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Evaluation of genetic relationship among some important Japanese and Thai soybean varieties using AFLP analysis

Aungkana Nimnual[1], Jutamas Romkaew[2], Ekachai Chukeatirote[1], and Somrudee Nilthong[1]*

[1]School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand
[2]Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

***Corresponding author: S.onto@sci.mfu.ac.th**

Abstract

Amplified fragment length polymorphism (AFLP) analysis was used to study the genetic relationship among 25 soybean varieties from Japan and Thailand. Seventeen AFLP primer pairs produced 317 polymorphic (44.90%) and 389 (55.10%) monomorphic AFLP fragments. The polymorphic information content (PIC) among varieties varied from 0.0357 (E-AAC/M-CAT) to 0.1464 (E-ACC/M-CTA) with an average of 0.1088. The Dice's similarity coefficients among soybean varieties ranged from 0.8822 (Fukuyutaka and CM60) to 0.9696 (SJ4 and SJ5), indicating a low genetic diversity among the 25 varieties. Soybean varieties from Japan and Thailand clearly classified into 3 groups based on UPGMA clustering analysis. The first group consisted of four varieties of Japanese soybeans. There were 20 varieties within the second group consisting of both Japanese and Thai soybeans. However, it should be noted interestingly that there was also two distinct internal clades of Japanese and Thai varieties within this second group. The third group clearly separated from other varieties consisted of only the Thai NS1 soybean. The analysis of molecular variance (AMOVA) demonstrated only 18% of the total diversity was attributed to differences among populations. Our data showed that AFLP could be used as an efficient marker for assessing soybean genotypes and relationship. Besides, our results will be useful for future application especially for the breeding program using these soybean varieties.

Keywords: AFLP; genetic diversity; *Glycine max* (L.) Merr.; soybean.
Abbreviations: AFLP_amplified fragment length polymorphism; AMOVA_analysis of molecular variance; PIC_polymorphism information content; RAPD_random amplified polymorphic DNA; RFLP_restriction fragment length polymorphism; SSR_simple sequence repeat.

Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the major crops important for human food and animal feed. It is a species of legume which has long been regarded as useful in crop rotation, effective nitrogen fixation, and soil improvement ability. Soybean albeit originated from Asia has been globally distributed providing its critical role as protein rich food and biofuel. According to the American Soybean Association (ASA, 2012), in 2011, soybeans represent 56% of world oilseed production in which 33% of those are produced in the United States. At present, the top three producers of soybean are the US, Brazil, and Argentina, producing more than 80% of the world's soybeans trade (USDA, 2012). As one of the important economic legume crops, many soybean cultivars have been preserved and generated in the germplasm bank of several countries. This is an important means to create diversity in soybean breeding. Additionally, it is expected that soybean yield and trait can be improved by the breeding programme. Traditionally, morphological and agronomic traits are the key information used in studying genetic diversity of soybean (Perry and McIntosh, 1991; Sneller, 1994). However, some factors especially those derived from environmental influence affect such results, and thus leading to uncertain, incomplete, and/or possible errors in the pedigree data. With the advent of the PCR and DNA sequencing techniques, several researchers have now used the DNA markers to identify and differentiate the genetic diversity of soybean varieties (Singh et al., 2010; Tantasawat et al., 2011). These molecular techniques are rapid and become a powerful tool for this purpose; these include restriction fragment length polymorphism (RFLP) (Diers et al., 1992; Zhang et al., 1999), random amplified polymorphic DNA (RAPD) (Brown-Guedira et al., 2000; Pham-Thi et al., 2003), amplified fragment length polymorphism (AFLP) (Mienie et al., 2002; Singh et al., 2010; Li and Cao, 2011), and simple sequence repeat (SSR) or microsatellites (Hudcovicova and Kraic, 2003; Singh et al., 2010;Wang et al., 2006). In Thailand, there are some research groups working on soybean genetic diversity (Chotiyarnwong et al., 2007; Tantasawat et al., 2011). These programs have been carried out with an aim to increase the production yield. However, the major drawbacks are a variety of soybean germplasm which is limited and a difficulty in accessibility of germplasm strictly obligated to a few governmental sectors. Besides, based on the work of Chotiyarnwong et al. (2007), a small genetic diversity of Thai soybean cultivars have been identified implying that they came from the closely related germplasm pool. Low genetic diversity among the Thai soybean varieties is problematic in breeding program. In this study, sixteen soybean varieties from Japan and nine from Thailand were selected and used as specimens to determine their genetic relatedness. All of these Japanese and Thai soybeans have been used in the comme-

Table 1. List of Japanese and Thai soybean varieties.

No.	Variety	Origin	No.	Variety	Origin
1	Akisengoku	Japan	14	Suzuotome	Japan
2	Akishirome	Japan	15	Tamahomare	Japan
3	Chadaizu	Japan	16	Tanbaguro	Japan
4	Fukuyutaka	Japan	17	CM2	Thailand
5	Himeshirazu	Japan	18	CM3	Thailand
6	Kodane	Japan	19	CM4	Thailand
7	Kosamame	Japan	20	CM60	Thailand
8	Kosuzu	Japan	21	NS1	Thailand
9	Nabeshima	Japan	22	SJ4	Thailand
10	Nattoshoryo	Japan	23	SJ5	Thailand
11	Shirotae	Japan	24	SK1	Thailand
12	Ootsuru	Japan	25	SK2	Thailand
13	Sachiyutaka	Japan			

rcial production. As part of the soybean breeding program, we aimed to address the parental origin of these soybeans and, more importantly, to provide useful data important for genetic improvement.

Results and Discussion

AFLP Profiles

Sixty-four *Eco*RI and *Mse*I primer pairs were screened for their ability to generate AFLP profiles of the 25 soybean varieties. Of these, seventeen primer pairs were selected for the analysis as they produced clearly amplified DNA bands (Figure 1 and Table 2). The scored fragment size ranged from 50 to 600 bp. Each primer pair produced 31-50 fragments with an average of 41.53 fragments per primer pair. A total of 706 AFLP fragments were identified of which 317 (44.90%) were polymorphic. The percentage of polymorphism per primer pair ranged from 36.96% (E-AAG/ M-CAG) to 64.86% (E-ACT/ M-CAA) with an average of 45.22%. The polymorphism information content (PIC) score of each primer pair ranged from 0.0357 in E-AAC/M-CAT to a maximum of 0.1464 in E-ACC/M-CTA with an average of 0.1088. The average PIC score was similar to an earlier study on soybean (Ude et al., 2003; Singh et al., 2010). Singh et al. (2010) studied on 44 soybean genotypes and found that 449 polymorphic bands showed the average PIC score for AFLP was 0.12. Thus, the polymorphism seen by AFLP efficiently disguised all of these soybean cultivars.

Phylogenetic Analysis

The analysis of molecular variant (AMOVA) showed that the majority of the genetic variation among the 25 soybean varieties was due to differences within population (82%) and the variance among populations was 18% (Table 3). There was a highly significant difference between Japanese soybean varieties and Thai soybean varieties ($P < 0.001$). All 706 AFLP fragments scored were used for the genetic diversity analysis. The Dice's similarity coefficients among soybean varieties showed a high genetic similarity ranged from 0.8822 (Fukuyutaka and CM60) to 0.9696 (SJ4 and SJ5), indicating a low genetic diversity among the 25 varieties. This is in agreement with Chotiyarnwong et al. (2007) who studied the genetic relationship among 149 indigenous and 11 recommended soybean varieties using 18 SSR markers and reported that only a small genetic diversity was found. However, the low genetic diversity among soybean cultivars may be caused by most of the Southeast Asian soybean accessions derived from the Chinese germplasm pool (Abe et al., 2003). A dendrogram was constructed based on genetic

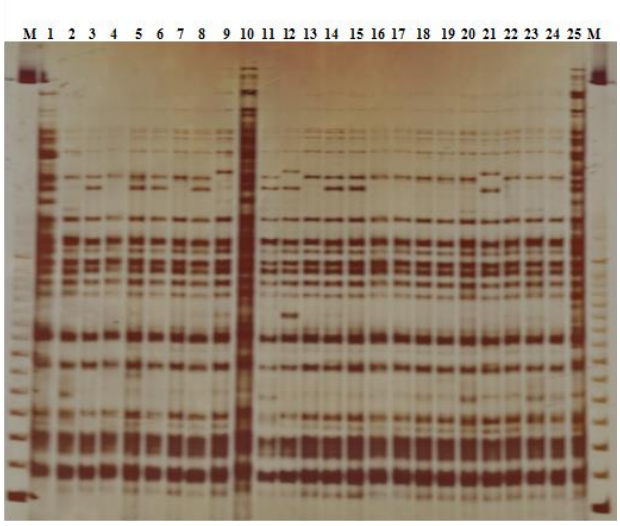

Fig 1. Representative of AFLP profile of 25 soybean varieties generated by primer E-AAC/ M-CAG. From left to right: M, 25 bp DNA ladder; 1 Akisengoku; 2, Akishirome; 3, Chadaizu; 4, Fukuyutaka; 5, Himeshirazu; 6, Kodane; 7, Kosamame; 8, Kosuzu; 9, Nabeshima; 10, Nattoshoryo; 11, Shirotae; 12, Ootsuru; 13, Sachiyutaka; 14, Suzuotome; 15, Tamahomare; 16, Tanbaguro; 17, CM2; 18, CM3; 19, CM4; 20, CM60; 21, NS1; 22, SJ4; 23, SJ5; 24, SK1; 25, SK2.

similarity data to evaluate the genetic relationships between Japanese and Thai soybean varieties using the UPGMA analysis (Fig 1). In the dendrogram, all soybean varieties were classified into three clusters. Cluster I composed of four varieties (Akisengoku, Akishirome, Chadaizu, and Fukuyutaka) from Japan. The Dice's similarity coefficient between Akisengoku and Akishirome was 0.9485, indicating that they vary genetically. Cluster II was the largest and the most diverse cluster consisting of 15 varieties of which twelve varieties were from Japan and three varieties were from Thailand. The CM2, CM3 and CM4 varieties from Thailand were grouped together in this cluster with the similarity of 95%. Dice's similarity coefficients of 'CM2' with 'CM3' and 'CM4' were 0.9527 and 0.9607, respectively. Such close similarity among these varieties may suggest that these three varieties may share the common pedigrees. Cluster III comprised only five Thai soybean varieties and both SJ4 and SJ5 varieties were in this cluster. Furthermore, SJ4 and SJ5 varieties showed the highest similarity (0.9696), the reason being that both SJ4 (Acadian x Tainung4) and SJ5 (Tainung4 x SJ2) varieties were progenies from crosses with Tainung4 from Taiwan as one of the

Table 2. Total number of AFLP fragments and the level of polymorphism detected among 25 soybeans varieties.

Primer pairs	Total no. of bands	Number of polymorphic bands	Percent polymorphic bands	Polymorphism information content (PIC)
E-AAC/M-CAG	54	21	38.89	0.1037
E-AAC/M-CAT	50	26	52.00	0.1361
E-AAC/M-CTC	41	18	43.90	0.1296
E-AAC/M-CAG	41	19	46.34	0.1252
E-AAC/M-CAT	50	21	42.00	0.0357
E-ACA/M-CAG	46	17	36.96	0.0949
E-ACC/M-CAA	31	13	41.94	0.1067
E-ACC/M-CTA	41	19	46.34	0.1464
E-ACG/M-CAC	41	20	48.78	0.1102
E-ACG/M-CTA	34	16	47.06	0.0988
E-ACG/M-CTC	32	15	46.88	0.1040
E-ACG/M-CTT	35	16	45.71	0.1247
E-ACT/M-CAA	37	24	64.86	0.1107
E-ACT/M-CTG	54	22	40.74	0.0805
E-AGC/M-CTT	43	17	39.53	0.1134
E-AGG/M-CAG	37	16	43.24	0.1059
E-AGG/M-CTA	39	17	43.59	0.1236

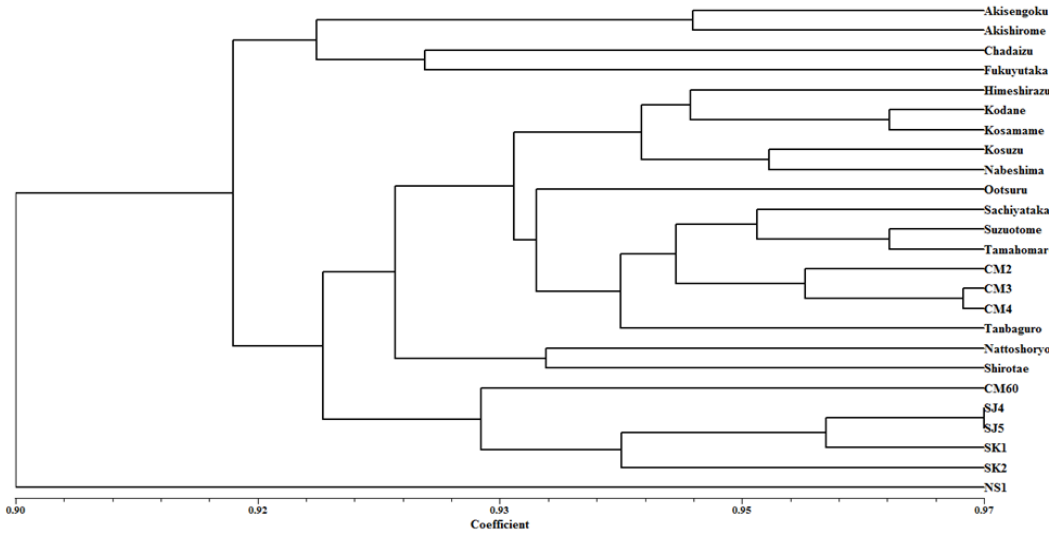

Fig 2. Dendrogram of 25 soybean varieties based on UPGMA cluster analysis.

parents. The NS1 variety did not cluster with any of the three clusters, as outlier in the dendrogram, suggesting its higher genetic divergence from others. From the above results indicated that the genetic diversity of soybean varieties released from Japan and Thailand is very low. This is in agreement with Thompson et al. (1998) who found that the genetic variation of soybean was low when composed with other self-pollinated crops in the same Fabaceac family because of the similarity and relationship of characteristics of parents (Chowdhery et al., 2002). The long history of domestication and commerce of soybean in Asia has contributed to the dispersion of alleles throughout the origin, reducing the influence of geography on patterns of variation among Asia soybean accession (Brown-Guedira et al., 2000).

Materials and Methods

Plant materials and DNA extraction

A total of 25 soybean varieties consisted of 16 Japanese varieties and 9 varieties from Thailand obtained from Department of Agriculture, Kasetsart University, Thailand were used in this study (Table 1). All soybean varieties were certified and used commercially in Japan and Thailand. The seeds of all varieties were germinated and genomic DNA was extracted from fresh young leaves of each soybean variety using the CTAB method previously described by Agrawal et al. (1992). Whole leaves were ground into find powder in liquid nitrogen and then suspended in 700 µl of DNA extraction buffer (2% CTAB, 1.4M NaCl, 20mM EDTA pH 8.0, 100mM Tris-HCl pH 8.0 and 0.2% 2-mercaptoethanol). The homogenates were incubated at 60°C for 60 min, followed by addition of equal volume of chloroform: isoamyl alcohol (24: 1), and DNA was precipitated with 10 µl of linear polyacrylamide and 2/3 volume of cold isopropanol at -20°C for 30 min. The pellets were washed with 70% ethanol and suspended in 500 µl of RNase buffer. RNase A (10 ng/ml) was added and the suspension was incubated at 37°C for 30 min. The suspension was extracted once with equal volume of phenol: chloroform: isoamyl alcohol (25: 24: 1) and once with chloroform: isoamyl alcohol (24: 1). The DNA was then pelleted by addition of 5 µl of polyacrylamide and 1 volume of ice-cold absolute ethanol and suspended in TE buffer (10mM Tris-HCl, 1mM EDTA (pH 8.0)). The DNA concentration was determined by NanoDrop 1000 spectrophotometer (Thermo Scientific).

Table 3. Analysis of molecular variance (AMOVA) of soybean varieties on the basis of AFLP data.

Source of variance	*df*	SS	V.P.	%	p value
Among Japanese and Thai soybean varieties	1	126.735	7.897	18	0.001
Within varieties	23	822.465	35.759	82	
Total	24	949.200			

SS: Sums of squares, V.P.: variance components, %: percentage of genetic variability

AFLP analysis

AFLP analysis was performed using the procedure of Vos et al. (1995). Genomic DNA (250 ng) was digested with two restriction enzymes (*Eco*RI and *Mse*I; Invitrogen, Corporation, CA) then ligated to *Eco*RI/*Mse*I adapters with T4 DNA ligase. After ligation, pre-amplification was performed with *Eco*RI and *Mse*I primers containing one selective nucleotide. The pre-amplification products were diluted (1: 20) with TE buffer, and an aliquot was used for selective amplification with the *Eco*RI and *Mse*I primers having three selective nucleotides at the 3´ end. Selective amplification products were separated on a 6% denaturing polyacrylamide gel electrophoresis and visualized by silver staining (Bassam et al., 1991). The AFLP fragment sizes were estimated in comparison with 25 bp DNA ladder (Invitrogen Corporation, CA).

Data analysis

The AFLP fragments were manually scored as present (1) or absent (0) for each primer across 25 soybean varieties. The total number of bands, the number of polymorphic bands and the percentage of polymorphic bands were determined for each primer. Genetic relationship among varieties was calculated according to Dice's coefficient (Dice, 1945) using NTSYSpc-2.01e (Rohlf, 1998). A dendrogram was constructed based on the resulting similarity matrix data using the unweighted pari-group method with the arithmetic averages (UPGMA) through the SHAN (sequenctial, hierarchical, agglomerative and nested clustering) module in the NTSYSpc-2.01e (Rohlf, 1998). Polymorphism information content (PIC), a measure or the allelic diversity at a locus, for each marker was calculated by using the following formula: $PIC = 2f_i(1 - f_i)$ where f_i is the frequency of the amplified fragments (Roldan-Ruiz et al., 2000). PIC was averaged over the fragments for each primer combination. Analysis of molecular variance (AMOVA) was used to estimate variance among and within populations using GenAlEx 6.4 software (Peakall and Smouse, 2006). Significance of variance was tested after 999 permutations.

Conclusion

The AFLP results presented here clearly indicated that the AFLP marker is a good choice for analysis the genetic diversity of soybean in agreement with previous reports (Ude et al., 2003; Singh et al., 2010). The genetic similarity among varieties established is important reference value for breeders to select parents in breeding program.

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