnology, Faculty of Biotechnology, Vietnam National University of Agriculture, Hanoi, Vietnam
[3]Detection Laboratory of Genetically Modified Organisms, Agricultural Genetics Institute, Pham Van Dong, Bac Tu Liem, Hanoi, Vietnam

Abstract

Background and Objective: Black soybeans [*Glycine max* (L.) Merr] are among the important crops, but the cultivated resources are normally low-yielding, susceptible to diseases and low profit. Therefore, it is necessary to evaluate the genetic diversity of black soybean germplasms for breeding programs. This study investigates the genetic diversity of 22 black soybean varieties by RAPD and ISSR markers. **Materials and Methods:** Twenty two black soybean genotypes in Vietnam were evaluated for genetic diversity by using Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) molecular markers. Data were scored following a binary matrix and analyzed using NTSYSpc 2.1 (Numerical Taxonomy and Multivariate Analysis System). **Results:** All 20 RAPD and 11 ISSR markers produced scorable bands. As 230 loci were investigated over the population, of which 107 were polymorphic, accounting for 46.5%. The collection of 22 black soybean varieties had a relatively close relationship with high genetic similarity coefficients, ranging from 0.71-0.99. Two main genetic clusters were classified. The RAPD markers showed better performance than ISSR markers in evaluating the genetic diversity of these 22 black soybean varieties. **Conclusion:** The results of this study display that 22 Vietnamese black soybean varieties are relatively identical in genetics. The study is suitable for breeding programs to improve black soybean varieties.

Key words: Black soybean, *Glycine max* (L.) Merr, molecular markers, genetic diversity, random amplified polymorphic DNA, inter-simple sequence repeat

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Corresponding Author: Thi Lieu Le, Detection Laboratory of Genetically Modified Organisms, Agricultural Genetics Institute, Pham Van Dong, Bac Tu Liem, Hanoi, Vietnam

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr] is one of the most important crops in the world, accounting for about 60% of global production[1]. Soybean is a valuable source of protein and vegetable oil that can be used popularly both for human consumption and animal feed. Soybeans are also rich in health-beneficial compounds such as isoflavone, tocopherols and saponin, antioxidants, minerals and vitamins, which have properties of anti-aging, anti-cancer, anti-bacterial and anti-diabetes[2,3]. Black soybean (black seed husk), the only soybean type contains anthocyanins, the important functional nutrients and antioxidants that benefit human health. Nowadays, the demand for black soybeans for producing food, especially for making medicine and functional foods is increasing. Therefore, the demand for the cultivation of good black soybean germplasms is rising.

In Vietnam, soybean is a traditional crop and plays a crucial role in agricultural production due to its high economic value and effective usage in crop rotation. Black soybeans are also highly expected for processing because of their health benefits. The cultivation of soybeans is mainly in the Northern Region of Vietnam, with higher production from the Northern Mountainous Region. The production scale is small at the householder level. Presently, the cultivation area of soybeans is decreasing due to the dominance of other industrial crops with higher economic efficiency. This decrease leads to a steady shortage in soybean output. The cultivation is normally exploited from local, old varieties that give low productivity, low economic efficiency. This consequence is due to the lack of good varieties, adaptable to ecological regions. Therefore, it is necessary to collect and evaluate the diversity of genetic resources of black soybeans to serve for the breeding of new improved varieties.

Genetic resources play an important role in crop improvement and sustainable agricultural production. The biodiversity of plant genetic resources is the key to assessing the potential value of germplasm, which then can be used as donors of resistant genes for breeding programs. The diversity of genetic germplasms can be characterized by many techniques, for example: Morphological traits, biochemical traits or molecular markers. Among those, a molecular marker is a powerful tool for the genetic characterization of plant resources. The RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter Simple Sequence Repeat) molecular markers have been considered reliable methods for evaluating genetic diversity in soybean[4-6] and other crops.

In Vietnam, some studies have been conducted to investigate the genetic diversity of soybean varieties but not for black soybean[7]. Therefore, this study aims to evaluate the genetic diversity among 22 black soybean varieties using RAPD and ISSR markers to conserve biodiversity and improve the black soybean varieties.

MATERIALS AND METHODS

Study area: The study was conducted at the Faculty of Agronomy and Biotechnology, Vietnam National University of Agriculture, Vietnam, from January to May, 2023.

Plant materials: The collection of 22 black soybean varieties was collected from some provinces in the Northern Mountain Region, Vietnam and was provided by the Plant Resources Center (Table 1). Molecular markers used for the evaluation of genetic diversity are RAPD and ISSR (Table 2-3).

Research procedure: Seeds of 22 black soybean varieties (Table 1) were planted to collect young leaves to extract DNA for genotyping.

DNA extraction and PCR amplification: The DNA was extracted from about 100 mg fresh young leaves, using CTAB method[6] with minor modifications. Briefly, fresh leaves were ground in nitrogen by crusher. Freshly add 10 µL of 2-mercaptoethanol and 15 mg PVPP to 1 mL of extraction buffer (per 100 mL: 0.5 g CTAB, 1 g EDTA, 2.5 g Trisbase and 5 g NaCl) and supplement this buffer to the tubes containing sample powder. Incubate the samples at 65°C for 10 min with gentle shaking. Then, centrifuged the samples, transferred the supernatants to new tubes and added an equivalent volume of phenol:chloroform:isoamyl alcohol (25:24:1) to the tubes to homogenize the samples. Second homogenization was done with chloroform:isoamyl alcohol (24:1). The DNAs were precipitated by an equivalent volume of cold isopropanol and washed twice with 70% ethanol. The dry pellet of DNAs was dissolved in RNase free water. The DNAs were quantified and qualified by 1% agarose electrophoresis and spectrophotometer measurement at 260/280 nm wavelength (Eppendorf BioPhotometer Plus).

The ISSR UBC primers (designed by the University of British Columbia, Canada) and RAPD decamer oligonucleotides (designed by Operon Technologies Inc. Alameda California USA) were used (Table 2-3). Amplification reactions were run using a MyTaq™ kit in a total volume of 20 µL, including 10 µL of master Mix 2X, 0.5 µM primer and 20 ng of genomic DNA. Amplification was done in T100 Thermal Cycler (Bio-Rad) with following programs: (1) Predenaturation at 94°C for 5 min, (2) Denaturation at 94°C

Table 1: List of 22 black soybean varieties

No	Code	Name of variety	Origin of variety
1	G1	Black soybean 1	Cao Bang
2	G2	Black soybean 2	Plant Resources Center
3	G3	Black soybean 3	Plant Resources Center
4	G4	Black soybean 4	Plant Resources Center
5	G5	Black soybean 5	Plant Resources Center
6	G6	Tau pau du	Plant Resources Center
7	G7	Black soybean 6	Plant Resources Center
8	G8	Tau pvieng	Plant Resources Center
9	G9	Black soybean 7	Son La
10	G10	Black soybean 8	Plant Resources Center
11	G11	Tau lu	Plant Resources Center
12	G12	Black soybean 9	Plant Resources Center
13	G13	Hoang tau	Plant Resources Center
14	G14	No-518937	Plant Resources Center
15	G15	Tau nhay 1	Plant Resources Center
16	G16	Black soybean 10	Plant Resources Center
17	G17	K92	Plant Resources Center
18	G18	Tau nhay 2	Plant Resources Center
19	G19	Black soybean 11	Plant Resources Center
20	G20	DN - black	Plant Resources Center
21	G21	Black soybean 12	Son La
22	G22	Tau nhay 3	Plant Resources Center

Table 2: Performance of RAPD primers on 22 black soybean varieties

Primer	Nucleotide sequence (5′-3′)	Annealing temperature (°C)	Amplified loci	Polymorphism (%)	Total bands/primer	PIC value	Band informativeness (I_B)	Resolving power (Rp)
OPC02	GTGAGGCGTC	34.0	7	57.14	102	0.81	1.32	9.27
OPC03	GGGGGTCTTT	34.0	10	50.00	188	0.89	1.71	17.09
OPC04	CCGCATCTAC	34.0	7	42.86	145	0.86	1.88	13.18
OPC05	GATGACCGCC	34.0	6	0.00	132	0.83	2.00	12.00
OPC08	TGGACCGGTG	32.0	12	50.00	221	0.91	1.67	20.09
OPC13	AAGCCTCGTC	32.0	6	33.33	93	0.77	1.41	8.45
OPC18	TGAGTGGGTG	32.0	10	30.00	187	0.89	1.70	17.00
OPB02	TGATCCCTGG	34.0	7	57.14	142	0.86	1.84	12.91
OPB03	CATCCCCCTG	34.0	8	50.00	164	0.87	1.86	14.91
OPB05	TGCGCCCTTC	34.0	7	28.57	132	0.84	1.71	12.00
OPB06	TGCTCTGCCC	34.0	13	84.62	164	0.90	1.15	14.91
OPS05	TTTGGGGCCT	32.0	6	16.67	112	0.81	1.70	10.18
OPO01	GGCACGTAAG	32.0	10	90.00	105	0.85	0.95	9.55
OPO02	ACGTAGCGTC	32.0	6	33.33	121	0.83	1.83	11.00
OPN01	CTCACGTTGG	32.0	10	30.00	195	0.89	1.77	17.73
OPN03	GGTACTCCCC	34.0	8	75.00	109	0.85	1.24	9.91
OPE04	GTGACATGCC	32.0	11	36.36	217	0.91	1.79	19.73
OPE07	AGATGCAGCC	32.0	9	33.33	159	0.87	1.61	14.45
OPA04	AATCGGGCTG	34.0	6	33.33	113	0.82	1.71	10.27
OPS10	ACCGTTCCAG	32.0	6	50.00	72	0.72	1.09	6.55
	Total		165		2873			
	Average/primer		8.25	44.08	143.65	0.85	1.60	13.6

for 30 sec, (3) Annealing at relevant temperatures of each primer as expressed in Table 2 and 3 for 30 sec and (4) Extension at 72°C for 2 min, repeat from step 2 to 4 for 35 cycles and final extension at 72°C for 10 min. Amplification products were analyzed by electrophoresis in 1% agarose gel containing stain solution Redsafe™ and photographed by UVP BioDoc-It Imaging Systems.

Data analysis: The amplified bands of the markers were scored manually following a binary matrix with 1 as present and 0 as absent. The data were analyzed by using NTSYSpc ver 2.1[8]. The polymorphism information content (PIC) value for each marker was calculated as:

$$PIC(i) = 1-\Sigma(P_{ij})^2$$

where, P_{ij} is the frequency of the i_{th} allele in the genotypes expressed by the j_{th} primer summed across all alleles of one locus. The resolving power (Rp) of a primer is calculated as:

$$Rp = \Sigma Ib$$

where, Ib stands for band informativeness and takes the value of 1-(2×(05-p)), with p representing the proportion of individuals containing the band. Clustering analysis was based on the unweighted pair group method with arithmetic (UPGMA) of the Sokal-Michener matrices similarity coefficient and the dendrogram was constructed with the TREE program. Clustering analysis was compared with analysis by Discriminant Analysis of Principal Components (DAPC) implemented in R package adegenet 2.1.3[9]. The find clusters function was used to determine the optimal number of genetic clusters (K) based on the Bayesian information criterion (BIC) with K varying from 2 to 12. Genetic distance matrix correlation between markers was estimated using a Mantel test in XLSTAT[10].

RESULTS AND DISCUSSION

Extracted DNAs of 22 black soybean varieties had bright and compact bands, as exemplified in Fig. 1. The numbers shown in the figure were the numerical order of black soybean varieties as expressed in Table 1.

The index of optical density (OD, ratio 260/280 nm) was in the range of 1.7-2.1. The extracted DNAs meet the quality and quantity requirements for PCR reactions and later genetic analysis.

Table 3: Performance of ISSR primers on 22 black soybean varieties

Primer	Nucleotide sequence (5'-3')	Annealing temperature (°C)	Amplified loci	Polymorphism (%)	Total bands/primer	PIC value	Band informativeness (I_B)	Resolving power (Rp)
ISSRT1	GTGTGTGTGTGTCC	50	4	25.0	76	0.73	1.73	6.91
UBC809	AGAGAGAGAGAGAGAGG	51.9	6	50.0	129	0.83	1.95	11.73
UBC824	TCTCTCTCTCTCTCTCG	50	6	66.7	116	0.83	1.76	10.55
UBC823	TCTCTCTCTCTCTCTCC	50	7	85.7	125	0.85	1.62	11.36
UBC812	GAGAGAGAGAGAGAGAA	50	5	40.0	100	0.79	1.82	9.09
UBC873	GACAGACAGACAGACA	45	4	75.0	74	0.74	1.68	6.73
UBC864	ATGATGATGATGATGATG	45	7	28.6	129	0.84	1.68	11.73
UBC826	ACACACACACACACACC	51.9	5	0.0	110	0.80	2.00	10.00
UBC836	AGAGAGAGAGAGAGAGYA	51.9	7	42.9	144	0.86	1.87	13.09
UBC811	GAGAGAGAGAGAGAGAC	50	7	28.6	140	0.85	1.82	12.73
UBC840	GAGAGAGAGAGAGAGAYT	51.9	7	57.1	102	0.81	1.32	9.27
	Total		65		1245			
	Average/primer		5.9	44.6	113.2	0.81	1.75	10.29

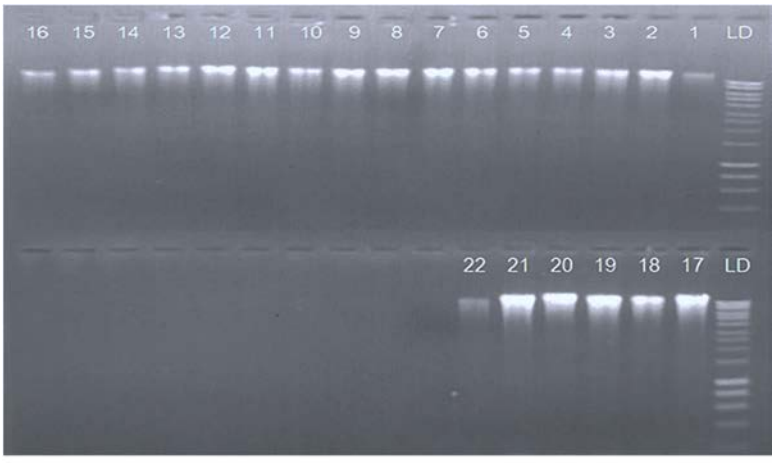

Fig. 1: Total genomics of extracted DNAs

LD: DNA ladder and Number 1-22: Numerical order of 22 black soybean varieties

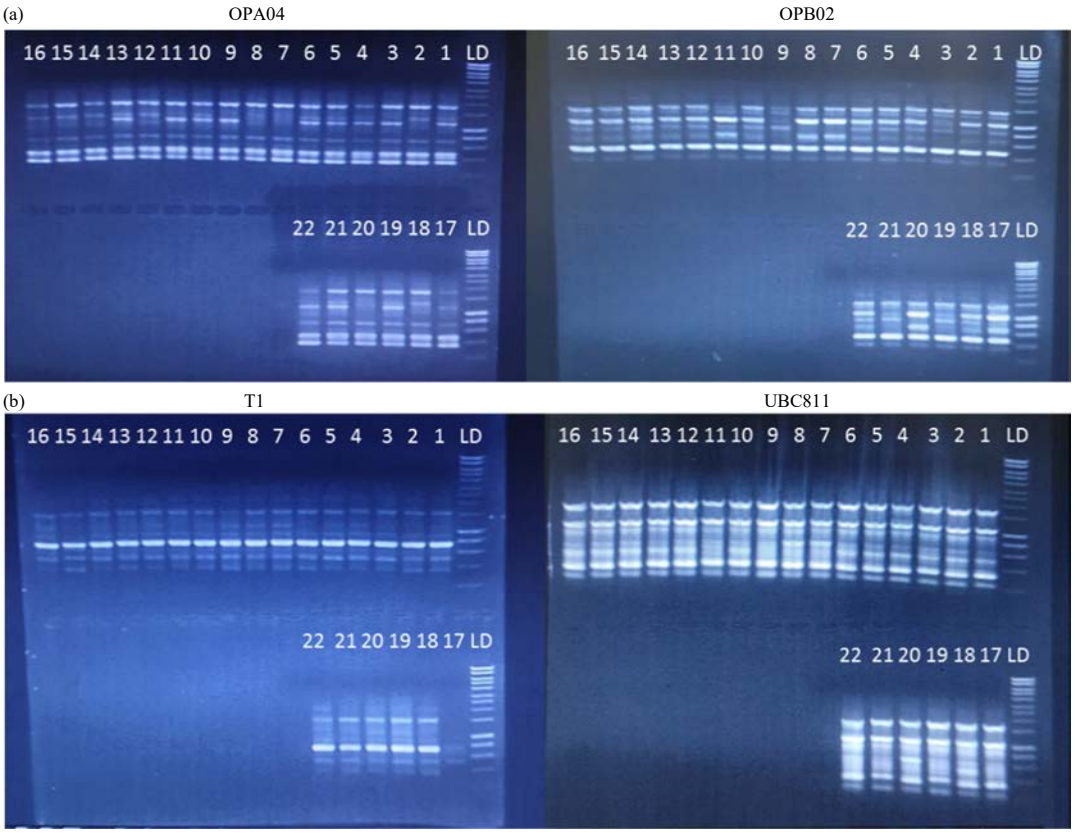

Fig. 2(a-b): Results of 22 Vietnamese black soybean accessions with (a) RAPD (OPA04 and OPB02) and (b) ISSR (T1 and UBC811) markers

Polymorphism of black soybean accession by the RAPD and ISSR markers: All 20 RAPD primers used in the study produced scorable bands. Two examples of RAPD profiles (OPA04, OPB02) were expressed in Fig. 2. A total of 2873 bands were detected for the whole accession of black soybeans with an average of 143.65 bands per variety. In which, 165 loci were observed, ranging from 6-13 loci per primer, averaged 8.25 loci/primer. Among the total detected loci, 77 loci were polymorphic and accounted for 46.67%. The polymorphic loci ranged from 0 (OPC05)-to 11 (OPO01), taken from 0 to 90%, respectively (Table 2). The PIC values were lowest for primer OPS10 (0.72) and highest for primer OPC08 and OPE04 (0.91), average value was 0.85 (Table 2). The resolving power of RAPD markers varied from 6.55 (OPS10) to 20.09 (OPC08), with an average of 13.06 for each primer.

Similarly, for 11 ISSR markers, they all produced scorable bands. The ISSR profiles were exampled by T1 and UBC811 as shown in Fig. 2. Sixty five loci were investigated from 11 ISSR markers. The amplified loci per primer ranged from 4-7, with an average of 5.9. Among the amplified bands, 29 loci were polymorphic and accounted for 44.6%. The UBC826 expressed no polymorphic bands, while UBC823 showed highly polymorphic among ISSR primers. The primer that showed a lower number of bands also expressed lower PIC values (ISSRT1 and UBC873), lower resolving power and vice versa (UBC823, UBC864, UBC836 and UBC811, Table 3).

In Vietnam, some studies on genetic diversity have been conducted in soybeans by molecular markers but have not been conducted in black soybeans. In this study, the percentage of polymorphism of RAPD markers (44.06%) was somehow similar to ISSR markers (44.6%) in the black soybean collection of 22 varieties. The report in this collection of the present study showed a relevant level of polymorphism to Egyptian soybean varieties (43.48%) by SDS-PAGE and ISSR markers[11], lower than Iran soybean collection (60%) by ISJ and RAPD markers[12], various genotypes from different countries by using RAPD markers[13], but much lower than Indian soybean collection (97.25%) by ISSR markers[14]. The difference in the polymorphic levels of Vietnamese and Indian soybean collections might come from the original diversity of used materials. In this present study, the PIC value of RAPD markers was higher than ISSR markers, this index provides more valuable information from RAPD markers on heterozygosity and differentiation among varieties. The PIC values of RAPD and ISSR markers in this study were higher than the value of SSR markers in the Vietnamese soybean collection[7].

The resolving power index of the markers indicates the correlation between genotypes and DNA markers, specifies the discriminatory potential of markers and estimates the ability to produce informative bands. The higher the Rp values, the more effective the marker is in genotyping. The resulting resolving power of RAPD markers was higher than this value of ISSR markers, this inferred the more effective RAPD than ISSR provides in genotyping. The usage of RAPD and ISSR markers in this black soybean collection is more effective than RAPD markers in the common bean collection with an average Rp value of 3.86[15].

Genetic relationship of black soybean accession: The genetic relationship of 22 black soybean varieties was based on the genetic similarity coefficient from NTSYS analysis, the higher the coefficient the closer the samples are and vice versa. Data were analyzed separately for RAPD, ISSR markers and both types of markers. The similarity coefficients between 22 samples ranged from 0.73 (G1 vs G20)-0.99 (G7 vs G8), 0.71 (G12 vs G22)-0.98 (G1 vs G2) by RAPD, ISSR markers, respectively (Table 4) and 0.76 (G12 vs G22) to 0.97 (G7 vs G8 and G2 vs G5) by both types of markers (data unpublished). The average of similarity coefficients was 0.85 for both analyses in separation and combination. The results showed a relatively close relationship among this collection of black soybeans expressed by RAPD and ISSR markers.

From the similarity coefficient, clustering of 22 black soybean varieties was conducted following the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method in NTSYSpc 2.1 software. The dendrogram showing the genetic relationship among varieties was plotted separately from RAPD, ISSR and both markers (Fig. 3a-c). To compare the clustering of genotypes in each group, genetic data were analyzed by DAPC (Discriminant Analysis of Principal Components) in R package adegenet 2.1.3[9]. This classification of genotypes was shown in Fig. 3d with K = 2. The assignment of varieties in each cluster was different from ISSR markers to RAPD and a combination of both markers. Both UPGMA clustering in NTSYS and DAPC in adegenet analyses gave similar results of varieties assignment from RAPD and a combination of RAPD and ISSR markers. Based on the similarity of RAPD markers and the combination of RAPD and ISSR markers, the assignment of varieties in each cluster was concluded.

At the similarity level of 81%, 22 black soybean varieties were divided into 2 main clusters. The cluster I and II had 16 and 6 black soybean varieties, respectively (Table 1, Fig. 3a-d). Based on high genetic similarity, G2 vs G5 and G7 vs G8 had the closest genetic relationship (Fig. 3a, c). To see the link between genotypic diversity the phenotypic variation, some main characteristics that contributed to the productivity of varieties have been scored (unpublished data). The result showed that black soybean 6 (G7) and Tau paving (G8) had relatively similar performances in almost all features. This close linkage from genotype to phenotype between black soybean G7 and G8 was seen clearer from RAPD analysis than ISSR or a combination of RAPD and ISSR.

Compared with the Vietnamese soybean collection which had a broad range of similarity coefficients (0.60-0.98), this black soybean collection had a narrower range (0.73-0.99) and higher similarity coefficients, which means the close genetic relationship in this black soybean varieties[7]. Indian soybean collection had also a broader range from 0.27-0.89 with an average of 0.58 value of similarity coefficients, which showed a higher level of genetic variation among those soybean genotypes[14]. In general, soybean genetic resources are reported to be less diverse[16] and therefore affect the ability to select original materials for breeding programs to improve varieties[17].

Comparative study of genetic diversity of RAPD- and ISSR-based: The use of different markers in combination will provide more precise information on studied genotypes[16]. Results from this study were in line with that opinion. The outcome of the analysis from the combination was appropriate with the analysis separation of RAPD markers. Moreover, the analysis by two different methods NTSYS and DAPC gave similar results. The different outcome from ISSR markers might be from the lesser number of used markers and lower values of resolving power of this marker type.

Investigating the arithmetic means of RAPD and ISSR markers from Table 2 and 3, data revealed that most indexes generated by RAPD expressed better than ISSR, for example, the loci amplified per primer (8.25 vs 5.9), the total bands amplified per primer (143.7 vs 113.2), the PIC value (0.85 vs 0.81) and the resolving power of primers (13.06 to 10.29). The index of band informativeness of RAPD markers was lower than ISSR (1.6 vs 1.75) markers and the index of polymorphic level showed a bit higher or equivalent (44.08 vs 44.6). These results suggested that RAPD markers were more efficient than ISSR markers in investigating the genetic diversity of black soybean collection. However, the ISSR markers were more effective in giving the band informativeness and contributing more information for genotype studying. The current results agreed with the study reported on Indian lettuce *Lactuca indica* and Indian *Mangifera indica* which showed more efficiency from RAPD markers by most of the indexes[18,19].

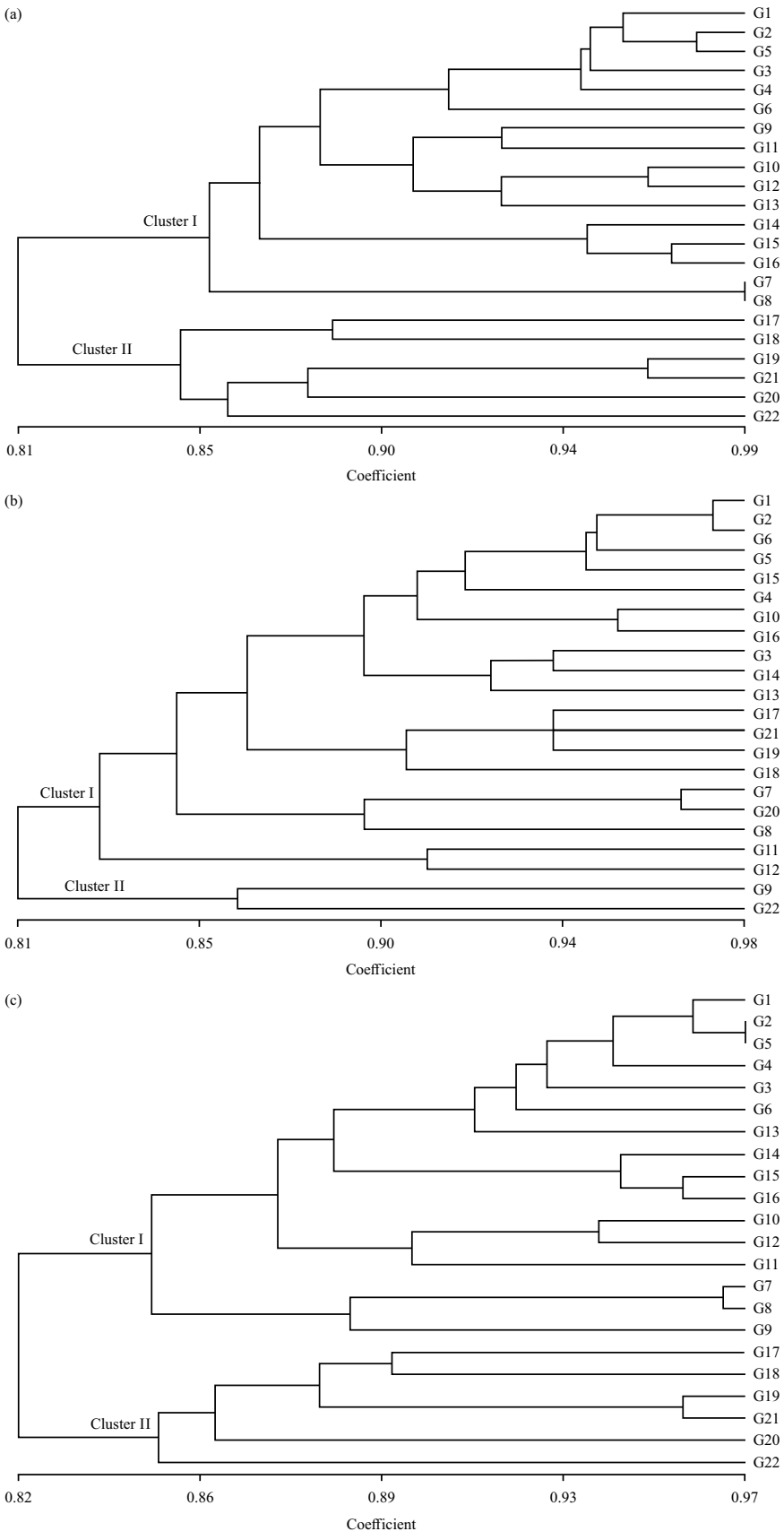

Fig. 3(a-d): Continue

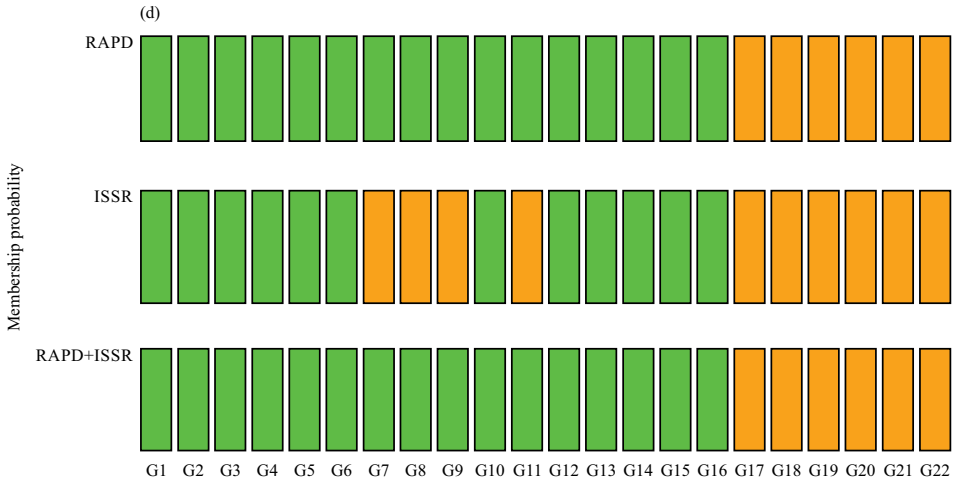

Fig. 3(a-d): Dendrogram of genetic relationship of 22 black soybean varieties constructed by UPGMA clustering from (a) RAPD, (b) ISSR, (c) Combined RAPD and ISSR markers and (d) by DAPC analysis

Table 4: Matrix of genetic similarity coefficient among 22 Vietnamese black soybean varieties analyzed by RAPD and ISSR markers

	ISSR																					
RAPD	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22
G1		0.98	0.91	0.92	0.94	0.98	0.89	0.80	0.82	0.91	0.78	0.85	0.91	0.91	0.94	0.89	0.92	0.91	0.86	0.89	0.86	0.80
G2	0.95		0.89	0.94	0.95	0.97	0.91	0.82	0.83	0.92	0.80	0.86	0.92	0.92	0.95	0.91	0.94	0.92	0.88	0.91	0.88	0.82
G3	0.95	0.95		0.83	0.88	0.89	0.80	0.83	0.75	0.85	0.85	0.88	0.91	0.94	0.88	0.86	0.83	0.85	0.80	0.80	0.86	0.74
G4	0.94	0.94	0.95		0.92	0.91	0.88	0.78	0.80	0.89	0.74	0.83	0.86	0.86	0.89	0.88	0.88	0.86	0.85	0.88	0.82	0.75
G5	0.96	0.98	0.96	0.96		0.95	0.89	0.80	0.82	0.91	0.75	0.85	0.94	0.91	0.94	0.92	0.89	0.88	0.89	0.89	0.86	0.80
G6	0.92	0.93	0.91	0.89	0.93		0.88	0.78	0.80	0.89	0.77	0.83	0.92	0.92	0.95	0.91	0.91	0.89	0.85	0.88	0.85	0.82
G7	0.82	0.84	0.83	0.84	0.85	0.87		0.91	0.89	0.89	0.77	0.83	0.83	0.83	0.89	0.85	0.91	0.86	0.88	0.97	0.85	0.88
G8	0.84	0.85	0.84	0.85	0.86	0.88	0.99		0.80	0.80	0.83	0.89	0.83	0.83	0.80	0.78	0.82	0.80	0.78	0.88	0.78	0.78
G9	0.85	0.84	0.85	0.87	0.87	0.88	0.90	0.91		0.85	0.78	0.72	0.75	0.78	0.82	0.80	0.86	0.78	0.86	0.86	0.86	0.86
G10	0.88	0.90	0.87	0.90	0.90	0.92	0.87	0.88	0.92		0.82	0.88	0.85	0.88	0.91	0.95	0.86	0.85	0.83	0.89	0.83	0.80
G11	0.85	0.84	0.85	0.88	0.87	0.87	0.90	0.90	0.93	0.92		0.91	0.82	0.85	0.82	0.83	0.86	0.82	0.80	0.77	0.86	0.74
G12	0.86	0.90	0.87	0.88	0.88	0.90	0.85	0.87	0.88	0.96	0.91		0.88	0.88	0.85	0.89	0.83	0.85	0.80	0.83	0.80	0.71
G13	0.90	0.91	0.92	0.92	0.93	0.91	0.87	0.88	0.88	0.93	0.91	0.93		0.94	0.91	0.86	0.86	0.88	0.83	0.83	0.86	0.77
G14	0.84	0.85	0.87	0.86	0.86	0.87	0.83	0.84	0.84	0.89	0.86	0.93	0.93		0.94	0.92	0.86	0.88	0.80	0.83	0.86	0.80
G15	0.84	0.87	0.87	0.87	0.88	0.88	0.81	0.82	0.81	0.85	0.83	0.88	0.90	0.96		0.92	0.92	0.91	0.86	0.89	0.89	0.86
G16	0.86	0.87	0.89	0.88	0.88	0.88	0.81	0.82	0.80	0.85	0.82	0.89	0.89	0.94	0.97		0.85	0.83	0.82	0.85	0.82	0.78
G17	0.81	0.84	0.84	0.84	0.84	0.84	0.80	0.81	0.82	0.85	0.84	0.85	0.86	0.84	0.83	0.85		0.92	0.94	0.91	0.94	0.85
G18	0.82	0.84	0.84	0.85	0.85	0.85	0.79	0.81	0.79	0.84	0.82	0.87	0.88	0.88	0.86	0.88	0.88		0.89	0.86	0.89	0.86
G19	0.78	0.78	0.81	0.81	0.81	0.83	0.79	0.80	0.82	0.80	0.83	0.80	0.84	0.81	0.82	0.81	0.87	0.87		0.88	0.94	0.85
G20	0.73	0.75	0.76	0.78	0.76	0.76	0.85	0.85	0.77	0.75	0.78	0.78	0.78	0.79	0.79	0.79	0.82	0.84	0.87		0.85	0.85
G21	0.77	0.77	0.80	0.79	0.79	0.81	0.79	0.80	0.81	0.79	0.82	0.79	0.82	0.79	0.79	0.80	0.87	0.86	0.96	0.89		0.85
G22	0.77	0.78	0.78	0.77	0.79	0.81	0.76	0.78	0.78	0.79	0.78	0.78	0.80	0.76	0.77	0.76	0.85	0.80	0.87	0.83	0.88	

Table 5: Result of mantel test between pair-type markers

Marker pair-type	Matrix correlation (r)	p-value (two-tailed)	Alpha
RAPD vs ISSR	0.391	<0.0001	0.05
RAPD vs RAPD and ISSR	0.952	<0.0001	0.05
ISSR vs RAPD and ISSR	0.648	<0.0001	0.05

To find the correlation between two types of markers, the Mantel test was done between coefficient matrixes of RAPD vs ISSR, RAPD vs RAPD+ISSR and ISSR vs RAPD+ISSR. The correlation value was 0.391, lowest between RAPD-ISSR and was 0.952, highest between RAPD and RAPD+ISSR (Table 5). The result of the Mantel test indicated a low correlation between RAPD and ISSR markers. However, the correlation between RAPD and combined RAPD+ISSR was high with r = 0.952 ($p<0.0001$) inferring the effectiveness of RAPD over ISSR in evaluating the genetic diversity of black soybean collection. The use of RAPD and ISSR has reported a poor correlation between two types of these markers in rice

resistant to brown planthopper[20], *Melocanna baccifera*[21] and lettuce (*Lactuca indica*)[19]. This difference inferred that the two types of markers investigate genetic variation differently.

CONCLUSION

The genetic diversity of twenty-two Vietnamese black soybean varieties was evaluated by RAPD and ISSR markers. The relatively close relationship among black soybean varieties was concluded with an average of similarity coefficient 0.85 by both types of markers. Two clusters were identified with the coincidence of NTSYS and DAPC analysis. Investigating the genetic diversity of black soybeans by RAPD markers is more effective than ISSR markers, however, ISSR markers provide more information that contributes to the more precise results. The outcome of the study provides useful information on different breeding programs.

SIGNIFICANCE STATEMENT

Genetic diversity of crop resources is critical for breeding programs to improve crop yield and quality. This study investigated the genetic diversity of 22 black soybean *Glycine max* L. genotypes using Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) markers. The result showed that this black soybean collection was genetically less diverse with two classified clusters. This is the first study of genetic diversity on black soybeans in Vietnam. The results of the study help select materials for breeding programs to develop a new valuable variety of black soybean *Glycine max* which can be used for genetic conservation of this important crop in Vietnam.

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