Korean J. Crop Sci. 46(2): 112~120(2001)

# Genetic Diversity Based on Morphology and RAPD Analysis inVegetable Soybean

**A. K. Chowdhury*, P. Srinives**†, P. Tongpamnak*** and P. Saksoong******

*University of Science and Technology, Patuakhali, Bangladesh
**†Department in Agronomy, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand
***Central Laboratory and Greenhouse Complex, Kasetsart University, Kamphaeng Saen, Thailand
****Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, Thailand

**ABSTRACT: Genetic diversity of 47 East-Asian vegetable soybean was characterized by means of agro-morphological traits and RAPD markers. A field trial was conducted to evaluate 14 agro-morphological traits. To study RAPD-based DNA analysis, a total of sixty 10-mer random primers were screened. Of these, 23 polymorphic markers in 16 varieties used for screening. Among 207 markers amplified, 48 were polymorphic for at least one pairwise comparison within the 47 varieties. A higher differentiation level between varieties was observed by using RAPD markers compared to morphological markers. Correspondence analysis using both types of marker showed that RAPD data could fully discriminate between all varieties, whereas morphological markers could not achieve a complete discrimination. Genetic distances between the varieties were estimated from simple matching coefficients, ranged from 0.0 to 0.640 with an average of 0.295 ± 0.131 for morphological traits and 0.042 to 0.625 with an average of 0.336 ± 0.099 for RAPD data, respectively. Cluster analysis based on genetic dissimilarity of these varieties gave rise to 4 distinct groups. The clustering results based on RAPDs did not match with those based on morphological traits. Geographical distribution of most varieties in each of the groups were not well defined. The results suggested that the level of genetic diversity within this group of East-Asian vegetable soybean varieties was sufficient for a breeding program and can be used to establish genetic relationships among them with unknown or unrelated pedigrees.**

*Keywords*: diversity, agro-morphological marker, RAPD marker, genetic relationship, vegetable soybean.

The vegetable soybean, *Glycine max* (L.) Merr., has been cultivated and consumed as snack or as component of a meal in China, Japan, Korea, Taiwan and Thailand. It is an important vegetable crop because of its high protein, fat, vitamin, fiber and mineral content. Its utilization is completely different from grain soybean. Green pods are harvested between the R6 and R7growth stages when the seeds have occupied about 80~90% of the pod cavity.

Information on genetic diversity and relationships in crop plants are important for efficient selection of parental lines in new crosses and preservation of germplasm by plant breeders (Tatineni *et al.*, 1996). Since genetic diversity of vegetable soybean was created through population structure and genetic recombination by plant breeders along the process of varietal improvement globally. In the process, plant breeders try to adjust the genotype to agricultural, social and economic environment. Some varieties were artificially selected by farmers to suit with different cultural and ethnic preferences. Some were naturally selected in response to geographical, climatic and edaphic features (Hawkes, 1983). Geographical origin and agro-morphological data of vegetable soybean are available in the Asian Vegetable Research and Development Center (AVRDC) collection and frequently serve as criteria for selection of genetically diverse parents.

Although Japan is the original source of vegetable soybean cultivars, the country is currently importing soybean from China, Korea, Taiwan and Thailand. Therefore, a new cultivar can be protected under the plant variety protection program. Traditionally, the genetic diversity of cultivars within *Glycine max* and the relationship among them are determined by a combination of morphological and agronomic traits or by biochemical tests. However, most commercial soybean cultivars arose from hybridization between members of an elite group of genotypes, and the amount of genetic variability among these cultivars is small. Such cultivars are often indistinguishable based on these agro-morphological traits or biochemical tests which are some times subjected to environmental influence interplaying with a number of genes and thus may not represent genetic divergence in the entire genome (Brown, 1979; Brown-Guedira *et al.*, 2000; Diwan & Cregan, 1997). A large number of polymorphic markers are required to measure genetic relationship and genetic diversity in a reliable manner. As a result, it is now widely accepted that information generated from DNA-based polymorphisms alone, or in tandem with

†Corresponding author: (Phone) 66-34-281267 (E-mail) agrpss@ku.ac.th
<Received May 9, 2001>

morphological analyses provide the best estimate of genetic diversity. The most common DNA-based techniques, such as RAPD, SSR and AFLP have been used in this assay. Among them, RAPD technique is simple, quick, inexpensive, no need prior DNA sequence, largely automatable, and require only tiny amounts of DNA (Karp *et al.*, 1996). In spite of these, inconsistency of results within and between laboratory is the main obstacle. This problem is much reduced as the mechanism of PCR generating RAPD fragments have been further understood (Weising *et al.*, 1995). A number of studies have been carried out to asses germplasm diversity and genetic structure in soybean. Brown-Guedira *et al.* (2000) Thompson *et al.* (1998), and Thomson & Nelson (1998) used RAPD markers to compare core and reserved samples of the USDA Soybean Germplasm Collection. To date, there is no report on utilizing RAPD primers and fragments to explore genetic diversity in East-Asian vegetable soybeans. The RAPD technique offers a potential to generate a large number of markers representing a random sample of the genome thereby presenting an advantage over agro-morphological traits.

The objective of this study was (1) to estimate the level of genetic diversity and relationships among 47 vegetable soybean accessions collected from East-Asian production regions, and (2) to compare the results using RAPD markers with those obtained using agro-morphological traits.

## MATERIALS AND METHODS

### Plant materials

Forty-seven East-Asian vegetable soybean cultivars, comprising 24 Japanese, 3 Korean, 14 Taiwan and 6 Thai, which appeared to be useful in breeding programs in Thailand were used in this study (Table 1). They were chosen based on their agronomic performance from the previous yield trials.

### Observation of morphological markers

The seeds of all cultivars were sown in the experimental field of the Asian Regional Center-Asian Vegetable Research and Development Center (ARC-AVRDC), Kasetsart University, Kamphaeng Saen, Thailand. Variety-wise, 14 agro-morphological characters were scored on plants as outlined by the International Board for Plant Genetic Resources, Descriptors for Soybean, Rome, 1984 in a field trial during 1998-1999 growing season.

### Bulk DNA isolation

Ten days after emergence, the first trifoliate leaves from eight young seedlings from each of the 47 cultivars were sampled and subjected to DNA extraction. Total genomic DNA was isolated from bulked leaf tissue using the protocol of Doyle and Doyle (1990). The presence of DNA was monitored by subjecting samples to 1% agarose gel electrophoresis in 1× TAE buffer (40 mM Tris-acetate, 1 mM EDTA) with 2 μL ethidium bromide (0.1 g/mL) and by visual assessment of band intensities compared with λ phage DNA standards. The exact DNA concentration and purity was determined by Spectrophotometry and the concentration was adjusted to 5 ng/μL.

### Individual plant DNA isolation

Plant-wise genomic DNA was isolated from 8 individual plants each of 10 selected varieties. Two varieties from Japan (Blue side & Fukura), two from Taiwan (Kaoshiung, and AGS 191) and 6 from seed companies in Thailand (ASB-02, E-H, E-O, E-M, Bangkok Land and E-K) were extracted for leaf DNA using the aforementioned protocol.

### PCR procedure

PCR reaction was performed in volume of 10 μL containing 2 μL extracted genomic DNA, 1 μL 10 × PCR buffer (100 mM Tris-Cl buffer, 500 mM KCl, 20 mM MgCl2, Gelatin 0.01%), 1 μL 1 mM dNTPs (Promega, USA), 1 μL 2 mM primer, 0.2 μL (1 unit/μL) Taq DNA polymerase (Promega) and 4.8 μL sterile water. Each tube was added with 30 μL of sterile mineral oil to seal the reaction mixture and to prevent evaporation. The PCR was carried out in a DNA Thermal Cycler (Bio Oven III) programmed to run the following temperature profile: 1 cycle of 1 min at 94°C; 44 cycles of 1 min at 91°C for denaturation; 1 min at 36°C for annealing; 2 min at 72°C for extension; 1 cycle of 7 min at 72°C as the final extension.

### Screening of primers

Sixty 10-mer oligonucleotide primers of Operon Technologies (USA) were initially screened against a subset of 16 selected varieties which were both morphologically and geographically diverse (see Table 1, variety no. 17~32). Primers that amplified only monomorphic fragments, produced weak banding patterns, or did not reproduce similar banding patterns when replicated at least twice were not used in further analyses.

### Electrophoresis

PCR products were size-separated by electrophoresis in

**Table 1.** Forty-seven vegetable soybean genotypes used in this study with their major agronomic traits.

| Variety no. | Variety name | AVRDC entry no. | Seed origin | Important Agronomic traits | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Days to flowerng | Days to maturity | Plant height (cm) | No. pod/ 500g | 100-seed weight (g) | Pod wt./ plant (g) |
| 1 | Oofurisode | GMa001 | Japan | 28 | 67 | 23.2 | 188 | 31.5 | 32.5 |
| 2 | Okuhara Wase | GMa002 | Japan | 24 | 67 | 21.6 | 200 | 28.5 | 31.6 |
| 3 | Ginsui | GMa003 | Japan | 25 | 67 | 23.6 | 175 | 30.0 | 27.9 |
| 4 | Shirofumi | GMa004 | Japan | 24 | 67 | 20.2 | 216 | 28.0 | 33.0 |
| 5 | Karitea | GMa005 | Japan | 28 | 68 | 24.8 | 180 | 30.0 | 32.2 |
| 6 | Tamasudare | GMa006 | Japan | 25 | 67 | 32.0 | 218 | 22.0 | 26.7 |
| 7 | Yukiyama | GMa007 | Japan | 31 | 68 | 28.0 | 201 | 31.5 | 27.0 |
| 8 | Kegon | GMa008 | Japan | 29 | 69 | 21.2 | 208 | 28.8 | 26.0 |
| 9 | Mikawashima | GMa009 | Japan | 30 | 69 | 18.2 | 215 | 25.0 | 25.5 |
| 10 | Hamanishiki | GMa011 | Japan | 31 | 67 | 28.2 | 213 | 35.0 | 34.0 |
| 11 | White Lion | GMa012 | Japan | 28 | 67 | 22.2 | 209 | 26.0 | 26.0 |
| 12 | Tsuronoko | GMa013 | Japan | 32 | 69 | 26.4 | 202 | 31.0 | 32.5 |
| 13 | Tamchon | GMa014 | Japan | 25 | 70 | 17.0 | 168 | 32.4 | 32.0 |
| 14 | Solunoko | GMa015 | Japan | 25 | 70 | 19.2 | 198 | 27.0 | 28.0 |
| 15 | Kahori | GMa017 | Japan | 28 | 70 | 33.2 | 201 | 28.0 | 27.0 |
| 16 | Tengamine | GMa018 | Japan | 29 | 67 | 28.8 | 211 | 27.5 | 29.5 |
| 17 | Blue Side | GMa019 | Japan | 31 | 70 | 29.0 | 162 | 36.0 | 33.5 |
| 18 | Wakakusha | GMa020 | Japan | 27 | 66 | 25.8 | 210 | 26.0 | 26.0 |
| 19 | Hatsutaga | GMa021G | Japan | 29 | 66 | 24.4 | 199 | 28.8 | 31.0 |
| 20 | Shironomai | Ma022 | Japan | 27 | 70 | 33.2 | 162 | 30.0 | 30.0 |
| 21 | ASB-02 | GMa036 | Seed co. | 23 | 70 | 27.4 | 165 | 30.5 | 32.0 |
| 22 | E-H | GMa031 | Seed co. | 23 | 68 | 28.6 | 172 | 27.5 | 25.0 |
| 23 | E-O | GMa032 | Seed co. | 24 | 69 | 23.2 | 168 | 32.0 | 30.0 |
| 24 | E-M | GMa033 | Seed co. | 29 | 68 | 26.4 | 179 | 29.0 | 26.6 |
| 25 | TMB | GMa034 | Japan | 28 | 70 | 28.8 | 181 | 28.0 | 27.0 |
| 26 | Bangkok Land | GMa024 | Seed co. | 27 | 70 | 28.2 | 178 | 29.0 | 25.5 |
| 27 | Suwon 155 | GMa025 | Korea | 29 | 70 | 25.8 | 162 | 30.0 | 32.8 |
| 28 | Suwon 156 | GMa026 | Korea | 29 | 70 | 24.2 | 164 | 29.5 | 33.5 |
| 29 | Suwon 157 | GMa027 | Korea | 30 | 70 | 22.6 | 162 | 29.0 | 33.0 |
| 30 | KVS 39 | GMa037 | Taiwan | 29 | 74 | 36.0 | 188 | 27.0 | 29.0 |
| 31 | KVS 124 | GMa038 | Taiwan | 29 | 70 | 27.0 | 184 | 30.0 | 31.0 |
| 32 | Kaoshiung | GMa039 | Taiwan | 31 | 70 | 32.2 | 167 | 24.0 | 31.0 |
| 33 | AGS 186 | GMa042 | Taiwan | 28 | 71 | 36.0 | 192 | 24.5 | 33.0 |
| 34 | AGS 187 | GMa043 | Taiwan | 29 | 70 | 27.2 | 177 | 26.0 | 35.6 |
| 35 | AGS 188 | GMa044 | Taiwan | 30 | 70 | 28.4 | 169 | 30.5 | 36.0 |
| 36 | AGS 189 | GMa045 | Taiwan | 24 | 70 | 35.4 | 179 | 28.0 | 29.5 |
| 36 | AGS 190 | GMa046 | Taiwan | 29 | 68 | 27.8 | 177 | 26.5 | 30.5 |
| 38 | AGS 191 | GMa047 | Taiwan | 30 | 68 | 32.0 | 173 | 29.0 | 29.0 |
| 39 | E-K | GMa030 | Seed co. | 25 | 68 | 22.4 | 196 | 25.8 | 27.0 |
| 40 | Taisho Shiroge | GMa048 | Japan | 29 | 62 | 29.4 | 166 | 34.4 | 36.5 |
| 41 | AGS 330 | GMa051 | Taiwan | 29 | 74 | 35.0 | 162 | 37.0 | 32.5 |
| 42 | AGS 331 | GMa052 | Taiwan | 29 | 74 | 26.8 | 171 | 33.0 | 31.0 |
| 43 | AGS 332 | GMa053 | Taiwan | 30 | 74 | 44.4 | 188 | 27.0 | 33.0 |
| 44 | AGS 333 | GMa054 | Taiwan | 29 | 72 | 34.6 | 161 | 37.0 | 36.6 |
| 45 | AGS 335 | GMa056 | Taiwan | 25 | 74 | 41.2 | 176 | 29.0 | 33.0 |
| 46 | TV1 | GMa035 | Japan | 28 | 70 | 33.2 | 169 | 31.0 | 35.0 |
| 47 | Fukura | GMa023 | Japan | 25 | 70 | 28.0 | 164 | 33.0 | 32.0 |
| Average±SD | | | | 27.78 ±2.37 | 69.3 ±2.35 | 27.92 ±5.67 | 183.6 ±17.77 | 29.36 ±3.24 | 30.64 ±3.19 |

1.6% agarose gel containing 0.6 mL 50 × TAE buffer and 1 μL ethidium bromide (10 mg/mL). Three μL of BFF (1.2 mg/mL bromophenol, 125 mg/mL Ficoll) was mixed with 10 μL of PCR products and the whole mixture was applied in each well of the gel. DNA molecular weight markers were added to each gel. The gels were run in 1× TAE buffer with 2 μL ethidium bromide at a current of 100 mA until the front marker of BFF had reached 1

cm from the end of the gel. The gels were further stained with ethidium bromide solution (0.08 mg/mL) for 5 min and then visualized by UV light transmitting Transilluminator and recorded with Bioprint-version 96.07 system. Polymorphisms at all loci were confirmed by three repeating tests at different times.

**Table 2.** Phenotypic characterization of 47 soybean genotypes scaled according to the guideline of International Board for Plant Genetic Resources (IBPGR), Descriptors for Soybean, Rome, 1984.

| Variety no. | Variety name | ------- Morphologic descriptors† ------- | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H |
| 1 | Oofurisode | 1 | 3 | 7 | 2 | 1 | 3 | 6 | 3 |
| 2 | Okuhara Wase | 2 | 7 | 9 | 3 | 1 | 5 | 3 | 2 |
| 3 | Ginsui | 1 | 3 | 7 | 1 | 1 | 3 | 2 | 3 |
| 4 | Shirofumi | 1 | 3 | 7 | 1 | 2 | 3 | 1 | 3 |
| 5 | Karitea | 1 | 3 | 7 | 1 | 2 | 3 | 1 | 3 |
| 6 | Tamasudare | 1 | 3 | 7 | 1 | 2 | 3 | 1 | 3 |
| 7 | Yukiyama | 1 | 3 | 7 | 1 | 1 | 3 | 3 | 3 |
| 8 | Kegon | 1 | 3 | 7 | 1 | 2 | 3 | 2 | 2 |
| 9 | Mikawashima | 1 | 3 | 5 | 1 | 2 | 3 | 1 | 2 |
| 10 | Hamanishiki | 1 | 3 | 5 | 1 | 2 | 3 | 1 | 3 |
| 11 | White Lion | 1 | 3 | 7 | 1 | 2 | 3 | 1 | 3 |
| 12 | Tsuronoko | 2 | 7 | 7 | 1 | 1 | 3 | 1 | 2 |
| 13 | Tamchon | 1 | 3 | 7 | 1 | 1 | 5 | 1 | 2 |
| 14 | Solunoko | 1 | 3 | 7 | 1 | 1 | 3 | 2 | 3 |
| 15 | Kahori | 1 | 3 | 7 | 1 | 1 | 3 | 1 | 3 |
| 16 | Tengamine | 1 | 3 | 7 | 1 | 1 | 3 | 8 | 3 |
| 17 | Blue Side | 2 | 7 | 7 | 2 | 1 | 3 | 8 | 3 |
| 18 | Wakakusha | 1 | 3 | 7 | 2 | 1 | 3 | 2 | 3 |
| 19 | Hatsutaga | 1 | 3 | 7 | 2 | 2 | 3 | 6 | 2 |
| 20 | Shironomai | 2 | 7 | 7 | 1 | 1 | 3 | 1 | 2 |
| 21 | ASB-02 | 1 | 3 | 7 | 1 | 1 | 3 | 3 | 3 |
| 22 | E-H | 1 | 3 | 7 | 2 | 2 | 3 | 8 | 2 |
| 23 | E-O | 1 | 3 | 7 | 3 | 2 | 3 | 6 | 3 |
| 24 | E-M | 1 | 3 | 7 | 2 | 2 | 5 | 2 | 2 |
| 25 | TMB | 2 | 7 | 7 | 1 | 1 | 3 | 3 | 2 |
| 26 | Bangkok Land | 1 | 3 | 7 | 1 | 2 | 3 | 1 | 3 |
| 27 | Suwon 155 | 1 | 3 | 5 | 2 | 2 | 3 | 7 | 8 |
| 28 | Suwon 156 | 2 | 7 | 5 | 2 | 2 | 3 | 7 | 8 |
| 29 | Suwon 157 | 2 | 7 | 5 | 2 | 1 | 3 | 7 | 8 |
| 30 | KVS 39 | 1 | 3 | 7 | 1 | 1 | 3 | 1 | 3 |
| 31 | KVS 124 | 1 | 3 | 3 | 2 | 1 | 3 | 1 | 2 |
| 32 | Kaoshiung | 2 | 7 | 5 | 2 | 3 | 3 | 8 | 2 |
| 33 | AGS 186 | 2 | 7 | 7 | 1 | 1 | 5 | 2 | 2 |
| 34 | AGS 187 | 2 | 7 | 7 | 1 | 1 | 3 | 2 | 2 |
| 35 | AGS 188 | 2 | 7 | 7 | 2 | 1 | 3 | 2 | 2 |
| 36 | AGS 189 | 2 | 7 | 7 | 1 | 1 | 3 | 2 | 2 |
| 37 | AGS 190 | 2 | 7 | 3 | 1 | 2 | 3 | 3 | 2 |
| 38 | AGS 191 | 2 | 7 | 7 | 1 | 1 | 3 | 3 | 2 |
| 39 | E-K | 1 | 3 | 5 | 1 | 1 | 3 | 1 | 2 |
| 40 | Taisho Shiroge | 2 | 7 | 7 | 2 | 2 | 3 | 2 | 2 |
| 41 | AGS 330 | 1 | 3 | 7 | 1 | 2 | 3 | 2 | 3 |
| 42 | AGS 331 | 1 | 3 | 7 | 1 | 1 | 3 | 2 | 3 |
| 43 | AGS 332 | 1 | 3 | 7 | 1 | 1 | 5 | 1 | 2 |
| 44 | AGS 333 | 2 | 7 | 7 | 1 | 1 | 5 | 3 | 3 |
| 45 | AGS 335 | 1 | 3 | 7 | 1 | 1 | 5 | 3 | 3 |
| 46 | TV1 | 2 | 7 | 7 | 1 | 1 | 3 | 2 | 2 |
| 47 | Fukura | 1 | 3 | 7 | 1 | 2 | 3 | 1 | 3 |

†A=Hypocotyl color : 1= Green, 2=Purple, B=Corolla color: 3=White, 7=Purple, C=Pubescence density: 3=Sparse, 5=Semi-sparse, 7=Normal, 9=Dense, D=Pubescence color: 1=Gray, 2=Light brown, 3=Tawny, E=Pubescence type: 1=Erect, 2=Semi-appressed, 3= Appressed, F=Stem determination: 3= Determinate, 5= Semi-determinate, G=Hilum color: 1=Clear, 2=Buff, 3=Brown, 6=Imperfect black, 7=Black, 8=Dark brown, H= Seed-coat color: 2=Yellow, 3=Green, 8=Black.

### Data analysis

Statistical analyses of the morphological and RAPD data were performed on a binary matrix. Each variable RAPD band was considered as a locus so that every locus had two alleles. For correspondence analysis, only polymorphic variables were kept. Data were analyzed with computer software packages (SPSS for Window, version 9.0). Simple matching (Puterka *et al.*, 1993) and similarity coefficient (Nei and Li, 1979) between all pairwise comparisons were calculated. Genetic dissimilarity were used to produce an average linkage cluster analysis dendrogram by the average linkage method, UPGMA (Sneath & Sokal, 1973). The method initially assumes each variety as a separated cluster and sequentially joins clusters based on increasing average distance between them.

## RESULTS

### Analysis of agro-morphological traits

Fourteen agro-morphological traits namely days to 50% flowering, days to maturity, plant height, number of marketable pods per 500 g, 100-seed weight (g), marketable pod yield/plant, hypocotyl color, corolla color, pubescence density, pubescence color, pubescence type, growth habit, hilum color and seed coat color were recorded and summarized in Table 1 and Table 2. Eight morphological traits, viz. hypocotyl color, corolla color, pubescence density, pubescence color, pubescence type, growth habit, hilum color and seed coat color were relatively free from environmental effects. Based of these traits, all cultivars were clearly separated into differential combinations of group. Three Korean varieties were especially characterized by black hilum and black seed coat color. On the other hand, all varieties of seed companies showed white corolla. The remaining morphological traits were scored in differential combinations of varieties. Individual identification of the varieties was not possible using all the morphological traits. Yet, the agronomic traits such as plant height, days to flowering, days to maturity and seed size are subjected to environmental as well as genetical influence.

**Table 3.** List of selected primer sequences and their RAPD analysis results in vegetable soybean. The RAPD fragments were named following the protocol outlined in *Crop Science* (Thomson & Nelson, 1998).

| Primer no. | Sequences 3'↔5' | No. scorable band | Name of polymorphic fragment | Polymorphism (%) |
|---|---|---|---|---|
| 1 | CATTCGAGCC | 6 | OK-$01_{1375}$ | 16.6 |
| 2 | CCGCCCAAAC | 9 | OK-$04_{564}$ | 11.1 |
| 3 | CCCAGCTGTG | 10 | OK-$17_{500}$ | 10.0 |
| 4 | GTGTCGCGAG | 7 | OK-$20_{1584}$ | 14.3 |
| 5 | GGCATGACCT | 9 | OL-$01_{1400}$, OL-$01_{1350}$, OL-$01_{1300}$ | 33.3 |
| 6 | TGGGCGTCAA | 6 | OL-$02_{1300}$, OL-$02_{1100}$ | 33.3 |
| 7 | CCAGCAGCTT | 9 | OL-$03_{1800}$, OL-$03_{1650}$, OL-$03_{1100}$ | 33.3 |
| 8 | GACTGCACAC | 4 | OL-$04_{500}$ | 25.0 |
| 9 | GTGACAGGC† | 10 | OL-$14_{1500}$, OL-$14_{850}$, OL-$14_{500}$ | 30.0 |
| 10 | AGCCTGAGCC | 8 | OL-$17_{1584}$, OL-$17_{500}$ | 25.0 |
| 11 | GTTGGTGGC† | 14 | OM-$01_{1584}$, OM-$01_{1300}$, OM-$01_{870}$ | 21.4 |
| 12 | ACAACGCCTC | 7 | OM-$02_{500}$ | 14.3 |
| 13 | GGGGGATGAG | 11 | OM-$03_{1584}$,OM-$03_{1300}$, OM-$03_{1100}$ | 27.3 |
| 14 | GGCGGTTGTC | 13 | OM-$04_{870}$ | 7.7 |
| 15 | CTGGGCAACT | 12 | OM-$06_{1100}$, OM-$06_{500}$ | 16.7 |
| 16 | GTCCACTGTG | 7 | OM-$11_{1584}$, OM-$11_{1350}$, OM-$11_{1100}$ | 42.9 |
| 17 | GGTGGTCAAG | 11 | OM-$13_{1350}$, OM-$13_{500}$ | 18.2 |
| 18 | AGGGTCGTTC | 9 | OM-$14_{870}$, OM-$14_{500}$ | 22.2 |
| 19 | GACCTACCAC | 15 | OM-$15_{1584}$, OM-$15_{1350}$ | 13.3 |
| 20 | ACCTCAGCTC | 6 | ON-$08_{500}$ | 16.7 |
| 21 | TCGCCGCAAA | 5 | ON-$11_{1050}$, ON-$11_{831}$, ON-$11_{600}$ | 60.0 |
| 22 | TCGTGCGGG† | 7 | ON-$14_{2000}$, ON-$14_{1350}$, ON-$14_{989}$, ON-$14_{750}$ | 57.1 |
| 23 | AAGCGACCT† | 10 | ON-$16_{1100}$, ON-$16_{870}$, ON-$16_{500}$ | 30.0 |
| Average ± SD | | 9.0 ± 2.57 | 2.08 ± 0.93 | 23.2 |

†Primer used for intra-varietal variation analyses.

### RAPD analysis

From a preliminary screening of 60 primers on 16 cultivars, a total of 23 primers produced polymorphic bands and thus used to evaluate the remaining varieties. A summary of all primers and amplified products from this study is shown in Table 3. Using 23 primers, a total of 207 fragments were amplified across the 47 varieties. Between 4 (OPL-04) and 15 (OPM-15) fragments per primer were scored with an average of 9.0 ± 2.75 bands per primer. Among the 207 fragments,118 (57%) were monomorphic, 48 (23.2%) were polymorphic while the remaining 41 (19.8%) were unstable between the repeated PCR amplifications, and thus excluded from further study. Bands amplified ranged in size from less than 500 bp to more than 2000 bp, although most (~80%) ranged between 800 and 1500 bp. The majority (~90%) of the unstable bands were size separated at either less than 500 bp or higher than 2000 bp. Only fragments with a high intensity and no doubt as to their presence or absence in the three RAPD replicates were taken into account. Each primers produced a different set of polymorphic bands. An example of a RAPD pattern generated by ON-11 primer on variety no. 17 to 32 is shown in Fig. 1. In this case, 5 specific fragments were amplified by the primer, the upper 2 bands are monomorphic in all varieties and thus not scored in data sheet while the three were scored as present or absent in different combinations of varieties. The mean number of selected polymorphic bands per primer was 2.08 ± 0.93 using this criterion. The number of polymorphic bands were not significantly correlated (r=0.119) with the total number of bands generated by each primer.

### Genetic distances

Eight morphologic traits described by 25 binary character states and 48 polymorphic markers of the 47 soybean varieties were separately used to calculate genetic distances for all 1,081 pairwise comparisons (Table not shown). Using morphological traits, the average genetic distance among 47 varieties was 0.295 ± 0.013, with the maximum value of 0.640 (Okuhara Wase vs AGS 330) and the minimum of 0.00. On the other hand, the RAPD based smallest genetic distance was found between the pairs Ginsui vs Tamasudare, Tamasudare vs Hamanishiki, Suwon 156 vs Suwon 157, AGS 190 vs AGS 191 and, AGS 333 vs AGS 335 all of which had a distance coefficient of 0.042 to 0.062 with at least two RAPD marker differences. The largest distance coefficient (0.625) was found between TMB vs Fukura with 28 marker differences. Substantial genetic variation exist among these vegetable soybean varieties with an average of 0.336 ± 0.099 (33.6%).

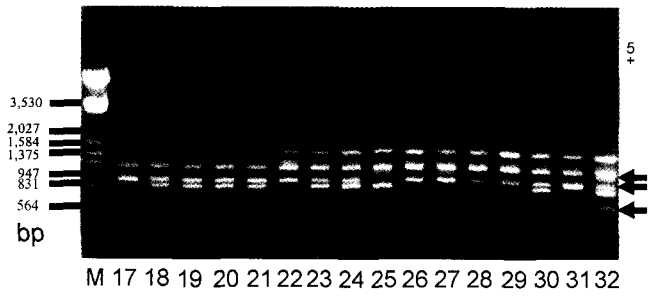


**Fig. 1.** RAPD fragment patterns of 47 East-Asian vegetable soybean varieties generated by the primer ON-11 (3' TCGCCGCAAA 5'). M is the DNA marker (l DNA digested with Hind III and EcoRI) Lanes 17 to 32 correspond to series number listed in Table 1. The polymorphic bands are marked by arrows.

### Cluster analysis

The distance matrix of simple matching coefficient from morphology and RAPD results were used for plotting the UPGMA based dendrogram (Fig. 2A and 2B). The morphological analyses resulted in differentiation of the soybean varieties into four main groups at about 22 distance scale level (Fig. 2A). The largest group (A) included 15 of 24 Japanese varieties, 5 of 14 Taiwan varieties and 4 of 6 Thai varieties with two sub-clusters. In the second group (B) a lone Taiwan varieties named KVS 124 was separated from the rests. The 3rd group (C) consisted of 5 Japanese and 6 Taiwan varieties. In the last group (D), the remaining 4 Japanese, 2 Taiwan, 2 Thai and all of the Korean varieties were clustered showing two distinct sub-clusters. One sub-cluster of this group contained all the Korean varieties. Thirty out of the 47 varieties (64%) were clearly discriminated showing differential genetic distances (>0) in the dendrogram (Fig. 2A).

On the other hand, cluster analyses based on 48 RAPDs, revealed differential associations (Fig. 2B). The vegetable soybean varieties fell into 4 groups at the distance scale of 22, containing 4, 40, 2 and 1 accessions, respectively. In the first group (E), only two closely related Taiwan varieties (AGS 190 and AGS 191) made association with two Japanese varieties (TMB and TV1). The largest group which was assigned as group F, consisted of 21 Japanese, 12 Taiwan, 6 Thai and 1 Korean accessions. All of these varieties can be further grouped into 5 sub-clusters containing differential geographical combination of vegetable soybean accessions. Four Thai varieties, namely ASB-02, E-H, E-O and E-K showed close proximity in one subcluster. Although one Taiwan varieties (Kaoshiung) made close relation with Thai varieties. In group G, 2 out of 3

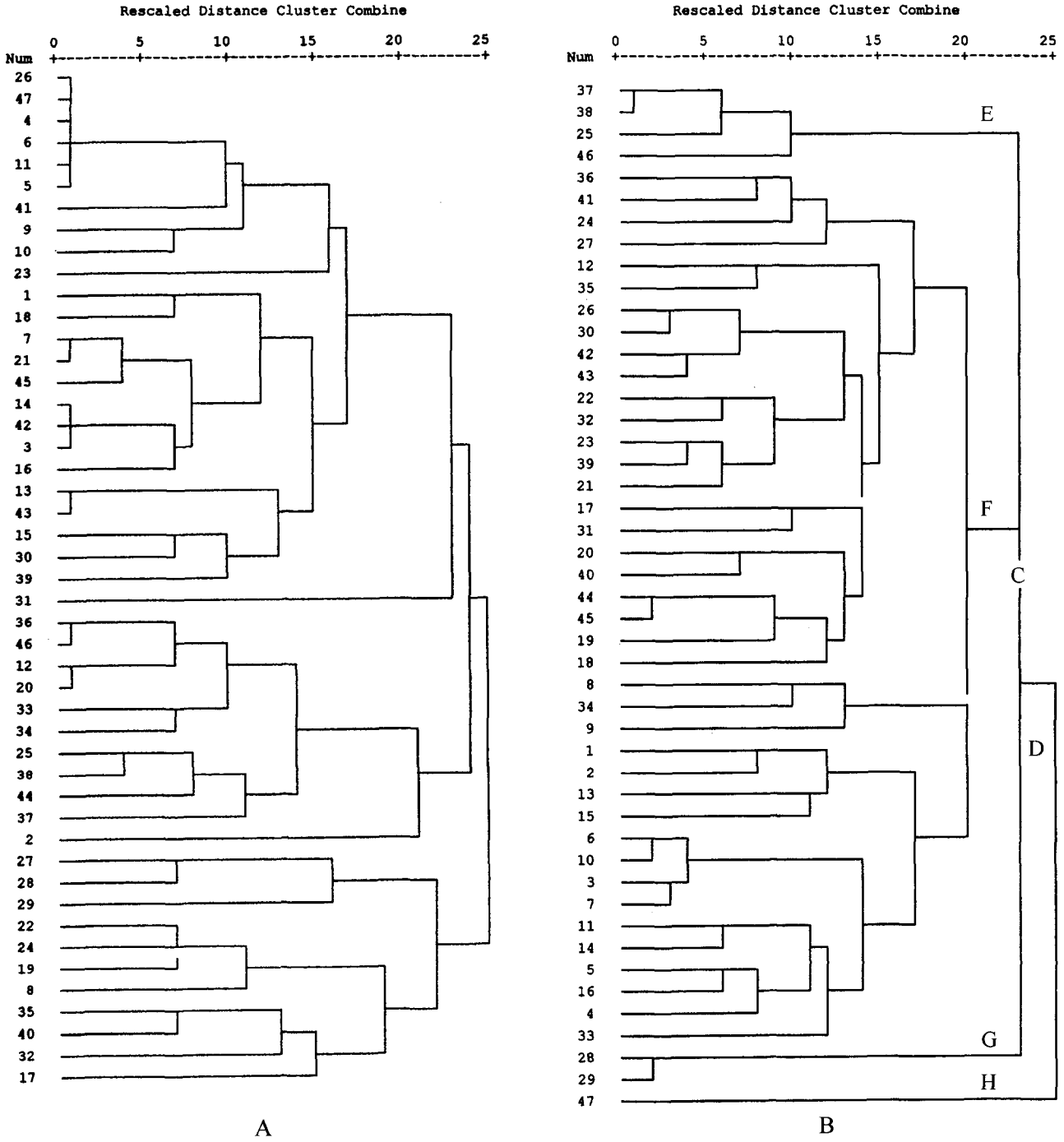


**Fig. 2.** A; Cluster analysis dendrogram of 47 soybean genotypes based on 8 morphological traits described by 25 binary character states listed in Table 2. The numerical scale indicates increasing genetic distance. Varieties numbers correspond to Table 1. B; Dendrogram illustrating genetic distance among 47 East-Asian vegetable soybean varieties generated by UPGMA cluster constructed from 48 RAPD markers. The numerical scale indicates increasing genetic distance. Varieties numbers correspond to Table 1.

Korean varieties formed a close association, whilst the remaining one was outside of this group but close to Taiwan accession KVS 39. The Japanese varieties Fukura was clearly distinguished from other varieties in the cluster analysis and assigned as group G (Fig. 2B). The cluster analysis of RAPD based variation did not separate these 47 varieties into groups in accordance with their geographic origins. But all of the varieties could be distinguished by at least one pair of RAPD marker difference with a genetic dissimilarity of 4% level.

### Analysis of intra-varietal variation

Two Japanese cultivars, 2 Taiwan cultivars and 6 Thai commercial cultivars were analyzed to assess their genetic uniformity and stability. Four primers, viz. OPL-14, OPM-1, OPN-14 and OPN-16 were chosen for the amplification of individual plant DNA (Table 2). Eight individuals of each varieties amplified only monomorphic band, thus revealed that no polymorphism was detected in this study.

## DISCUSSION

This study is among the first one trying to detect genetic diversity of East-Asian vegetable soybean varieties based on comparative analysis of agro-morphological traits and RAPDs. Analysis of morphological traits revealed that the specificity of these markers for variety discrimination was very limited. For example, the 47 varieties could be classified into two classes when using all possible combinations of two morphological characters such as hypocotyl and corolla color. Agro-morphological comparisons based on plant height, days to flowering and maturity, and seed size have limitations including subjectivity in assigning values to each character and the influence of management practices on the characters. Moreover, limited diversity among varieties with highly similar pedigrees and confining of expression of some diagnostic characters to a particular stage of development, such as corolla color on flowering stage, pubescence traits on immature pod and hilum color on mature dry seed are another limitation. These morphological traits have been used traditionally to distinguish varieties. But out of fourteen, eight characters were highly heritable and stable. Using these morphological traits, it was possible to trace those varieties that were at the largest genetic distance. Therefore, the importance of scoring the morphological traits with molecular data cannot be underestimated. The varieties included in this study were chosen to represent the major regions of vegetable soybean production. However, there was no clear-cut discernable geographical patterns of variation found in this study. Griffin and Palmer (1995) assumed that the long history of domestication and commercialization of soybean in East-Asia has contributed to the dispersion of alleles throughout the region, lessening the influence of geography on patterns of variation among them.

The average number of polymorphic bands per primer of 2.1 obtained in vegetable soybean is relatively low compared to that observed in wild soybean (Hu *et al.*, 1997) and Azuki bean (Yee *et al.*, 1999) but similar (2.0 bands/primer) with USDA-ARS soybean germplasm reported by Brown-Guedira *et al.* (2000). This difference can be attributed to the genotypes evaluated, the primers used, and to the selection of scorable bands in each study (Cansian & Echeverrigaray, 2000). The large number of monomorphic bands (57%) were produced across all varieties during RAPD analysis. This is an evidence for a high degree of similarity among the varieties. Matching coefficients were, for some parts, higher than similarity coefficients because both shared absence and presence of bands as indication of similarity in the matching coefficients. There was a slight ranking difference between simple matching and similarity averages, since matching coefficients have better discriminatory power than similarity coefficients between closely related genotypes (Hilu, 1994). Therefore, simple matching coefficients were used for the estimation of genetic distance.

Both morphological and RAPD dendrograms did not indicate as clear pattern of division among the vegetable soybean varieties based on putative geographic origin, as seen in some other crops (Spooner *et al.*, 1996; Metais *et al.*, 2000). Originally, Japanese accessions have been highly utilized as parental materials in breeding programs of other East-Asian countries. Hence the genepool contributing to cultivated vegetable soybean is rather restricted. In RAPD dendrogram, Japanese variety Fukura was excluded from the rests. However, it is rather difficult to conclude whether this was due to morphological difference, as it had no genetic distance and was closely clustered with Shirofumi, Karitea, Tamasudare, White Lion and Bangkok Land in morphological dendrogram. Similarly, the Japanese variety Okuhara Wase and Taiwan variety KVS 124 were quite different in morphological dendrogram, but both of them made a close relation with Oofurisode and Blue Side in RAPD dendrogams, respectively. The grouping based on RAPD data has nothing to do with morphological grouping, but most likely related to their genetic resemblance, *i.e.*, due to level of RAPD marker difference (Debener *et al.*, 1996; Liu, 1997). Japanese varieties Ginsui vs Tamasudare, Tamasudare vs Hamanishiki, Korean varieties Suwon 156 vs Suwon 157 and Taiwan varieties AGS 190 vs AGS 191 and AGS 333 vs AGS 135 revealed low genetic distance and showed close association with the same geographical originated varieties in the dendrogram. Theoretically, varieties chosen through line selection from the same cross should be clustered closely with each other due to the close genetic relationship. It is not surprising that the grouping based on RAPD variation did not fully agree with that based on morphological traits. RAPD analysis estimates overall dissimilarity at DNA level with each PCR product potentially representing a different locus (Liu, 1997). However, varieties possessing traits of interest in wide genetic diversity should be good sources of variation which are essential for progress of breeding programs. Therefore, RAPD markers can be employed to select the perspective parents. Conse-

quently, varieties number 46 in group E, 27, 18, 9, 13, 15 and 33 in group F, and 47 in group H were identified and could be prescribed as potential parents for crossing programs in the future. Among them, varieties 13, 27, 33, 46 and 47 yielded more than average marketable pods (30.6 g per plant). Therefore, considering RAPD based potentiality and the economic trait, namely yield per plant, the above mentioned cultivars would be prescribed in crossing programs.

It can be concluded that RAPD is a valuable tool for assessing genetic diversity levels in vegetable soybean. It generated polymorphism at the DNA level and thus more efficient in discrimination among varieties. The future utility of genetic distance estimation is to identify genetic variation and to find linkages between RAPD markers and agro-morphological traits of interest. This information, together with quantitative genetic studies of economically important traits will help the breeder making his final choice of the parental lines, and employing appropriate marker-assisted selection.

## ACKNOLEDGEMENT

This work is a part of Ph. D. thesis of the first author and was supported by the Agricultural Research Management Project (ARMP), Bangladesh, Kasetsart University and the Thailand Research Fund (TRF).

## REFERENCES

Brown, A. H. D. 1979. Enzyme polymorphism in plant populations. *Theor. Appl. Genet.* 15 : 1-42.

Brown-Guedira, G. L., J. A. Thomson, R. L. Nelson and M. L. Warburton. 2000. Evaluation of genetic diversity of soybean introductions and North American ancestors using RAPD and SSR markers. *Crop Sci.* 40 : 815-823.

Cansian, R. L. and S. Echeverrigaray. 2000. Discrimination among cultivars of cabbage using randomly amplified polymorphic DNA markers. *Hort Sci.* 35(6) : 1155-1158.

Debener, T., C. Bartels and L. Mattiesch. 1996. RAPD analysis of genetic variation between a group of rose cultivars and selected wil rose species. *Molecular Breeding* 2 : 321-327.

Diwan, N. and P. B. Cregan. 1997. Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. *Theor. Appl. Genet.* 95 : 723-733.

Doyle, L. J. & J. J. Doyle, 1990. Isolation of plant DNA from fresh tissue. *Focus* 12 : 13-14

Griffin, J. D. and R. G. Palmer. 1995. Variation of thirteen isozyme loci in the USDA soybean germplasm collections. *Crop Sci.* 35 : 897-904.

Hawkes, J. G. 1983. The Diversity of Crop Plants. Harvard University Press, MA.

Hilu, K. W. 1994. Evidence from RAPD markers in the evolution of *Echinochloa* millets (Poaceae). *Plant Syst. Evol.* 189:247-257.

Hu, Z. A., M. Zhong, W. Wei and H. X. Wang. 1997. Detection of DNA diversity of wild soybean (*Glycine soja*) in natural populations by new procedure of RAPD and RFLP. *Soybean Genet. Newsl.* 24 : 37-38.

IBPGR. 1984. Descriptors for Soybean. International Board for Plant Genetic Resources, Rome, Italy. 38p.

Karp, A., O. Seberg and M. Buiatti. 1996. Molecular techniques in the assessment of botanical diversity. *Ann. Bot.* (London) 78 : 143-149.

Liu, C. J. 1997. Geographical distribution of genetic variation in *Stylosanthes scabra* revealed by RAPD analysis. *Euphytica* 98 : 21-27.

Metais, I., C. Aubry, and R. Jalouzot. 2000. Description and analysis of genetic diversity between commercial bean lines (*Phaseolus vulgaris* L.) *Theor. Appl. Genet.* 101 : 1207-1214.

Nei, M. and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. (USA).* 76 : 5269-5273.

Puterka, G. J., W. C. Black IV, W. M. Steiner, and R. L. Burton. 1993. Genetic variation and phylogenetic relationships among worldwide collections of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), inferred from allozyme and RAPD-PCR markers. *Heredity* 70 : 604-618.

Sneath, P. H. A. and R. R. Sokal (1973). Numerical taxonomy. W.H. Freeman. San Francisco.

Spooner, D. M., J. Tivang, J. Neinhuis, J. T. Miller, D. S. Douches and A. Contreras-M. 1996. Comparison on four molecular markers in measuring relationships among wild potato relatives *Solanum* section *etuberosum* (subgenus *Potatoe*). *Theor. Appl. Genet.* 92 : 532-540.

Tatineni, V., R. G. Cantrell and D. D. Davis. 1996. Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPDs. *Crop Sci.* 36 : 186-192.

Thompson, J. A., R. L. Nelson and L. O. Vodkin. 1998. Identification of diverse soybean germplasm using RAPD markers. *Crop Sci.* 38 : 1348-1355.

Thompson, J. A. and R. L. Nelson. 1998. Core set of primers to evaluate genetic diversity in soybean. *Crop Sci.* 38 : 1356-1362.

Weising, K., H. Nybom, K. Wolf and W. Mayer. 1995. DNA fingerprinting in plants and fungi. CRC Press, Inc. London.

Yee, E., K. K. Kodwell, G. R. Sills, and T. A. Lumpkin. 1999. Diversity among selected *Vigna angularis* (Azuki) accessions on the basis of RAPD and AFLP markers. *Crop Sci.* 39 : 268-275.