Analysis of Powdery Mildew Resistant Characteristic and Genetic Relationship of Cultivated Soybean, *Glycine max*, in Vietnam for Parental Selection

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Manuscript submitted March 7, 2016; accepted May 25, 2016.
doi: 10.17706/ijbbb.2016.6.3.105-113

Abstract: Soybean, *Glycine max*, is an important crop in Vietnam. The yield of this crop can be reduced up to 35% due to powdery mildew (PMD), a common disease in soybean. In the present study, we analyzed the PMD resistance of soybean accessions for two seasons and subsequently used SSR markers to determine the genetic diversity of these accessions. Twenty three SSR markers were used to amplify polymorphic SSRs from all of these genotypes in eighteen linkage groups. By analyzing 69 alleles for all accessions, the number of alleles ranged from 2 to 7 with an average of 4.3 at each locus. The polymorphic information content of each marker ranged from 0.104 (Sct_187) to 0.818 (Satt009) with a mean of 0.578. The pairwise coefficients of genetic similarity (Jaccard's coefficients) among all genotypes ranged from 0.28 to 0.98 with an average of 0.61. Unweighted pair-group method arithmetic average and principal coordinate analysis separated the accessions into two major groups containing 28 and 6 accessions. The results of this study suggested some appropriate parent pairs based on genetic relationship as well as the yield and resistant characteristics of soybean accessions for breeding soybean accessions with PMD resistance and high yield characteristic.

Key words: *Glycine max*, genetic variations, Microphaera diffusa, SSR markers, PMD resistance.

1. Introduction

Glycine max, commonly called the soybean, is one of the most popular and important crops all over the world. Soybean is exclusively planted in America and Asia for food and animal feed. Soybean seeds are rich source for protein and oil production. Worldwide, soybean is planted on 111.269 million hectares and average yield is 2.4841 tons/hectare. In Vietnam, soybean is a traditional crop for providing important protein in daily meals. However, soybean harvested areas are small with 117.191 hectares and the average yield is 1.4361 tons/hectare [1]. Low yield is a consequence of several main reasons including low seed quality, lacking of effective farming techniques and diseases in which PMD caused by *Microphaera diffusa* is a common soybean disease [2]. It has been recorded that the yield of soybean reduced up to 35% on susceptible cultivars [2], [3]. The breeding is of significant importance in order to improve yield, and quality and to diseases resistance [4], [5].

One of the important tools to evaluate genetic diversity in plants is DNA marker such as RAPDs, RFLPs, SNPs and SSRs [6]-[13]. SSR markers have been used successfully to evaluate the genetic diversity for a

wide range of plant species [14]-[16]. Moreover, SSR markers also provided the high level of polymorphism in soybean [8]-[10]. For instance, Hwang *et al.* (2008) used 377 SSR markers to assess genetic diversity of 87 Japanese elite cultivars and wild soybean in 20 linkage groups [17]. Tantasawat *et al.* (2011) evaluated genetic diversity and relationship in 25 soybean genotypes by 11 SSR markers [18]. Recently, Kumar *et al.* (2014) screened the soybean genotypes for yellow mosaic virus (YMV) disease resistance and their molecular characterization using SSRs markers [19]. The genetic diversity of soybean relating PMD resistance in Vietnam, however, has never been investigated.

In the present study, we assessed the PMD resistance and evaluated the genetic diversity of 34 soybean accessions in Vietnam by using SSR markers. This study may provide the crucial information for genetic improvement of *Glycine max* population via parental selection.

2. Materials and Methods

2.1. Plant Material and PMD Assessment

Thirty four soybean accessions were assessed for genetic diversity including 21 local accessions from Vietnam and 13 foreign accessions (from Australia, China, USA, Thailand and Taiwan). Plants were scored for PMD disease for two seasons based on the scale of 0 to 5 grades [20]. Grade 0 = no leaf symptoms, 1 = 10% of the leaf surface with symptoms, 2 = 11 to 25% of the leaf surface with symptoms, 3 = 26 to 50% of the leaf surface with symptoms, 4 = 51 to 75% of the leaf surface with symptoms, 5 = more than 75% of the leaf surface with symptoms. In this study, plants scored as grades 0, 1, 2, 3 were considered PMD resistant and plants scored as grades 4 and 5 were considered PMD susceptible.

2.2. DNA Extraction

Total genomic DNA was extracted from bulk three of young leaves of each soybean accession using the CTAB method [21]. Extracted DNA samples were treated with RNase at 37^0C in one hour, quantified by Nanodrop Lite (Thermo Scientific) and adjusted to final concentration of 50 ng/µL.

2.3. SSR Analysis

PCR reactions were performed using 16 SSR primers mapped on 14 linkage groups of soybean [22] with 34 soybean accessions in PCR PTC – 100 (MJ Research Inc, USA). PCR were conducted in 25 µl of a reaction mixture containing: 1X polymerase chain reaction buffer (75mM Tris-HCl, pH 9.0, 50 mM KCl, and 20 mM $(NH_4)_2SO_4$), 0.25 mM dNTPs, 2 mM $MgCl_2$, 0.2 µM primer F, 0.2 µM primer R, 1 U Taq DNA polymerase, 100 ng DNA template, and sterile ultrapure water. PCR thermal cycles consist of several steps including: 94ºC for 4 min, followed by 35 cycles of denaturation at 92ºC for 45sec, annealing at 45^oC to 55^oC for 45sec and extension at 72ºC for 45sec, final extension at 72ºC for 10 min and 4ºC for 30 min. Amplified products were separated on 6% denaturing polyacrylamide gels in 0.5X TAE (Tris-acetate-EDTA). The gel was stained with silver nitrate [23], and the bands were revealed and photographed after staining.

2.4. Data Analysis

The results were scored and recorded in a data. Score – "1" was given for the presence and score – "0" was given for the absence of band from each SSR primer. The PIC was calculated for each SSR marker locus by the formula: PIC = $1-\sum Pij^2$, *Pi* is the frequency of the i^{th} allele of SSR locus [24]. Genetic similarity among accessions was calculated on the basis of NTSYS-pc version 2.10m [25]. NTSYSpc software was also used to assess principal coordinate analysis (PCoA) to establish two dimensional dendrograms.

3. Results and Discussion

3.1. The Reaction of 34 Soybean Accessions for PMD Disease

The results have showed that six accessions including SV1, SV7, SV9, SV12, SV14, and SV29 accessions gave the high yield with more than 2.5 tons/ha, meanwhile SV2, SV4, SV6, and SV17 accessions had the low yield from 1.4 to 1.7 tons/ha (Table 1).

Table 1. Signal, Origin, Yield, and PMD Resistance of 34 Soybean Accessions

Sign	Origin	Yield (tons/ha)	PMD resistance grade scale	Sign	Origin	Yield (tons/ha)	PMD resistance grade scale
SV1	Vietnam	2.5	1	SV18	China	2.2	5
SV2	USA	1.7	0	SV19	Vietnam	2.2	4
SV3	Thailand	2.4	1	SV20	Vietnam	2.0	5
SV4	USA	1.6	1	SV21	China	1.9	5
SV5	Australia	2.4	5	SV22	Vietnam	2.1	4
SV6	USA	1.7	0	SV23	China	2.3	4
SV7	Vietnam	2.5	2	SV24	Vietnam	2.0	2
SV8	Vietnam	2.2	5	SV25	Vietnam	1.9	5
SV9	Vietnam	2.5	4	SV26	Vietnam	2.2	2
SV10	Australia	2.4	5	SV27	Taiwan	1.8	2
SV11	Vietnam	2.3	2	SV28	Vietnam	2.1	2
SV12	Vietnam	2.7	3	SV29	Vietnam	2.5	3
SV13	Vietnam	2.4	2	SV30	Vietnam	2.2	4
SV14	Taiwan	2.8	1	SV31	Vietnam	2.3	4
SV15	China	2.3	4	SV32	Vietnam	2.4	5
SV16	China	2.2	4	SV33	Vietnam	2.4	3
SV17	Vietnam	1.4	1	SV34	Vietnam	2.0	5

Twenty four remain accessions had medium yield which ranged from 1.8 to 2.4 tons/ha. Moreover, among resistance accessions, six accessions including SV1, SV2, SV3, SV4, SV14, and SV17 showed strong resistance for PMD (Table 1). Furthermore, in the total of 34 soybean accessions, two accessions have the highest yield (SV12 and SV14) and get 2.7 to 2.8 tons/ha. Ten accessions (SV1, SV3, SV5, SV7, SV9, SV10, SV13, SV29, SV32 and SV33) have the high yield with more than 2.4 tons/ha. In total 12 high yield accessions, there are three accessions (SV1, SV3 and SV14) which have strongest resistant characteristic (grades 0 and 1), five accessions (SV7, SV12, SV13, SV29 and SV33) are resistant (grades 2 and 3) and four accessions (SV5, SV9, SV10 and SV32) are susceptible to PMD. Moreover, in seven strongest PMD resistance accessions, three accessions have high yield (SV1, SV3 and SV14) and four remaining accessions have low yield (SV2, SV4, SV6 and SV17).

3.2. Polymorphism of SSR loci

SSR analysis identified totally 69 alleles with 16 markers with an average of 4.3 (Table 2). This value was higher than what has been reported in previous study of Hwang *et al.* (2008) (3.7) [17] and lower than study of Tantasawat *et al.* (2011) (4.82) [18]. The numbers of alleles in each marker ranged from 2 (Sct_187, Satt321, Satt573) to 7 alleles (Satt009, Satt373 and Satt431). The PIC values showing the diversity of allele were very high among 16 SSR markers, ranged from 0.104 to 0.818 with an average of 0.578.

The PIC values of alleles were very high among 16 SSR markers, ranging from 0.104 to 0.818 with an average of 0.578. This average value was lower than that in the study of Abe *et al.* (2003) (0.782) [26]. However, it seem to be higher than the average PIC value reported in the studies of Hwang *et al.* (2008)

(0.44) [17] and Tantasawat *et al.* (2011) (0.60) [18]. These results may be caused by the differences in selected of plant materials, the set and number of markers as well as the various distribution of these markers in soybean genome [18], [27], [28]. Fourteen SSR markers had PIC value higher than 0.3 which are useful for genetic diversity of soybean accessions using RFLP, RAPD and AFLP [11], [12], [24], [29], [30]. Fig. 1 revealed that the DNA profiles were assessed by Sct_394 markers with 4 alleles.

Table 2. Primer Sequences, Type of SSR, Linkage Groups, Numbers of Alleles, and PIC of SSR Primers

SSR primers	Primer sequence 5' → 3'	Type of SSR	Linkage group	Numbers of alleles	PIC
Sat_137	GATGACGCCGCAGGTTTCTCC GGTGCGGTTCCACAGTTTTTT	(AT)16	A1	3	0.565
Satt329	GCGCATTGGAGTTTTTGCTTTT GCGGGACGCAAAATTGGATTTAGT GCGCCGAATAAAACGTGAGAACTG	(TAT)24	A2	5	0.620
Satt197	CACTGCTTTTTCCCCTCTCT AAGATACCCCCAACATTATTTGTAA	(ATT)20	B1	5	0.697
Satt294	GCGTAGGCACACTTCGTTGTTTACT GCGGGTCAAATGCAAATTATTTTT GCGCTCAGTGTGAAAGTTGTTTCTAT	(TAT)23	C1	5	0.620
Satt681	GCGGTGCACTTGTCAATCTGTT GCGGTGAGGCATATGTCAGTC	(TTA)20	C2	5	0.721
Satt321	CACCGTCGTAAAAACTGTGTCGT GCGTGTCAAAGAGTTTTAGACATC	(TAA)14	D1a	2	0.438
Satt698	GCGCAAATTTCGTTCTTATTA GCGCAATCGCTTCTTTAGAT	(TTA)17	D1b	3	0.588
Satt256	GCGATGCATAAATTAGACACAT CCACTGCTTCATCACATTCACAC	(ATT)10	D2	3	0.549
Satt573	GCGGATTTCGATTTGAATATACTTAC CCTGTGGCTGTTATACTATGCATATA	(ATT)10	E	2	0.360
Sct_187	GCGGCGAAACCCACAAAGCATA CATGCTCCCATTCTCT AACATTGGCTTTTTACTTAG	(CT)10	G	2	0.104
Sct_065	CCCTGTGTTTCCCTCT GAAAAGTTTTATGTTCTGAGTG	(CT)16	J	3	0.236
Sat_394	GCGTTGGCGGTAAGAGCACTATA GCGGACAGTGTGCTCCTCATATAATAG GCGTGACTCGGACTTGAAGATAATAATG	(AT)36	J	4	0.573
Satt431	GCGTGGCACCCTTGATAAATAA GCGCACGAAAGTTTTTCTGTAACA	(AAT)21	J	7	0.779
Satt373	CCGTCGATTCCGTACAA TCCGCGAGATAAATTCGTAAAAT GGCCAGATACCCAAGTTGTACTTGT	(ATT)21	L	7	0.805
Satt150	AAGCTTGAGGTTATTCGAAAATGAC TGCCATCAGGTTGTGTAAGTGT	(ATT)20	M	6	0.783

Satt009	CCAACTTGAAATTACTAGAGAAA CTTACTAGCGTATTAACCCTT	(ATT)14	N	7	0.818
Total				69	
Average				4.3	0.578

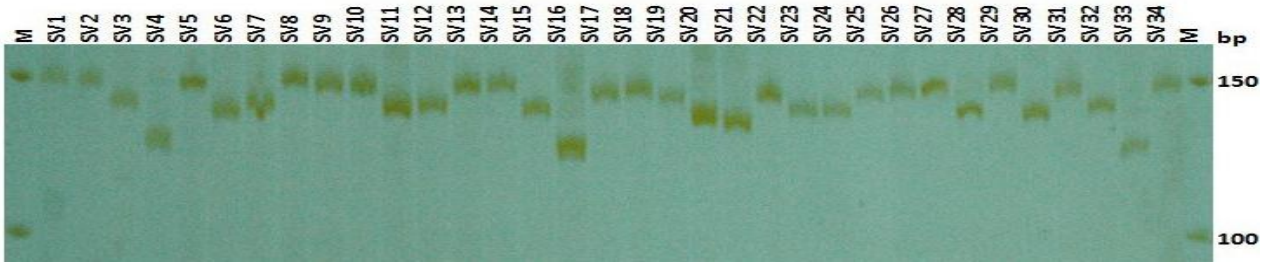

Fig. 1. The SSR profiles of 34 soybean accessions with Sat_394 loci; M: DNA ladder marker 50 bp.

3.3. Genetic Diversity and Clustering of 34 Soybean Genotypes

Pairwise of Jaccard's similarity coefficients ranged from 0.28 to 0.945 with an average of 0.61 (results not showed). The dendrogram showed two major clusters and some subclusters (Fig. 2).

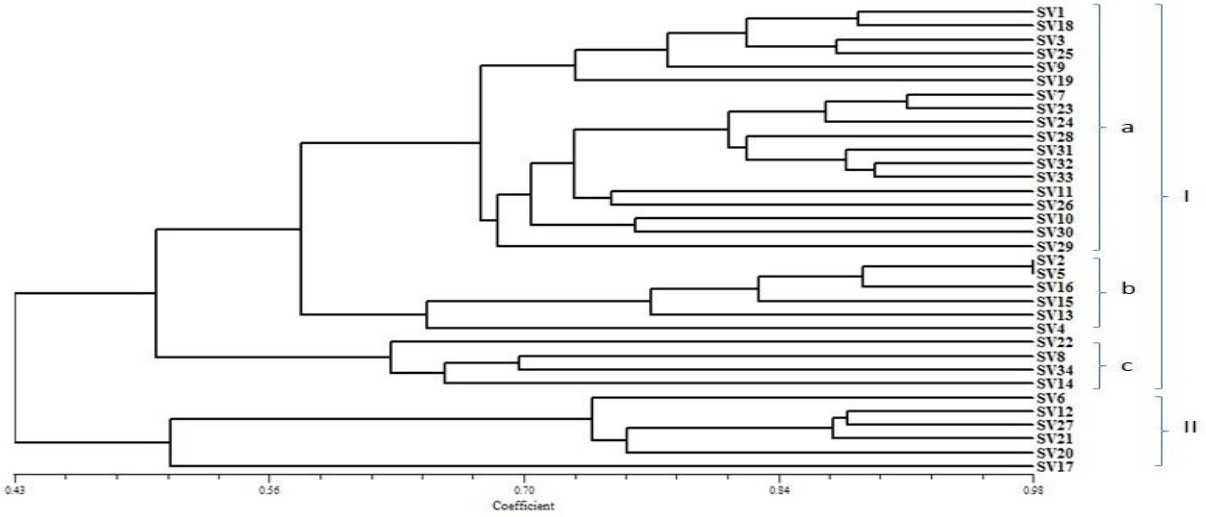

Fig. 2. Dendrogram for similarity coefficients of 34 soybean accessions based on UPGMA clustering analysis.

Cluster I including 28 soybean accessions was the biggest cluster. It further divided into 3 subclusters with different origins. Subcluster Ia consisted of 18 cultivars including 15 Vietnamese accessions, one Chinese accessions, one Australia accession and one Thai accession with genetic similarity of 67.2%. Subcluster Ib had six accessions which have only one accession from Vietnam, two accessions from USA, two accessions from China and one accession from Australia with genetic similarity of 64.4%. Subcluster Ic included 4 cultivars consisting of three Vietnamese accessions and one Taiwan accession with genetic similarity of 62.0%. By comparison, Cluster II had genetic similarity of 50.4% involving 6 cultivars from four different origins including one from China, one from USA and one from Taiwan and three from Vietnam (Table 3).

Based on PCoA analysis support for UPGMA dendrogram, a dendrogram was established (Fig. 3). When compared with dendrogram that was established based on UPGMA analysis, the two dendrograms were similar in general (Fig. 3, Table 3).

Table 3. The Difference and Similarity of UPGMA and PCoA Separation among 34 Accessions

Sign	UPGMA analysis	PCoA analysis	Sign	UPGMA analysis	PCoA analysis
SV1	Ia	I	SV18	Ia	I
SV2	Ib	I	SV19	Ia	I
SV3	Ia	I	SV20	II	II
SV4	Ib	II	SV21	II	II
SV5	Ib	I	SV22	Ic	I
SV6	II	II	SV23	Ia	I
SV7	Ia	I	SV24	Ia	I
SV8	Ic	I	SV25	Ia	I
SV9	Ia	I	SV26	Ia	I
SV10	Ia	I	SV27	II	II
SV11	Ia	I	SV28	Ia	I
SV12	II	II	SV29	Ia	I
SV13	Ib	I	SV30	Ia	I
SV14	Ic	I	SV31	Ia	I
SV15	Ib	I	SV32	Ia	I
SV16	Ib	I	SV33	Ia	I
SV17	II	II	SV34	Ic	I

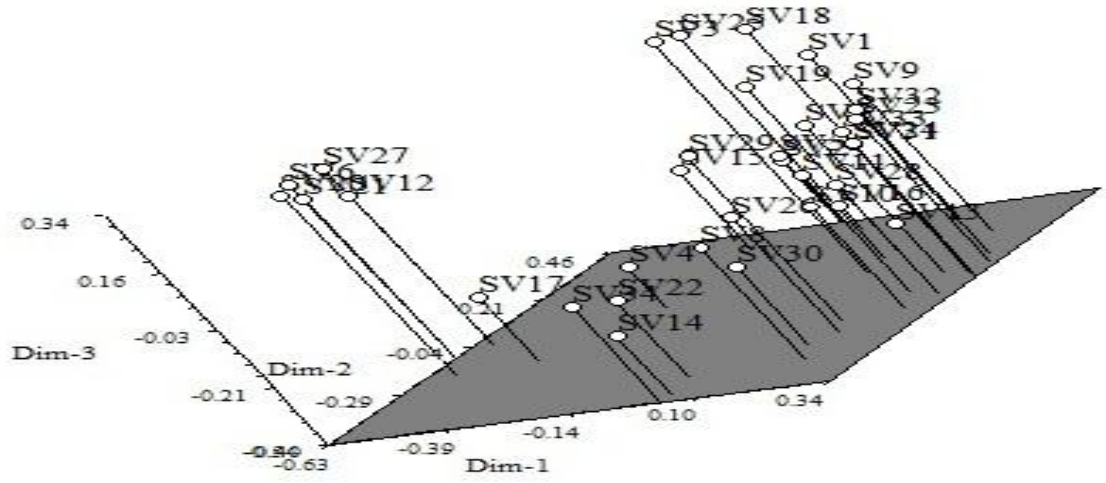

Fig. 3. Three-dimensional distribution of 34 soybean accessions was revealed by principal coordinate analysis (PCoA).

This observation confirmed that SSR markers are particularly useful for conducting diversity analysis or genotyping for proprietary purposes in soybean [15], [17], [18], [31]. Moreover, the dendrogram established based on UPGMA analysis showed two clusters (Fig. 2). The Chinese accessions were separated in all studied clusters. The reason may be due to that the soybean cultivars of Southeast, Southern and central Asia, America and Europe are thought to have originated from China [26], [30]. Our result is in the same line with the results of Hwang group's study [17] in which cultivars in all studied groups revealed to have close relations to Chinese cultivars.

4. Conclusion

In conclusion, three PMD strongest resistant accessions with high yield including SV1, SV3 and SV14 can be used to create the populations of cultivar. This study suggested some significant hybrid combinations, for instance, the PMD susceptible accessions i.e SV6 with high yield can crossbreed with some strongest PMD resistant accessions with low yield i.e. SV5, SV9, SV10 and SV32 to create good-trait cultivars.

Acknowledgement

This work was supported by the Institute of Biotechnology, Vietnam Academy of Science and Technology and the Legumes Research and Development Center, Field Crops Research Institute, Vietnam Academy of Agricultural Sciences.

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