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Analysis of zymogram variations on cultivated soybean (*Glycine max* L. Merr.) of Taiwan

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Abstract. Fifteen enzymes and one protein, including AP, ADH, β-AM, DIA, ENP, EST, EU, GOT, IDH, LAP, MDH, EP, PGD, SDH, XDH and trypsin inhibitor (TI) were assayed for their zymogram variations on 104 accessions of cultivated soybean germplasm collected in Taiwan. From a total of 46 loci studied, about 30% of these isozymic loci were proved to be polymorphic (based on the frequency of most frequent allele being less than 99%). The average number of alleles per locus was 1.348 and the gene diversity was about 0.115. It was indicated that the polymorphic loci occurred on enzymes such as DIA, IDH, ENP, EU, and PGD etc., while no variation was observed on GOT, MDH, SDH and XDH etc.. Furthermore, intra-varietal variations were frequently observed on accessions with the same name but collected from different resources. Results of this study provided information on the basic genetic structure of isozymic patterns for Taiwan's soybean resource.

Key words: Genetic variation; *Glycine max* L. Merr.; Inter- and intra-varietal variation; Isozymes; Polymorphic loci.

Introduction

In recent years, many plant geneticists and plant breeders are interested in using protein and isozyme markers in their research and breeding programs because the biochemical loci have generally codominant alleles, easy and rapid assaying methodology and inexpensive equipments for the standard electrophoretic analysis. The application of protein and isozyme electrophoresis has facilitated the systematic studies on plant taxonomy, cultivar identification, genetic variation, gene mapping, evolutionary and breeding studies. (Kiang and Gorman, 1983; Tanksley and Orton, 1983; Pollak *et al.*, 1984; Kahler and Wehrhahn, 1986). Soybean isozyme studies, like any other important agronomic crops such as corn (*Zea mays*) (Cardy and Kannenberg, 1982), wheat (*Triticum aestivum* L.) (Cox and Worrall, 1987), barley (*Hordeum vulgare*) (Nielsen and Johansen, 1986) and rice (*Oryza sativa* L.) (Glaszmann, 1987), have prevailed in the past decade. Kiang and Gorman (1983) summarized different zymogram variants of 19 enzymes, including glucose-6-phosphate dehydrogenase, lipoxygenase, mannose-6-phosphatase isomerase, phosphoglucose isomerase, phosphoglucomutase, and urease etc., among cultivated soybean and some other wild species. Studies concerning genetic and linkage analysis of these soybean protein and isozyme loci have also been published (Chiang and Kiang, 1987; Kiang, 1987; Kiang and Chiang, 1985; Kiang and Gorman, 1985; Kiang *et al.*, 1985; Kiang *et al.*, 1987a; Kiang and Chiang, 1986; Griffin and Palmer, 1987). Protein and isozyme loci available in soybean were recently reviewed by Palmer and Kilen (1987). Thus, utilization of protein and isozyme loci for studies of genetic variation on soybean should efficiently gain new information. Nevertheless, the protein and isozyme zymogram information on Taiwan's soybean

resources is very limited. Kiang *et al.* (1987b) compared the genetic variation of cultivated soybean germplasm originated from China, Japan, South Korea, Taiwan, USA and USSR with only 11 cultivars from Taiwan were included. Genetic variation is the foundamental resource for crop improvement. Recently, Delannay *et al.* (1983) reported a narrow genetic base for present day soybean cultivars in North America. With modern breeding programs, we believe that Taiwan's soybean resource may have the same trend in reduction of genetic variation. The main objective of this investigation, therefore, is to estimate genetic structure by examining isozyme variation and distribution of variant types in Taiwan's soybean germplasm. Genetic variation based on the observed information will also be discussed.

Materials and Methods

Cultivated soybean seeds were obtained from the following three sources: 1) Taiwan Agricultural Research Institute (TARI), 2) Asian Vegetable Research and Development Center (AVRDC), and 3) Kaoshiung District Agricultural Improvement Station (Kaoshiung DAIS). A total of 104 accessions commonly used as breeding stocks or cultivars in Taiwan were included in this study. Among 104 accessions, 44 were duplicates of 16 varieties (Table 3), however, we considered each accession individually after our enzyme assay.

Horizontal slab gel electrophoresis was used to screen the zymograms of 15 enzymes and one protein on these soybean accessions. Principal electrophoretic procedures followed Gorman and Kiang (1977) and Kiang and Gorman (1983) with a little modification. A small piece of cotyledon from dry soybean seed was ground with 2-3 drops of 5 mM L-histidine-HCl buffer (pH 7.0). Whatman No. 3 filter paper, 3mm x 3mm, was used as a wick to absorb the extract sample by placing it on the ground sample. The sample saturated paper wick was then inserted in the gel for electrophoresis. The gel types, time for electrophoretic run and stain recipes for each enzyme were listed in Table 1. The enzymes studied were acid phosphatase (AP), alcohol dehydrogenase (ADH), β-amylase (AM), diaphorase (DIA), endopeptidase (ENP), esterase (EST), urease (EU), glutamate oxaloacetic transaminase (GOT), isocitric dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), peroxidase (EP), phosphogluconate dehydrogenase (PGD), shikimic dehydrogenase (SDH), trypsin inhibitor (TI), and xanthine dehydrogenase (XDH). The enzyme commission numbers were also listed in Table 1. For each enzyme at least 6-8 seeds per accession were assayed. Gels were electrophoresed at 4°C.

For estimation of genetic variation, three parameters were calculated: percentage of polymorphic loci (with the most frequent allele being less than 99%), the average number of alleles per locus and average expected heterozygosity. The expected heterozygosity (gene diversity) for each locus was calculated following Nei (1973) and Kiang *et al.* (1987b). The formula is $Hexp = 1 - \Sigma Xi^2$, where Xi is the frequency of the ith allele. The average expected heterozygosity $\bar{H}exp$ is the average of the Hexp over all loci.

Results and Discussion

Zymogram variations tended to vary from enzyme to enzyme, however, some enzymes did exhibit more zymogram types than the others. From our study, EST, GOT, MDH, SDH, and XDH showed a unique zymogram type (monomorphic) while ADH, AM, AP, ENP, EU, LAP, EP and TI had two, PGD had three, and five different zymograms were observed for both IDH and DIA from the 104 cultivated soybean accessions (Table 2, Fig. 1). The zymogram types of each enzyme in this study will be briefly described and disscussed in the following.

Alcohol Dehydrogenase (ADH)

Two ADH zymogram types were observed in this study (Fig. 1 ADH). Four to three bands exhibiting stain activity of ADH were recorded. Majority of Taiwan's cultivars had Type 2 zymogram and only three accessions with Type 1 (Table 2). As shown in Fig. 1, the only difference of these two types is the lack of Band 3 at Rf 0.42 in Type 2. Five to seven bands in ADH zymograms were reported in cultivated and wild soybean previously (Gorman and Kiang, 1977; Gorman and Kiang, 1978 and Kiang and Gorman, 1983). Three bands existing simultaneously in radicle, root nodules and secondary roots of all varieties were also noted in their studies. Samples of our study was exclusively from a piece of dry seed cotyledon and only three to four bands were realized. The inability to identify the 7

Table 1. *Enzymes, gel types, running hours of electrophoresis and stain recipes for soybean isozymes.*

Enzyme	E.C. No.[a]	Gel Type[b]	Running[c] hour	Stain recipe or reference
Alcohol dehydrogenase (ADH)	EC 1. 1. 1. 1	7%P+2%S	6.5	Gorman and Kiang (1977)
β-amylase (AM)	EC 3. 2. 1. 2	7%P	4.5	Gorman and Kiang (1977), Chen et al. (1987b)
Acid phosphatase (AP)	EC 3. 1. 3. 2	7%P	8	Gorman and Kiang (1977), Chen et al. (1987b)
Diaphorase (DIA)	EC 1. 6. 4. 3	7%P+2%S	5.5	Chen et al. (1987b)
Endopeptidase (ENP)	EC 3. 4.11. 1	7%P+2%S	7	Chen et al. (1987b), Doong and Kiang (1987a)
Esterase (EST)	EC 3. 1. 1. 2	7%P	4.5	100mg fast blue RR salt 3ml 1% α-naphthyl butyrate in 50% acetone 100ml 0.1 M NaH_2PO_4 incubate at 30°C for 4 to 8 hrs
Urease (EU)	EC 3. 5. 1. 5	7%P	7	Chen et al. (1987b), Buttery and Buzzell (1971)
Glutamate oxaloacetic transaminase (GOT)	EC 2. 6. 1. 1	7%P	6.5	Kiang et al. (1987)
Isocitrate dehydrogenase (IDH)	EC 1. 1. 1.42	7%P+2%S	7.5	Kiang and Gorman (1985), Chen et al. (1987b)
Leucine aminopeptidase (LAP)	EC 3. 4. 1. 1	7%P+2%S	6.5	Kiang et al. (1985), Chen et al. (1987b)
Malate dehydrogenase (MDH)	EC 1. 1. 1.37	7%P+2%S	6.5	35mg MTT, 60mg NAD, 5mg PMS in 100ml stain buffer (0.01M L-malic acid + 0.025M Tris, pH 7.0)
Phosphogluconate dehydrogenase (PGD)	EC 1. 1. 1.44	6%P+4%S	7	Chiang and Kiang (1987)
Peroxidase (EP)	EC 1.11.1. 7	7%P+2%S	6.5	Brewbaker et al. (1968)
Kunitz trypsin inhibitor (TI)		9%P	4.5	Kiang and Chiang (1986)
Shikimic dehydrogenase (SDH)	EC 1. 1. 1.25	7%P+2%S	7.5	Chen et al. (1987b)
Xanthine dehydrogenase (XDH)	EC 1. 2. 1.37	7%P+2%S	6.5	60mg NAD, 30mg NBT, 50mg PMS, 3.0ml 1M Hypoxanthine, 2.0ml 0.5M Tris-HCl (pH 7.0), 77.0ml H_2O, incubate at 37°C for 2hrs.

[a] Enzyme Commission numbers

[b] P-Polyacrylamide (95% of acrylamide + 5% bis-acrylamide)
S-Starch (S 4501, Sigma)

[c] The applied voltage for electrophoresis was 160V.

Table 2. *Summary on frequencies and zymogram types of cultivated soybean in Taiwan for 15 enzymes and one protein.*

	Enzymes[b]															
Band[a] type	ADH	AM	AP	DIA	ENP	EST	EU	GOT	IDH	LAP	MDH	PGD	EP	SDH	TI	XDH
1	3[c]	103	96	36	53	104	82	104	8	102	100	47	96	104	34	102
	(2.9)[d]	(99.1)	(92.3)	(34.6)	(51.0)	(100)	(78.9)	(100)	(7.7)	(98.1)	(100)	(45.2)	(96.0)	(100)	(32.7)	(100)
2	101	1	6	3	46		18		9	2		29	2		60	
	(97.1)	(0.9)	(5.8)	(2.9)	(44.2)		(17.3)		(8.7)	(1.9)		(27.9)	(2.0)		(57.7)	
3				2					38			16				
				(1.9)					(36.6)			(15.4)				
4				25					47							
				(24.1)					(45.2)							
5				36					1							
				(34.6)					(0.9)							
Mixed			2	2	5		4		1			12	2		10	
			(1.9)	(1.9)	(4.8)		(3.8)		(0.9)			(11.5)	(2.0)		(9.6)	

[a] Band types referred to Figure 1.

[b] Enzyme full name referred to Table 1.

[c] Number of accessions.

[d] Numbers within parenthesis indicated percentage.

to 5 band zymograms as those reported by Kiang's group might be due to the incomplete separation of bands. Because the applied voltage and running hour are different in comparision with those of Kiang's studies.

β-Amylase (AM)

Single anodic band was observed on β-amylase zymogram with a mobility of Rf 0.32 in most accessions. Only one accession with exception showed a single band type with mobility slower than the others (Fig. 2 A, Table 2). This accession was originated from a breeding line of Kaoshiung DAIS. There are three loci encoding for amylase. The *Am1* and the *Am2* loci were known to control α-amylase formation and the *Am3* for β-amylase (Kiang, 1981; Kiang and Gorman, 1983). The β-amylase in cultivated soybean was controlled by a single locus with four alleles *Am3*-f, *Am3*-s, *Am3*-sw and *Am3*-n (Kiang and Gorman, 1983; Palmer and Kilen, 1987). Most Taiwan's soybean cultivars have β-amylase zymogram with allele of fast mobility (*Am3*-f). Alleles of both the fast and the slow moving bands are known to be codominant.

Acid Phosphatase (AP)

Zymogram of acid phosphatase of cultivated soybean is generally composed of a cluster of three bands with another band in the front toward anode (Fig. 1 AP). Most variations lie on the front band. From our study, two types of zymogram were found. The cluster of three bands were invariable in both types, while mobility of the single front bands were different from each other (Fig. 2 B). From the 104 accessions screened, 96 accessions had Type 1 with a faster front band, 6 accessions had the slower front band and the other 2 accessions had shown both types in their seed samples examined. Variant types on AP zymograms in *G. max* and *G. soja* were reported by Gorman and Kiang (1977) and Doong and Kiang (1987b). Four zymogram types, differing in mobility of the fastest migrating band, were noted in their studies. We are able to identify our Type 2 being their slow type, and Type 1 is possibly of medium type (Type 2) in Doong and Kiang's (1987b) report. Kiang and Gorman (1983) suggested that there were at least four AP loci in soybeans, each probably producing a single electrophoretic band. Presently, only the genetic control of the front fastest band has been realized.

Diaphorase (DIA)

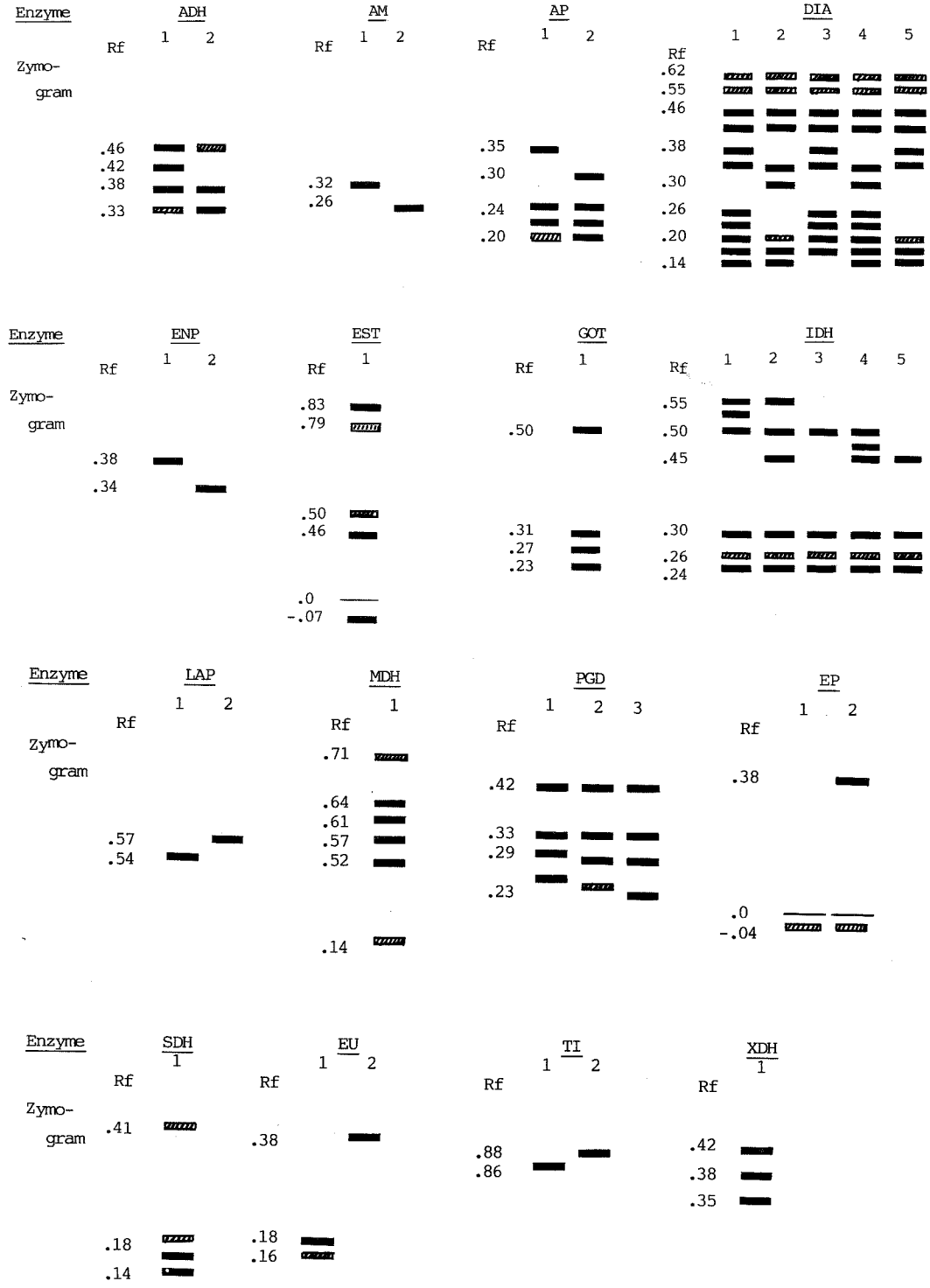

Fig. 1. Summary of zymogram types of fifteen enzymes and one protein from the cultivated soybeans (*Glycine max* L. Merr.) of Taiwan. ADH-Alcohol dehydrogenase, AM-β-Amylase, AP-Acid phosphatase, DIA-Diaphorase, ENP-Endopeptidase, EST-Esterase, EU-Urease, GOT-Glutamate oxaloacetic transaminase, IDH-Isocitric dehydrogenase, LAP-Leucine aminopeptidase, MDH-Malate dehydrogenase, PGD-Phosphogluconate dehydrogenase, EP-Peroxidase, TI-Trypsin inhibitor, SDH-Shikimic dehydrogenase, and XDH-Xanthine dehydrogenase.

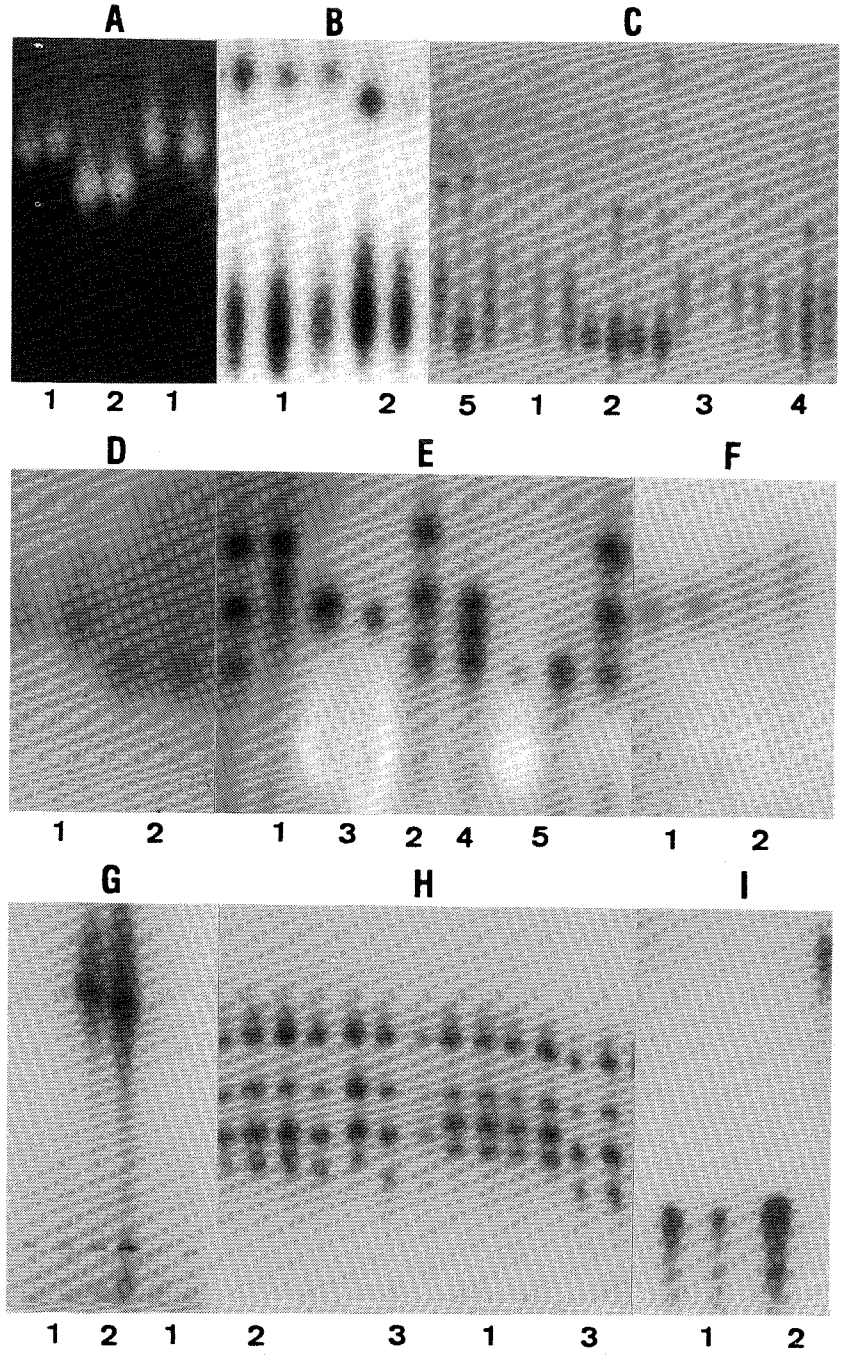

Fig. 2. Zymogram variations of A (AM-β-Amylase), B (AP-Acid phosphatase), C (DIA-Diaphorase), D (ENP-Endopeptidase), E (IDH-Isocitric dehydrogenase), F (LAP-Leucine aminopeptidase), G (EP-Peroxidase), H (PGD-Phosphogluconate dehydrogenase) and I (EU-Urease) on cultivated soybean in Taiwan.

Zymograms of DIA is the most complicate one found in this study. At least five different types were noted (Fig. 1 DIA & 2 C). The genernal structure of the DIA zymograms is a cluster of 3 to 5 bands at the bottom in between Rf 0.14 to 0.26, followed by four separated bands and another two faint bands right in front at Rf about .55 and .62. As shown in Fig. 1 DIA & 2C, the major distinctions among the five types are on the cluster of five to three bands and difference in mobility of the followed two bands next to the cluster. In between Rf .14 to .26, Type 1 has a cluster of five bands. Type 2 has only three bands of the cluster in the bottom and the followed two bands had a relative slow mobility in comparison with Type 1. Type 3 is characterized by missing of the slowest band in the cluster and the mobility of other bands is similar to Type 1. Type 4 shows the same cluster of five bands as Type 1 but the followed two bands has mobility same as Type 2. Type 5 has the same cluster of three bands as Type 2 but the followed two bands have same migration rate as Type 1 instead of Type 2. The distribution of these five types of zymograms are listed on Table 2. No variation was observed at the top two faint bands. Gorman *et al.* (1983) hypothesized that the five band cluster were resulted from a fixed homo-heterotetramer complex and produced by two interacting loci. The difference in zymograms of this cluster is thought to be caused by incompletely dominant weak (*Dia1*-n) and normal (*Dia1*-+) activity or production alleles (Kiang and Gorman, 1983). Variations in migration rate of the followed two band types were known to be controlled by another locus with two codominant alleles (*Dia2*-s and *Dia2*-f) corresponding to the two slow and fast migration bands. Variant type at Rf above 0.38 were not found in this study. However, Gorman *et al.* (1983) defined a *Dia3* locus with dominant and recessive alleles.

Endopeptidase (ENP)

Two zymogram types, both with a single anodal band but different in their mobility (Fig. 2 D), were observed among the 104 accessions. The fast migration band had a Rf 0.38, while the slow one had a Rf 0.34 (Fig. 1 ENP). Doong and Kiang (1987a) reported four homozygous types in ENP. Genetic study of these vari-

Table 3. *Zymogram variations on varieties with the same name collected from different resources.*

Name of variety	No. of entries	Enzyme[a]															
		ADH	AM	AP	DIA	ENP	EST	EU	GOT	IDH	LAP	MDH	EP	PGD	SDH	TI	XDH
Black bean	3	—[b]	—	*	—	—	—	—	—	—	—	—	—	—	—	—	—
Chunghsing 2	2	—	—	—	*	—	—	—	—	—	—	—	—	—	—	—	—
Chunghsing 3	4	—	—	—	*	—	—	—	—	*	—	—	—	*	—	—	—
Hwa Yen	2	—	—	—	*	—	—	*	—	—	—	—	—	—	—	—	—
Kaohsiung 3	4	—	—	—	—	—	—	*	—	*	—	—	*	*	—	—	—
Kaohsiung 8	2	—	—	—	—	*	—	—	—	—	—	—	—	—	—	—	—
Kaohs. Sel. 1	2	—	—	—	—	*	—	—	—	*	—	—	—	*	—	—	—
Kaohs. Sel. 10	2	—	—	—	—	*	—	—	—	—	—	—	—	—	—	—	—
KS-478	2	—	—	—	—	*	—	—	—	—	—	—	—	—	—	—	—
Mao-205	2	—	—	—	*	—	—	*	—	*	—	—	—	*	—	*	—
Shih-Shih (Ten stone)	4	*	—	—	*	*	—	—	—	*	—	—	—	*	—	*	—
Summ. Soybean	2	—	—	*	—	—	—	*	—	*	—	—	—	*	—	—	—
Tainung 3	2	—	—	—	—	—	—	—	—	—	—	—	—	*	—	*	—
Tainung 4	5	—	—	—	—	*	—	—	—	*	—	—	*	*	—	*	—
Tai-Kaohs. 5	4	—	—	—	*	*	—	*	—	*	—	—	—	*	—	—	—
Ta-lein Tu	2	—	—	—	—	*	—	—	—	—	—	—	—	—	—	—	—

[a]Enzyme full names referred to Table 1.

[b] "—", lack of variation; "*", with zymogram difference; "*", zymogam difference even within entry.

ant types were also noted in their report. Alleles, controlling this ENP locus, are known to have codominant effects. From the accessions screened in this study, frequencies of these two variant types were similar. (Table 2). Verification of these allele types requires the direct comparison of the zymograms with the reference varieties of Doong and Kiang (1987a).

Esterase (EST)

Four anodal bands and one cathodal band were recorded in EST zymogram in the accessions screened. These four anodal bands were located at Rf about 0.46, 0.50, 0.79 and 0.83 respectively and looked like two groups of two bands with Band 2 and 3 having a relative light intensity (Fig. 1 EST). Ferrer-Monge (1973) reported zymogram patterns from different tissues of soybean. Three anodic bands were detected with both α- and β-naphthyl acetate as substrates and three cathodic bands were also noted when α-naphthyl acetate was used as substrate (Ferrer-Monge, 1973). Bult and Kiang (1989) observed two cathodal mobility variant bands, which was controlled by a single esterase locus *Est1*. The two alleles were designated as *Est1-a* and *Est1-b* (Bult and Kiang, 1989). We didn't find any variant type in Taiwan's cultivated soybean in the anodal band types and variation in cathodal band was not checked.

Glutamate Oxaloacetic Transaminase (GOT)

As shown in Fig. 1 GOT and Table 2, only one zymogram type were found in GOT. General character of this zymogram is one cluster band at Rf around 0.23 -0.31 together with another single band at Rf about .50. From our study, it is difficult to tell whether 2 or 3 bands are actually involved in the cluster band. However, Kiang *et al.* (1987a) indicated a four-band zymogram in GOT with most zymograms being composed of a cluster of three bands and one single band and the variation resulted from difference in mobility of the single band. Three alleles were noted in this variable locus of GOT in their study. Thus, the cluster band we found must have three bands. The inability to resolve the three bands in the cluster might be due to the difference in electrophoretic conditions used between laboratories. The electrophoretic conditions used in our laboratory were 160V for 6.5h run on 7% polyacrylamide gel, while Dr. Kiang's labortoary used 7% polyacrylamide + 2% starch gel with 200V for 13h run. (Kiang, *et al.*, 1987a).

Isocitrate Dehydrogenase (IDH)

The distinct variation in IDH zymogram of this study was the mobility difference in the top three bands (Fig. 1 IDH & 2 E). Type 5 is a sort of variant which has not been reported. We found this variant in the TN2R accession. Accession TN2R was originated from a irradiated population and generally had a high flower and pod abortion rate. The other four types are same as those described by Kiang and Gorman (1985), except variation in the bottom two bands (close to the origin) is generally difficult to distinguish in our study. Frequencies of Type 1 to Type 4 are 7.7%, 8.7%, 36.6% and 45.2%, respectively. Type 3 and 4 seem more frequent than the other three types in our materials. As pointed out by Kiang and Gorman (1985), there were possibly four loci involving in controlling the IDH zymograms in soybean, two coded for cytosol-associated IDH, the other two coded for mitochondrial associated IDH. The variant types found in this study is believed to be cytosol-associated. The Type 5 with a single band at Rf 0.45 might be a variant with a nuIl allele. Presently, the genetic control of Type 5 variant is under study.

Leucine Aminopeptidase (LAP)

Two single band types with different migration rates were noted in LAP activity staining from this survey (Fig. 2 F). However, the faster band type was observed only in two accessions (Table 2). Among these two, one accession named Fu-Wei-Chin-Pe-Tu was a native cultivar collected in Taiwan, the other is from a breeding line. Four types of LAP zymograms in *G. max* and *G. soja* had been reported by Kiang and Gorman (1983). Genetic analysis and linkage studies indicated that *Lap1*-b, representing the fast type, is the most common allele in both *G. max* and *G. soja*. Another allele (*Lapl*-a) at the same locus had a relative slow migrating band. Judging from Kiang and Gorman's information, our zymograms is apparently belonged to *Lap1* locus. However, when checking the mobility with reference variety of *Lap1*-b allele, it was demonstrated that our slow type LAP zymogram actually had the same mobility as that of homozygous *Lap1*-b allele. Thus, Type 2 LAP zymogram of this study should have a migration rate even faster than that of *Lap1*-b type.

Malate Dehydrogenase (MDH)

No variation in zymogram types in MDH from our survey was found. The general character of MDH zymogram was shown in Fig. 1 MDH. A total of 6 bands were frequently observed with first band (Rf .14) and the sixth band (Rf .71) lightly stained. In between these two faint bands, four bands with Rf .52, .57, .61, and .64, respectively were observed. Doong and Kiang (1987b) also indicated the same zymogram type in *G. max* and *G. soja* accession. Kiang and Gorman (1983) reported three of the observed NAD active bands were associated with cytosol and three with mitochondria. The other two probably associated with microbody.

6-Phosphogluconate Dehydrogenase (PGD)

All the PGD zymogram types consisted of four anodal bands, however, mobility of Band 1 and 2 seems to be associated each other. Fig. 1 PGD indicated three variant types and the relative migration rate of Band 1 and 2 is Type 1 > Type 2 > Type 3 (Fig. 2 H). Inheritance and linkage relationships of 6-PGD had been studied by Chiang and Kiang (1987). There are three loci controlling the four 6-PGD bands. Band 1 and Band 3 were realized to be homodimers, whereas Band 2 was the inter-locus heterodimer of Band 1 and Band 3, which were encoded by *Pgd1* and *Pgd2* loci (Chiang and Kiang, 1987). The variant types we observed seems all associated with the *Pgd1* locus. Chiang and Kiang (1987) also indicated that both *Pgd1* and *Pgd2* loci have three codominant alleles and each allele specified an isozyme band. Two types of the fourth band resulting from the controlling of two codominant allele at the *Pgd3* locus were also noted (Chiang and Kiang, 1987). Frequencies of three PGD types in Taiwan's cultivated soybean are 45.2, 27.9 and 15.4 individually and 11.5% of accession showed intravarietal variations (Table 2). The possible explanation will be discussed in a later section.

Peroxidase (EP)

Both anodal and cathodal bands are frequently observed in peroxidase gels (Fig. 2 G). About 96% accessions had a single cathodal band (Rf -0.04), while 2% of accessions had one cathodal band and one anodal band. Two accessions showed mix of the two types. Previous studies on peroxidase activity is mostly on high and low peroxidase activity (Kiang and Gorman, 1983; Buzzell and Buttery, 1969). However, Brim *et al.* (1969) reported large differences in peroxidase zymograms among different soybean tissues. Presently, information on the zymogram type variations in soybean EP is very limited. Genetic study of these two variant types of EP is undertaken now.

Shikimate Dehydrogenase (SDH)

The only zymogram type observed was four anodal bands with a cluster of three bands and one faster single band. The single band (Rf .41) and the top band (Rf .18) of cluster band had a relatively faint color when compared to the others. Doong and Kiang (1987b) also reported only one homozygous SDH zymogram type with three anodal bands in their study. No genetic data is available now for SDH.

Urease (EU)

A fast type and slow type of EU isozyme were observed (Fig. 1 EU), the slow type occassionally showed a two-band structure, while the fast type had a single band. The slow type zymogram was more frequent than the fast type in the accession screened (Table 2). Genetic study for urease isozyme had been reported (Buttery and Buzzell, 1971). The fast and slow types were known to be controlled by a single loci with the fast dominant over the slow. Buttery and Buzzell (1971) also suggested that the slow type is a dimeric form of fast type and the gene is controlling the degree of polymerization of the molecule. The observation of two bands in the slow type (Fig. 2 I) might correspond to this explanation.

Kunitz Trypsin Inhibitor (TI)

Two types of single band zymograms were observed in TI. Type 1 had the single band at Rf .86, Type 2 band had a relative fast migration rate than Type 1. (Fig. 1 TI). Previous studies on variants of TI have indicated three mobility variants with three codominant alleles to a null variant TI (Kiang and Gorman, 1983; Doong and Kiang, 1987b). From the accessions screened, 32.7% had Type 1 zymogram, 57.7% exhibited Type 2 and about 9.6% showed the mix type. (Table 2).

Xanthine Dehydrogenase (XDH)

XDH shows monomorphic zymogram in our screening. A three-band zymogram was observed at Rf .35, .38 and .42 respectively. The isozymic variant of this enzyme was not found so far in *Glycine max.*

Intra-varietal Zymogram Variations

Among the accessions collected, 16 cultivars or varieties have duplicates. The duplicates were either from the same distribution or different resources. However, only few accession with the same varietal name showed all identical zymogram types of the 15 enzymes and one protein studied. There were only two black bean and two Kaohsiung 3 accessions showed the exact same zymogram patterns in all 15 enzymes and one protein. Other accessions with the same name all showed variation in at least one or two enzymes. Within the same accession, occasionally mixed types of zymogram patterns were also noted. Table 3 shows the same varieties with zymogram variations. Numbers of accessions with the mixed types of zymograms are also listed in Table 2. For the enzymes screened, at least 6-8 seeds were assayed. The observation of mixed type zymograms at the same accession, generally, were not on only one seed out of 6 or 8 seeds. Some enzyme such as PGD, TI even have about 10% of accessions with mixed types. From the total of 44 accessions representing 16 cultivars (Table 3), intra-accession variations were also observed. Although the mechanical mixture of seed source was a possible explanation for the occurrence of mixed type zymograms, however, intravarietal variation and the stability of enzyme patterns might have other possibilities. Soybean is a highly selfing crop. The natural crossing rate was believed to be from $< 0.5\%$ to about 1%. (Carlson and Lersten, 1987). Since soybean seeds is generally maintained by the breeders of different institutes in Taiwan, variation in enzyme patterns of the same variety can not exclude the possibility of natural crossings. Nevertheless, variety registration is generally based on selection for agronomic important traits without conscious selection for isozyme loci. Thus, heterogeneous origin on enzyme patterns of certain cultivars should not be excluded. Intravarietal variations in isozyme patterns have been reported in self-pollinating species such as barley (Nielsen and Johansen, 1986; Almgard and Landegren, 1974; Fedak and Rajhathy, 1972), wheat (Cox and Worrall, 1987) and oats (Almgard and Clapham, 1975; Singh *et al.,* 1973). Chen *et al.* (1987a) also found two accessions of rice variety - Dular, originated from Pakistani, with two distinct types of seed protein zymograms. Baily (1983) pointed out that occurrence of intravarietal variations can be quite extensive within line cultivars. This is not a surprise because line cultivars are developed by evaluating and selecting toward morphologically and physiologically uniform. However, there is no correlation between morphological uniformity and isozymic uniformity (Baily, 1983). Soybean cultivars in Taiwan are mostly line cultivars. Intravarietal variations in isozyme patterns in cultivated varieties might be due to lack of selection on isozyme loci during variety development process. Anyway, this information is important for soybean breeders especially when isozyme markers are intended to apply for the breeding program. The occurrence of intravarietal variations suggested a selection for isozyme loci on single plant basis is prerequisite for the utilization of these isozyme loci.

Genetic Variations of Cultivated Soybean in Taiwan

Percentage of polymorphic loci, number of allele/locus and gene diversity (the average expected heterozygosity) were estimated for studies of genetic variation on these cultivated soybean source. Each accession was considered as an individual genotype in calculating genetic variation. From a total of 15 enzymes and one protein screened representing a total of 46 loci, 30.4% of loci exhibited polymorphism (the most frequent allele being less than 99%), the average number of alleles is 1.348 and the average expected heterozygosity is 0.115. Kiang *et al.* (1987b) reported Taiwan cultivated soybean had 34.8% loci exhibiting polymorphism; the average number of alleles/locus is 1.304 and the average expected heterozygosity is 0.118 based on 11 cultivars data. Our estimated values are closed to those of Kiang's, but the sample size is almost 10 times greater. This might be due to the narrow genetic background in cultivated soybeans of Taiwan. The obtained value is slightly lower than those of Japan, Korea, China and USA sources (Kiang *et al.,* 1987b). Nevertheless, our estimation of genetic variation may be underestimated because several accessions with the same name but different in isozyme patterns are considered as different individuals.

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台灣栽培種大豆之酶譜變異分析

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蛋白質與酶電泳法之酶譜分析是一用來檢視遺傳變異之有效方法，其主要之優點爲分析所需之組織材料量少、準備容易且花費少。本研究即利用同功異構酶電泳法進行分析台灣栽培種大豆間之遺傳變異。所分析之酶包括：乙醇脫氫酶、β-澱粉酶、酸性磷酸酶、黃遞酶、肽鏈內切酶、酯酶、異檸檬酸脫氫酶、穀氨酸草醯乙酸轉氨酶、尿素酶、亮胺酸胺肽酶、蘋果酸脫氫酶、過氧化酶、磷酸葡萄糖酸脫氫酶、莽草酸脫氫酶、黃嘌呤脫氫酶及胰蛋白酶抑制劑等十六種，計檢視了 104 個搜集系之酵素酶譜。在所分析之十六種酶代表了 46 個基因座之酶譜，約有 30% 之異構酶基因座具有多形性，其平均每基因座所具有之對偶因子約爲 1.348 個，而基因之歧異性約爲 0.115。具有多形性基因座之酶包括黃遞酶、異檸檬酸去氫酶、磷酸葡萄糖脫氫酶等，而不具變異者有酯酶、蘋果酸脫氫酶、莽草酸脫氫酶、黃嘌呤脫氫酶等。此外品種內之酶譜變異亦時有發現。此分析之結果提供了台灣栽培種大豆其異構酶類型之基本資料，有利於日後大豆育種、遺傳上之應用。