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Polymorphic differentiation and genetic variation of soybean by RFLP analysis

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Abstract. Twenty soybean accessions, representing local varieties, improved cultivars, plant introductions, breeding lines, two pairs of isogenic lines with different seed coat colors, and one *Glycine soja* accession were used for detecting genetic differentiation by restriction fragment length polymorphism (RFLP) markers. Seventeen probes which are believed to be primarily single copy sequences derived from a soybean genomic library of *Pst*I digests were used. Eleven of these seventeen probes were proved to yield polymorphisms in one- or two-enzyme digestion. From a total of fifty-three probe -enzyme combinations, fifteen demonstrated polymorphism among accessions. DNA digested with *Hind*III showed a higher percentage of polymorphisms. The number of polymorphic alleles estimated from this study was forty-three alleles over twenty-five loci. Among these, 20% of loci have *G. soja* specific differences. Both dominant and codominant loci were noted, with 36% and 64% respectively. Alleles occurring in one or two accessions are considered rare. Fifty-six percent of polymorphic loci predominantly had rare allele, and only 44% of loci were informative. Variations within accessions were also noted with some probe-enzyme combinations. An average of 0.236 heterozygosity per locus was obtained from these RFLP marker loci. Dendrograms based on the coefficient of genetic similarity derived from this RFLP data classified the twenty accessions into five groups. Genetic differentiation could be achieved in eighteen of twenty soybean accessions by eleven probe-enzyme combinations. However, difficulty in distinguishing one pair of isogenic lines remained.

Key words: Average heterozygosity; Genetic similarity; *Glycine max* (L.) Merr.; Informative loci; Variety.

Introduction

Until recently the genetic mapping of most crop species progressed slowly, due to a shortage of available genetic markers. The exploration of molecular markers threw a light on gene mapping, leading to a complete linkage map with markers all over the linkages and spreading along each chromosome arm. Selection of quantitative traits, which account for most agriculturally important traits, will be possible with the assistance of these markers (Lande and Thompson, 1990; Lander and Botstein, 1989; Stuber *et al.*, 1987).

Molecular markers have an advantage over morphological markers in several ways: 1) genotype determination is not restricted to certain developmental stages; 2) a relatively large number of marker loci can be found; 3) usually no deleterious effects are associated with alternate alleles; 4) most alleles have the codominant gene action facilitated genotypic identification; and 5) fewer epistatic or pleiotropic effects are observed with molecular loci (Tanksley, 1983). Presently, isozyme and DNA markers are two of the most common types of marker for plant genetics and breed-

ing studies. Isozyme analysis provides a very simple and inexpensive method for these studies. Unfortunately, isozyme markers suffer the drawback of limited number. The restriction fragment length polymorphism (RFLP), a type of DNA marker believed to be sufficiently abundant to saturate the genome, was first proposed by Bostein *et al.* (1980) for mapping the linkages of the human genome. This stimulated the use of RFLP markers in crop improvement studies (Soller and Beckmann, 1983; Helentjaris *et al.*, 1985; Tanksley *et al.*, 1989).

RFLP markers in soybean were first reported by Apuya *et al.* (1988), who used them to identify widely distant cultivars of *Glycine max.* (L.) Merrill and wild perennial relatives of soybean (Menancio *et al.*, 1990). Keim *et al.* (1989) surveyed fifty-eight soybean accessions from the genus *Glycine*, subgenus *soja* with seventeen RFLP markers, but only low levels of molecular diversity were noted. RFLP markers of mitochondria DNA were used for the identification of cytoplasmic background difference in a subclass of Mandarin soybean (Grabau *et al.*, 1989). Keim and Shoemaker (1988) generated a random recombinant DNA library enriched with single copy DNA. These probes have been used to construct an RFLP genetic linkage map, to map disease-resistance traits (Diers, 1992b), and in near-isogenic line mapping studies (Muehlbaner *et al.*, 1991). The association between RFLP marker loci and quantitative traits in soybean was also addressed (Keim *et al.*, 1990a,b; Diers *et al.*, 1992a). Recently, linkage relationships between RFLP markers and *Phytophthora* resistance loci of soybean was noted (Diers *et al.*, 1992b).

Soybean is a self-pollinated plant, grown from the northern temperate zone to the tropics, with at least thirteen photoperiod-response maturity groups (Palmer and Kilen, 1987). The objective of this study was to explore RFLP marker loci for genetic differentiation, and to understand the genetic variation of cultivated soybean in Taiwan's breeding resource. Soybean DNA probes, obtained from Dr. Shoemaker at Iowa State University, Ames, Iowa in the USA, were used to differentiate land varieties, improved cultivars, isogenic lines, and plant introduction accessions. The polymorphic structure, genetic similarity, and efficiency of probe sources for differentiation are discussed.

Materials and Methods

Plant Materials

A total of twenty soybean lines, representing three local land varieties from Taiwan, four improved cultivated varieties, two pairs of isogenic lines derived from F5 selfed progeny, eight plant introduction or breeding lines, and one *soja* accession (PI245331), were obtained from the Taiwan Agricultural Research Institute. Seeds of soybean were germinated and grown under hydroponic culture in a growth chamber that maintained a 28°C daytime temperature and a 24°C nighttime temperature.

The nutrient components in the hydroponic cultures were 2.5 mM KNO_3 and $Ca(NO_3)_2$, 0.5 mM KH_2PO_4, 1.0 mM $MgSO_4$, 20 ppm FeNaEDTA, and microelements (Barrentine *et al.*, 1976). Trifoliolate leaves at the V3 stage (Fehr and Caviness, 1977) were sampled from at least four to five plants in each accession for DNA isolation.

DNA Extraction, Enzyme Digestion and Southern Blotting

The procedure for DNA isolation was similar to that of Murrary and Thompson (1980). In general, about 3 g of fresh or frozen leaves were ground to a fine powder in liquid N_2 with a chilled mortar and pestle. Nine ml of preheated extraction buffer [50 mM Tris-HCl (pH 8.0), 0.5% N-lauroylsarcosine, 1.2% SDS (sodium dodecyl-sulfate) and 250 mM EDTA (pH 8.0)] was then added and the mixture incubated for 2 h at 60°C. Leaf residues were then discarded by centrifuging at 15,000 rpm (17,600 x g) for 15 min. The supernatant liquid was adjusted to contain 0.7 M NaCl and 1% CTAB (cetyltrimethylammonium bromide) followed by extraction with an equal volume of chloroform/isoamylalcohol (24:1, v/v). DNA extraction in 1% CTAB and 0.7 M NaCl with an equal volume of chloroform/isoamylalcohol was repeated at least three times. DNA was precipitated by adding 0.6 volumes of isopropanol, washed two times with 80% ethanol containing 15 mM ammonium acetate, and dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). The DNA was then treated with RNase (50 μg/ml), and proteinase K (100 μg/ml), in 0.5% SDS at 37 °C, for 2 h respectively. A phenol/chloroform (1:1, v/v) extraction followed. One-tenth volume of 3M sodium acetate (pH

6.1) was added, and then the DNA was precipitated with two volumes of absolute ethanol. The DNA was washed with 80% ethanol containing 15 mM ammonium acetate, and then dissolved in TE buffer. The DNA was digested with several restriction enzymes (Table 1). In general, about 10 units of endonuclease per ug of DNA were used for a 5 h digestion in 200 μl of incubation buffer. The DNA fragments were electrophoresed in 0.8% agarose gel, with 8-10 μg of DNA per lane. TBE (89 mM Tris base, 89 mM boric acid, and 2 mM EDTA) served as the running buffer. After electrophoresis, the DNA was transferred to Hybond-C extra membrane or Hybond-N membrane (Amersham Inter- [illegible] Southern (1975).

Probes Preparation and Random Primed Labeling

A total of eighty-five soybean probes, which were primarily single copy sequences from a *Pst*I genomic library (Keim and Shoemaker, 1988), were kindly supplied by Dr. Randy C. Shoemaker of Iowa State University, USA. From these, seventeen soybean probes: PA7, PA36, PA65, PA73, PA85, PA86, PA109, PA110, PA111, PA117, PA122, PA129, PA136, PA170, PA233, PA256, and PK3, were randomly selected and screened for polymorphic loci with various endonuclease (Table 1) on eight - nine selected soybean accessions from different sources. For those probes showing polymorphism, studies were then extended to other soybean accessions. Plasmid DNA was isolated by the mini alkaline lysis method (Maniatis *et al.*, 1982), and then digested with *Pst*I in preparation for isolation of the inserts with electrophoresis and NA45 membrane (Schleicher and Schnell, NA45) interception. The inserts were labeled with α-^{32}P-dCTP by the random primed method (Feinberg and Vogestein, 1983), and purified in a Sephadex G-50 column.

Hybridization Analysis

Membranes were prehybridized in 10 - 20 ml of 20 mM phosphate buffer (pH 6.8), 1X Denhardt's solution (Denhardt, 1976), 5X SSC (20X SSC is 3M NaCl, 0.3 M sodium citrate, adjusted to pH 7.0), 0.1% SDS, and 200 μg/ml calf thymus DNA for 4 - 5 h at 65°C. The labeled probes were then directly injected into the hybridization bags, and hybridized overnight at 65°C. The membranes were then washed twice in 2X SSC and 0.1% SDS at room temperature, once for 30 min in 1X SSC and 0.1% SDS at 65°C, and once for 30 min in 0.1X SSC and 0.1% SDS at 65°C. Autoradiography was carried out at −70°C, with Kodak X-Omat AR film and two sheets of DuPont Cronex Hi-Plus intensifying screen (Chen *et al.*, 1990).

Table 1. *Summary of number of probes tested for polymorphisms on soybean accessions for each enzyme*

Enzyme	With variation	Without variation	T[illegible]

Estimation of Genetic Parameters

Genetic similarity between soybean accessions was calculated according to Nei and Li (1979), i.e., S(xy) = 2Nxy/(Nx+Ny), where S(xy) is the value of genetic similarity between lines x and y, Nxy is the number of bands common to lines x and y, and Nx+Ny is the sum of the number of bands for lines x and y. A dendrogram was derived by average linkage (UPGMA) cluster analysis with the coefficient of genetic similarity by SAS (Statistical Analysis System, Cary, NC). The percentage of polymorphic loci was calculated based on probe-enzyme combinations with polymorphism. The number of polymorphic alleles in each probe-enzyme com-

ived from the *Pst*I digestion library. However, variations were noted at no more than two - three loci in a single probe-enzyme combination (Fig. 1). The allelic relationships between polymorphic bands were inferred from their distribution among the soybean accessions. Fragments which were seldom observed together in a single accession, though every accession had one or the other, were classified as codominant loci. Fragments which were present or absent with no exclusory relationships with other fragments were classified as dominant loci (Keim *et al.*, 1989). Tentative classifications

estimated. The average expected also calculated according to Chen nate the genetic diversity.

encies among the Probe-Enzyme

accessions representing different s were used to determine the probe-ons that could serve for further tudies. From a total of fifty-three binations (Table 1), fifteen (28.3%) w polymorphic patterns in at least cific differences between the *soja*) and other *G. max* accessions were *nd*III, PA110-*Hind*III, and PA110-s. Sixty-five percent of the probes proved to be polymorphic. As shown nzymes examined, *Hind*III had the of detection of polymorphisms h different probes. Of a total of thir-eight indicated variations among n the total DNA was digested with digestion, only 33.3% of the probes phisms. Enzymes such as *Taq*I, *Eco*RV showed little or no variation. rns and genetic similarities were the fifteen probe-enzyme combina-

rphic Patterns

patterns were frequently observed A was hybridized with probes der-

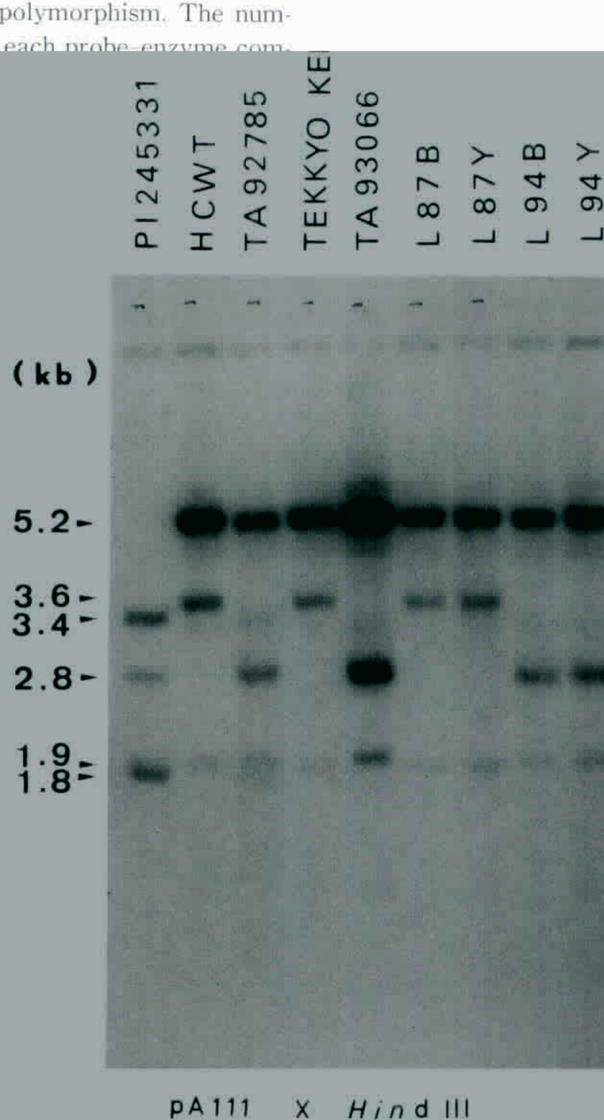

Fig. 1. Autoradiogram of restriction fragments from *Hind*III digestion of nine soybean genomic DNAs, hybridized with PA111 probe. PI245331 is a *G. soja* accession.

bination was also heterozygosity wa *et al.* (1989) to esti

Results

Polymorphic Freq Combinations

Eight soybean genetic backgroun enzyme combinati genetic variation s probe-enzyme con were found to sho one accession. Sp accession (PI24533 noted in PA36-*Hi* *Eco*RI combinatio used for this study in Table 1, of the highest percentag when combined wi teen probes tested the accessions whe *Hind*III. In *Eco*RI exhibited polymo *Bam*HI, *Dra*I, and Polymorphic patte then estimated for tions.

Structure of Polym

Multiple band when digested DN

uencies of soybean

A- 30 6	TA- 932- 35	L87 -B	L87 -Y	L94 -B	L94 -Y	Allele freq- uency
+	−	+	+	+	+	0.60
−	+	+	+	−	−	0.75
+	+	+	+	+	+	0.95
−	−	−	−	−	−	0.05
+	+	+	+	+	+	0.90
−	−	−	−	−	−	0.15
+	+	+	+	+	+	0.85
+	+	+	+	+	+	0.95
−	−	−	−	−	−	0.05
+	+	+	+	+	+	0.90
+	+	+	+	+	+	0.95
−	−	−	−	−	−	0.10
−	−	−	−	−	−	0.10
−	−	−	−	−	−	0.20
+	+	+	+	+	+	0.80
−	−	−	−	−	−	0.15
+	+	+	+	+	+	0.85
−	−	−	−	−	−	0.05
+	−	−	+	+	+	0.70
−	+	+	+	−	−	0.30
−	−	−	−	−	−	0.05
−	−	−	−	−	−	0.05
+	+	+	+	+	+	0.95
+	+	+	+	+	+	0.95
−	−	−	−	−	−	0.05
+	+	+	+	+	+	0.95
+	+	+	+	+	+	0.90
−	−	+	+	−	−	0.45
−	−	−	−	−	−	0.10
+	+	−	−	+	+	0.60
−	−	−	−	−	−	0.10
−	−	+	+	+	+	0.70
[illegible]	+	−	−	−	−	0.30
−	+	−	−	−	−	0.45
−	−	−	−	−	−	0.05
−	−	+	+	+	+	0.70
−	+	−	−	−	−	0.35
−	−	−	−	−	−	0.05
−	+	+	+	+	+	0.80
−	−	−	−	−	−	0.20
−	−	−	−	−	−	0.05
−	+	+	+	+	+	0.95
−	−	−	−	−	−	0.10

ıeterozygous within accession was noted.

Table 3. *Genetic similarity between soybean accessions estimated by RFLP markers*[a]

	AGS-129	HCWT	FWCP	TN15	HL-1	KS10	G2120	PI245-331	SKT	BPCL	TEKKYO KERO	TA92-785	TA92-616	TA93-005	TA93-066	TA93-235	L87-B	L87-Y	L94-B	L94-Y	AVERAGE Band No.
AGS-129		30/44	36/45	28/42	28/41	44/45	34/41	22/43	36/43	32/41	32/41	32/42	32/42	30/41	32/41	36/42	38/42	38/43	36/41	36/41	33.3±4.8
HCWT	68.2		30/45	36/42	36/41	30/45	26/41	12/43	30/43	28/41	28/41	26/42	36/42	22/41	32/41	32/42	30/42	32/43	28/41	28/41	29.1±5.5
FWCP	80.0	66.7		28/43	28/42	36/46	32/41	24/44	34/44	30/42	32/42	30/43	32/43	26/42	30/42	30/43	34/43	36/44	34/42	34/42	31.4±3.4
TN15	63.6	85.7	65.1		36/39	28/43	24/38	10/41	30/41	26/39	28/39	22/40	36/40	26/39	30/39	32/40	28/40	30/41	26/39	26/39	27.9±5.8
HL-1	68.3	87.8	66.7	92.3		28/42	26/37	10/40	30/40	28/38	30/38	24/39	36/39	24/38	32/38	32/39	30/39	32/40	28/38	28/38	28.7±5.8
KS10	97.8	66.7	78.2	65.1	66.7		32/41	22/44	36/44	32/42	32/42	32/43	32/43	30/42	32/42	36/43	38/43	38/44	36/42	36/42	32.6±5.0
G2120	80.0	60.0	78.0	63.2	70.3	78.1		16/39	32/39	28/37	34/37	28/38	28/38	24/37	28/37	28/38	30/38	32/39	32/37	32/37	28.7±4.4
PI245331	51.2	27.9	54.6	24.4	25.0	50.0	41.0		18/42	18/40	16/40	22/41	14/41	18/40	14/40	14/41	18/41	18/42	18/40	18/40	16.9±4.0
SKT	83.7	69.8	77.3	73.2	75.0	81.8	82.1	42.9		32/40	32/40	30/41	32/41	26/40	32/40	34/41	32/41	34/42	34/40	34/40	31.5±4.0
BPCL	78.0	68.3	71.4	66.7	73.7	76.2	75.7	45.0	80.0		28/38	34/39	26/39	26/38	34/38	30/39	30/39	32/40	34/38	34/38	29.6±4.0
TEKKYO-KERO	78.0	68.3	76.2	71.8	78.9	76.2	91.9	40.0	80.0	73.7		28/39	32/39	28/38	28/38	28/39	34/39	36/40	32/38	32/38	30.0±4.2
TA92785	76.2	61.9	69.8	55.0	61.5	74.4	73.7	53.7	73.2	87.2	71.8		26/40	30/39	30/39	26/40	30/40	32/41	34/39	34/39	28.9±3.8
TA92616	76.2	85.8	74.4	90.0	92.3	74.4	73.7	34.2	78.0	66.7	82.1	65.0		26/39	30/39	32/40	32/40	34/41	30/39	30/39	30.3±5.0
TA93005	73.2	53.7	61.9	66.7	63.2	71.4	64.9	45.0	65.0	68.4	73.7	76.2	66.7		22/38	26/39	32/39	32/40	26/38	26/38	26.3±3.5
TA93066	78.1	78.1	71.4	76.9	84.2	76.2	75.7	35.0	80.0	89.5	71.8	75.0	75.0	56.4		34/39	30/39	32/40	34/38	34/38	30.0±4.8
TA93235	85.7	76.2	69.8	80.0	82.1	83.7	73.7	34.2	82.9	76.9	71.8	65.0	80.0	66.7	85.0		32/40	32/41	30/39	30/39	30.2±4.8
L87-B	90.5	74.4	79.1	70.0	76.9	88.4	79.0	43.9	78.0	76.9	87.2	75.0	80.0	82.1	75.0	80.0		40/41	34/39	34/39	31.9±4.6
L87-Y	88.4	79.1	81.8	73.2	80.0	86.4	82.1	42.9	80.9	80.0	90.0	78.0	82.9	80.0	78.0	78.0	97.6		38/40	38/40	33.5±4.8
L94-B	87.8	68.3	81.0	66.7	73.7	85.7	86.5	45.0	85.0	89.5	84.2	87.2	76.9	68.4	87.2	76.9	87.2	95.0		38/38	31.7±4.9
L94-Y	87.8	68.3	81.0	66.7	73.7	85.7	86.5	45.0	85.0	89.5	84.2	87.2	76.9	68.4	87.2	76.9	87.2	95.0	100.0		31.7±4.9
AVERAGE																					29.7±3.5
(%)	78.6	69.2	73.0	69.3	73.2	77.0	74.5	41.1	76.5	75.4	76.5	72.0	75.6	67.0	76.5	75.1	79.5	81.6	80.8	80.8	73.7±8.6
±S.D.	±10.8	±13.4	±8.0	±14.5	±14.8	±10.5	±11.4	±9.0	±9.7	±10.5	±11.0	±9.9	±12.0	±8.8	±12.5	±11.6	±10.9	±11.5	±12.5	±12.5	

LSD = 5.3

a: Numbers in the up right orthogonal triangle indicated number of bands for the estimation of genetic similarity (2Nxy/Nx+Ny) as described in the materials and methods; numbers in the down left orthogonal triangle indicated percentages of genetic similarity.

for the locus types based on the allelic relationships between polymorphic bands from this study are indicated in Table 2. About twenty-five loci were distinguishable from this classification. Both dominant and codominant alleles were noted in this study (Table 2). Rare alleles which were variety or accession specific were noted in about 56% of loci. For example, the 1.5 kb band of PA117-*Hind*III in KS-10, the 1.5 kb of PA136-*Eco*RV in HCWT, the 2.8 kb band of PA233-*Dra*I in SKT, the 8.0 kb band of PA36-*Eco*RI, the 2.4 kb band of PA36-*Hind*III, the 23.1 kb band of PA109-*Eco*RI, the 7.0 and 3.9 kb bands of PA110-*Hind*III, and the 3.0 kb band of PA110-*Eco*RI were only found in PI245331, the *soja* accession. Polymorphisms with more than one enzyme were noted in probes PA36, PA85 (Fig. 2), PA110, and PA136. As indicated in Table 2, some heterozygous band patterns were also observed in accessions such as TA92785, L87Y, SKT, and FWCP in loci of c4, c7, c14, c1, and c12 respectively. It is indicated that 20% of polymorphic loci are the result of the difference between *G. max* and *G. soja*. From the total of forty-three fragment types observed, 79.1% were in the form of codominant allele and 20.9% were in dominant form. In terms of loci, 64% of the loci were codominant types and 36% were dominant types. Approximately 56% of loci were classified as uninformative, due to the presence of rare allele which were sources of polymorphism among accessions.

Genetic Variation among Accessions and Cluster Analysis

Genetic similarity and average heterozygosity were estimated for the study of genetic variation among the selected accessions. The PI245331 accession had the lowest average genetic similarity to other accessions. The number of bands in common varied from eleven to nineteen among the *Glycine max* accessions, and the average similarity ranged from 67.0% to 81.6% (Table 3). Cluster analysis based on the coefficient of genetic similarity has subdivided the nineteen *Glycine max* accessions into four groups (Fig. 3). Group I includes the two pairs of isogenetic lines (L87Y, L87B, L94Y, and L94B), and KS10, AGS129, TEKKYOKERO, G2120, and FWCP; Group II includes TA93006, BPCL, TA93235, and SKT; Group III includes HL-1, TN15, TA92616, and HCWT; Group IV includes TA93005, and TA92785. PI245331 is apparently distinct from the others. The three local lines, FWCP, HCWT, and BPCL were distributed in three different main clusters. The observed polymorphic alleles were not able to distinguish between isogenic lines L94B and L94Y, nor between lines L87Y and L87B (which were the F6 seeds selected from F5 segregation families), except that heterozygous alleles in locus c7 were observed in the L87Y accession. Nevertheless, the L87 pair and the L94 pair can be distinguished by PA7-*Hind*III and PA111-*Hind*III. Among improved varieties, KS-10 is actually the same variety as AGS-129; however, in the probe-enzyme combination screening, a difference was noted in a single band at the d7 locus. The average heterozygosity estimated from this study is 0.236, and the average allele per locus is 1.72.

Discussion

Source and Structure of Polymorphism

Probes for this study were derived from a random recombinant library constructed by ligating *Pst*I-digested soybean genomic DNA into plasmid vector pBS+, and transforming into *Escherichia coli* strain DH5 alpha, which is believed to consist primarily of low-copy sequences (Keim and Shoemaker, 1988). In this study, the hybridized bands detected by most probes were multiple band types (two - eight bands), though variations were generally found in only one or two bands (Figs. 1 and 2). The results of this study indicated that 28.3% of the probe-enzyme combinations were capable of detecting polymorphism in the accessions tested. Apuya *et al.* (1988) indicated that about 20% of the probes revealed a polymorphism. Keim *et al.* (1990b) reported a 40% chance of detecting polymorphism between *G. max* and *G. soja* accessions in *Pst*I digested clones. It was found that of a total of seventeen probes screened, ten (58%) probes could detect polymorphism among the *G. max* accessions, and one to two probes were specific only for the *G. soja* accession. For example, PA110 only distinguished PI245331 (*soja* accession) from others, with both *Hind*III and *Eco*RI digestions. The same results were found with the PA36-*Hind*III combination, but the PA36-*Eco*RI combination could also detect differences among *G. max* accessions at the c2 locus (Table 2). This verified that these probes, constructed by Keim and Shoemaker (1988), are a very useful marker source for soybean mapping (Keim *et al.*, 1990b). Our study found that various restriction enzymes had differing abilities to detect

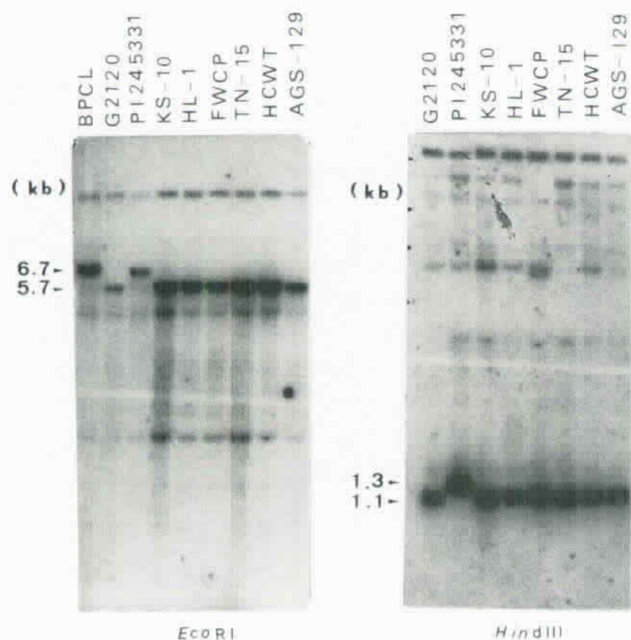

Fig. 2. Autoradiogram of restriction fragments from *Eco*RI and *Hin*dIII digestions of eight soybean genomic DNAs, hybridized with PA85 probe. PI245331 is a *G. soja* accession.

polymorphism. Probes hybridized with *Hin*dIII-digested DNA had the highest frequency of detection. Graner *et al.* (1990) indicated that efficiency in detecting polymorphism in barley tended to vary with the restriction enzyme. Landry *et al.* (1987) reported that enzymes recognizing four base pairs display fewer polymorphisms. In this study, most endonuclease were six-bp-recognizing enzymes, and half of the polymorphisms were detected with *Hin*dIII digestion. Polymorphisms with more than one enzyme occurred in probes PA36, PA85 (Fig. 2), and often in PA110 and the *soja* accession. Apuya *et al.* (1988) suggested that changes in a specific cleavage site will alter only the fragments produced by that specific enzyme, whereas rearrangement of DNA will alter the size of fragments produced by several different restriction enzymes. This provides evidence that genomic difference between the *G. max* and *G. soja* might involve genomic rearrangement in some linkage groups.

A total of forty-three alleles were observed at an estimated twenty-five loci, predominantly with two alleles per locus. Alleles with a fragment present or absent, which are considered dominant types, were observed in nine loci. This number is high in comparison with that reported by Keim *et al.* (1990b), who found that only 10% of the RFLP markers in one cross population were of dominant type. Our study indicated that 64% of loci were codominant, with the others being dominant. Heterozygosity in RFLP band patterns was also noted in some accessions, such as FWCP, TA92785, SKT, and L87Y. This is not surprising, since heterozygosity in isozyme patterns was noted in some cultivars during a previous study (Chen *et al.*, 1989). McCouch *et al.* (1988) indicated RFLP variations in inbred lines of rice. Roth *et al.* (1989) proposed that inbred soybean cultivars might have certain mechanisms to generate genetic variation in a controlled manner. Whether the observation of heterozygous alleles in self-pollinated cultivars corresponds to this phenomenon is not known.

Genetic Classification among Accessions

Variety specific band patterns among *G. max* were detected at the 1.5 kb band of probe PA117-*Hin*dIII in KS-10 and the 1.5 kb band of PA136-*Eco*RV in HCWT, while the 5.7 kb band of PA7-*Hin*dIII in FWCP was absent (Table 2). Most accessions could be distinguished from each other by one or two probes. However, the two pairs of isogenic lines were indistinguishable from each other, excepting that both heterozygous alleles (17.1 kb and 11.8 kb bands of PA109-*Eco*RI) were noted in the L87Y accession. A one-allele difference was noted between accessions AGS-129 and KS-10, which by pedigree are the same of variety (Table 2). Our previous studies with isozymes indicated that variety, under the same name, also had differences in some isozyme patterns. As shown in Table 3, the average similarity between the accessions examined was about 73.7%, ranging from 67.0% to 81.6% among the *G. max* accessions. PI245331, the only *soja* accession, had an average similarity to the other accessions of 41.1%. The two pairs of isogenic lines derived from the same cross, and differing in the color of the seed coat, showed high similarity. The dendrogram (Fig. 3) obtained from cluster analysis clearly subdivided the nineteen *G. max* accessions into four groups. The eight plant-introduction accessions were distributed among all four groups. The local lines FWCP, BPCL, and HCWT each belong to a different group. This is an indication of genetic diversity among these lines. The two isogenic lines and the four cultivars are clustered as a group of two, as shown in Figure 3. Although FWCP was classified as Group I, its looks are distinct

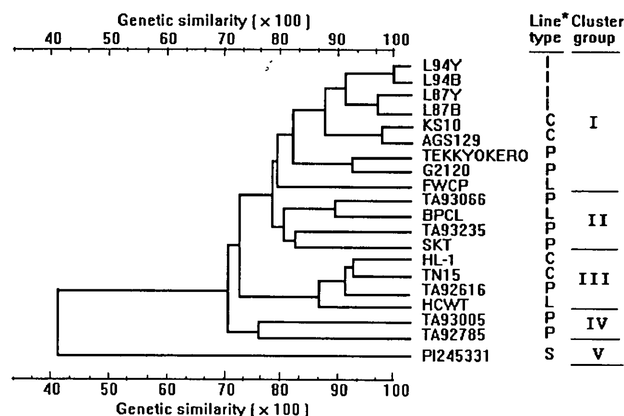

Fig. 3. Dendrogram of 20 soybean accessions based on average percent of genetic similarity from RFLP analysis. *: I- isogenic line; C: cultivar; P: plant introduction or breeding line; L- local line; S- *G. soja* accession.

from those of other members within the cluster. The *G. soja* accession did not cluster with the others.

Exploration of genetic variations by molecular markers shows differences between the cross-pollinated and inbreeding species that have been noted in several studies (Paterson *et al.*, 1991; Helentjaris *et al.*, 1985). Relatively little variation in genetic markers among cultivars was also noted (Paterson *et al.*, 1991; Keim *et al.*, 1989; Helentjaris, 1985; Graner *et al.*, 1990). Most of the accessions used in this study were cultivated lines, and among *G. max* accessions an average of 0.236 heterozygosity per locus was estimated with these RFLP markers. Although 65% of the probes were able to detect polymorphism, only 28.3% of the probe-enzyme combinations revealed polymorphisms, with an average of 1.72 alleles per locus. Our previous estimates of genetic variations in Taiwan's soybean accessions indicate that in cultivated soybean, 30.4% of isozyme loci exhibit polymorphisms, the average number of alleles/locus is 1.348, and the average expected heterozygosity is 0.115. It seems that the number of polymorphic loci found using RFLP or isozyme markers is similar (28.3% vs 30.4%), but the average heterozygosity and number of allele per locus are higher with RFLP markers than with isozyme markers. Nevertheless, the RFLP method is more profound, laborious, and time consuming than the isozyme method. Wang and Tanksely (1989) used ten selected probes to distinguish fifty-eight of seventy rice varieties from one another, by combining all fifty probe-enzyme combinations. Genetic variation was found with twenty-eight of the fifty (56%) probe-enzyme combinations. This is about twice the percentage found in our study, in which eighteen of twenty soybean accessions could be distinguished from one another by eleven probe-enzyme combinations. The difference in relative frequency of occurrence of polymorphism may be due to the breadth of the lines selected, and the probe source. Landry *et al.* (1987) pointed out that polymorphism was detected 2.5 times more frequently with cDNA probes than with random genomic probes. Presently, most RFLP mapping includes the parent of *G. max* and *G. soja* accessions, due to a lack of marker loci among *G. max* accessions. However, Griffin and Palmer (1987) have pointed out that the recombination distances between genetic markers are shorter in *G. max* × *G. max* than in *G. max* × *G. soja*. Thus, where one population produces a saturated map, another may not (Keim *et al.*, 1990b). The determination of more marker loci is a prerequisite for the application of RFLP to the improvement and selection of cultivated species of *G. maxis*.

Although RFLP markers have been utilized for the genetic mapping of several plant species, current procedures are tedious, time consuming, laborious, and expensive (Paterson *et al.*, 1991). If the relative ability of RFLP to detect polymorphisms can not be increased, its use will diminish. We have adopted the random amplified polymorphic DNA (RAPD) method (Pang *et al.*, 1992) which is derived from arbitrarily primed polymerase chain reactions (Williams *et al.*, 1991). It looks more promising because of its speed and efficiency in handling large volumes of samples.

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大豆核酸限制酶酶譜多形性之遺傳辨識

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利用分別代表本省大豆在來種、經品種選拔或改良之栽培種、外國引入品系、及育種用品系、二對近同源系及一栽培大豆近源種系（*Glycine soja*）共 20 個搜集系進行核酸限制酶酶譜多形性之遺傳辨識。所利用之探針係導自由限制內切酶 *Pst*I 切割片段所構成之基因組庫篩選而得，且一般認爲係低套數之核酸序列片段。由 17 個測試之探針中，11 個顯示在一或二個限制內切酶組合中可檢測出多形性。從總共 53 個探針與限制內切酶組合檢定中，15 個可顯現搜集系間之多形性、在所利用之內切酶酵素中 *Hind*III 出現多形性之機會最高。由此實驗中總計約 43 個多形性因子型及 25 個因子座被估算出來，其中約 20%多形性因子座爲因栽培種及近緣種間之不同而有。顯性及共同顯性之因子座各佔 36%及 64%。多形性因子僅在 1 或 2 個搜集系出現者被視爲稀有因子，在所發現之多形性因子座間約有 56%因子座具有稀有因子，而因子型頻率出現較平均而有分者只佔 44%左右。搜集系內之變異性亦偶有發現。平均而言，每一因子座之異質性約爲 0.236。利用群叢分析可

將參試之十九個栽培大豆品系區分成四次群叢以爲遺傳相似性群之參考。而在參試的 20 個搜集系中，18 個可利用 11 個探針與內切酶組合分辨出來，然而有二個近同源系無法利用上述探針及酵素組合分辨出。