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The relationships of cultivated soybeans and their wild relatives collected from Taiwan: revealed by seed proteins

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Abstract. We conducted molecular studies of the taxonomic relationships between cultivated soybean, *Glycine max*, and its wild relatives, *G. soja*, *G. tomentella*, and *G. tabacina*, collected in Taiwan and the nearby islands. The *G. max* and *G. soja* are annual and the others are perennial. We used protein gels and Western blot to study their seed proteins—including two major storage proteins, lectin, and several seed maturation proteins. We performed RFLP analysis using soybean seed maturation protein cDNA clones as the probes. The data were used to assess the degree of genetic variability and similarity existing between and within different species of *Glycine* collected in Taiwan. The results confirm and extend the present model of the taxonomic relationships. Seed maturation proteins may serve as a marker that reveals relationships between the member species of the *Glycine* taxon.

Keywords: Coefficients of genetic similarity; *Glycine* species; Polyacrylamide gel electrophoresis; Restriction fragment length polymorphism; Soybean seed lectin; Soybean seed maturation proteins; Soybean seed storage proteins; Western blot.

Abbreviations: **GS**, coefficients of genetic similarity; **RFLP**, restriction fragment length polymorphism; **SDS-PAGE**, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; **Ta**, *Glycine tabacina*; **To**, *Glycine tomentella*; **W82**, Williams' 82.

Introduction

The cultivated soybean, *Glycine max* (L.) Merr., is a major crop and an important plant material for studies in morphology, taxonomy, physiology, and molecular biology.

The genus *Glycine* Willd., as currently circumscribed, consists of many species in two subgenera, *Soja* and *Glycine* (Newell and Hymowitz, 1983). The subgenus *Soja* includes the diploid (2n=40) cultivated soybean, *G. max* (L.) Merr., and its wild relatives, *G. soja* Sieb. and Zucc. Both *G. max* and *G. soja* are annual, and intercross freely (Palmer et al., 1987). The subgenus *Glycine* includes about 15 described species, and all species within the subgenus are perennial (Hymowitz and Singh, 1987). The distribution of these wild soybeans include the USSR, Korea, Japan, mainland China, Taiwan, the Philippines, South Pacific islands, and Australia.

A wide variety of systematic approaches, including cytogenetics (Singh and Hymowitz, 1985), morphology (Constanza and Hymowitz, 1987), isozyme (Kiang et al., 1987), and RFLP (Menancio et al., 1990), have been used to define relationships within the taxon. Genetic variations within or among cultivated or wild soybeans have been examined by protein profile in many studies (e.g. Kiang and Gorman, 1983; Grant et al., 1984). Kiang and his colleagues used 857 lines of *G. max* and 136 accessionss of *G. soja* collected from China, Japan, Korea, Taiwan, the USA, and the USSR to study genetic variations by examining 15 enzymes and one protein representing a total of 46 loci (Kiang et al., 1987). Cultivated and wild soybean had higher genetic variation than other reported selfing annual species, and wild soybean germplasm had a greater genetic variation than the cultivate soybean. In the present study, we used two widely-cultivated soybean varieties, one from Taiwan and one from the USA, and their wild relatives, *G. soja*, *G. tomentella*, and *G. tabacina*, collected from Taiwan and the nearby islands. We use protein gels and Western blot to study their seed proteins, including the major storage proteins, lectin, and several seed maturation proteins. We performed RFLP analysis using soybean seed maturation protein cDNA clones as probes; results were used to assess the degree of genetic variability and similarity existing between and within different species of *Glycine* collected in Taiwan.

Materials and Methods

Plant Materials

With the exception of cultivated varieties, seeds of all accessions used in this study were collected from Taiwan and the nearby islands by Drs. J. S. Hsieh and Y. C. Huang.

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Table 1. Accessions of the wild *Glycine* species used in the study and their places of collection.

Species	Accession No.	Locations of collection
Glycine soja Sieb. & Zucc.	S001	Shimen, Taoyuan
Glycine tomentella Hayata	To029	Maubito, Pintung
	To034	Dakwung, Pintung
	To037	Haiko, Pintung
	To039	Tungho, Taitung
Glycine tabacina (Labill.) Benth.	Ta004	Holiaw, Penghu
	Ta016	Gibay, Penghu
	Ta019	Oonie, Penghu
	Ta064	Chimay, Penghu

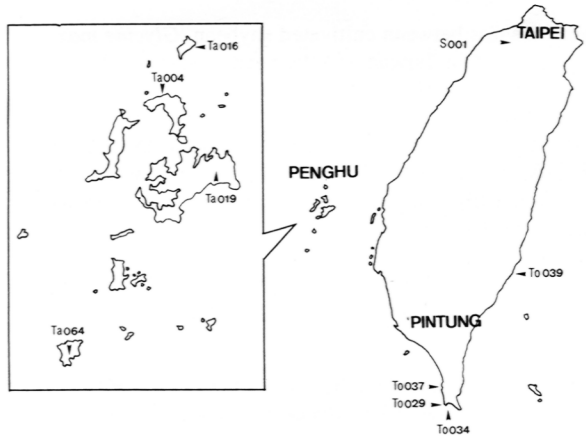

Figure 1. Distribution of *Glycine* accessions used in the present study.

Table 1 and Figure 1 identify the accession number and locations of collection used in the study. Seeds of Shi-shi were provided by Kaoshiung Agricultural Experimental Station, Taiwan, and those of Williams' 82 by Dr. R. L. Bernard, USDA, Department of Agronomy, University of Illinois, USA. The plants were grown to maturity in the greenhouse. Plant tissues, including seeds and leaves, were frozen in liquid N_2 immediately after harvesting and stored at -70°C prior to extraction.

Seed Protein Preparations

Seeds were homogenized with mortar and pestle in ice-cold grinding buffer consisting of 63 mM Tris-HCl (pH 7.8), 20 mM $MgCl_2$, 10 mM 2-mercaptoethanol, and 1 mM PMSF (phenylmethyl-sulphonyl fluoride). Following homogenization, an equivalent volume of Laemmli protein solubilization buffer (Laemmli, 1971) was added. The slurry was transferred to a microfuge tube and incubated at 100°C for 10 min. Proteins were separated by one-dimensional 12.5% SDS-polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue R-250.

Western Blot Immunodetection

Western blotting was performed as described by Towbin et al. (1979). Four kinds of primary antisera were used. The anti-130-kDa soybean seed maturation protein serum was prepared by immunizing mice with seed 130-kDa protein (Hsing et al., unpublished data). The anti-GmPM1 soybean seed maturation protein serum was prepared by immunizing rabbits with purified fusion GmPM1 protein (Hsing et al., unpublished data). The anti-carrot embryonic protein DC8 (Franz et al., 1989) antibody was provided by Dr. Z. R. Sung, University of California at Berkeley, USA. The anti-lectin antibody was purchased from Sigma Company. For the secondary antiserum, goat anti-mouse or goat anti-rabbit IgG conjugated to alkaline phosphatase (AP) was used, and nitroblue-tetrazolium was used as the chromogenic substrate.

Genomic DNA Preparation and Southern Hybridization

Total genomic DNA was extracted from young leaves of *Glycine* species as described by Junghans and Metzlaff (1990). Four restriction enzymes were used—EcoRI, EcoRV, HindIII, and XbaI. DNA prepared from the 11 different accessions were digested with each of the four enzymes and hybridized with 4 GmPM probes. The digested DNA samples were resolved on agarose gels and transferred to nylon membranes. DNA labeling was performed with α-[^{32}P]dCTP using a modified oligolabeling method (Hodgson and Fisk, 1987). Hybridization was performed at 65°C for 16 h. The membrane was then washed in a series consisting of SSC buffer (1×SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0)—5×SSC buffer containing 0.5% SDS at room temperature for 20 min, 1×SSC containing 0.5% SDS at 37°C for 20 min, 1×SSC containing 0.1% SDS at 65°C for 20 min, and then 0.1×SSC containing 0.1% SDS at 65°C for 20 min.

Statistical Analysis

Coefficients of genetic similarity (GS) between pairs of accessions based on RFLP data were calculated according to the following equation (Nei and Li, 1979):

$$GS = 2N_{xy} (N_x + N_y)^{-1}$$

where GS is the similarity coefficient between accessions x and y, N_{xy} is the number of bands common to x and y, and N_x and N_y are the total number of bands detected in x and y, respectively. GS reflects the proportion of RFLP bands that cannot be distinguished between two

accessions. Hence, a GS value of 1 indicates maximum similarity between two accessions, while a GS value of 0 indicates maximum diversity.

Results and Discussion

Soybean Major Seed Storage Protein Analysis

There are two major storage proteins in cultivated soybean seeds. One is glycinin, also known as legumin or 11S storage protein, which consists of acidic subunits and basic subunits (Kitamura et al., 1976). The other is conglycinin, also known as vicilin or 7S storage protein, which is a glycoprotein composed of three major subunits, α', α, and β (Thanh and Shibasake, 1977). Together, glycinin and conglycinin constitute approximately 70% of the total seed protein at maturity.

SDS-PAGE analyses of *Glycine* seeds showed no change in the banding patterns of glycinin and conglycinin in *G. soja* seeds when compared with those in cultivated species, but the polypeptide content decreased and the molecular weight of each of these two proteins changed slightly in both *G. tomentella* and *G. tabacina* (Figure 2).

Soybean Seed Lectin Analysis

There are three minor soybean seed storage proteins—urease, lectin, and trypsin inhibitor. Lectin normally accounts for up to 5% of the total seed protein (Vodkin and Rhodes, 1986). In the present study, we used Western blotting to investigate the presence of lectin in several *Glycine* species (Figure 3). The two cultivated varieties and the *G. soja* accession all contained lectin in their seeds. In a separate study, five out of 12 *G. soja* accessions screened did not have lectin (Hsieh et al., unpublished data). There was no lectin in the *G. tomentella* and *G. tabacina* seeds.

The seed lectin of *G. max* lines has been extensively surveyed. Five out of 102 screened lines lacked lectin protein (Pull et al., 1978). In *G. soja*, approximately 50% of the plant introductions contained seed lectin (Stahlhut et al., 1981). The lectin-negative trait, *Le*⁻ or null, was inherited as a single gene (Orf et al., 1978). The lectin-nega-

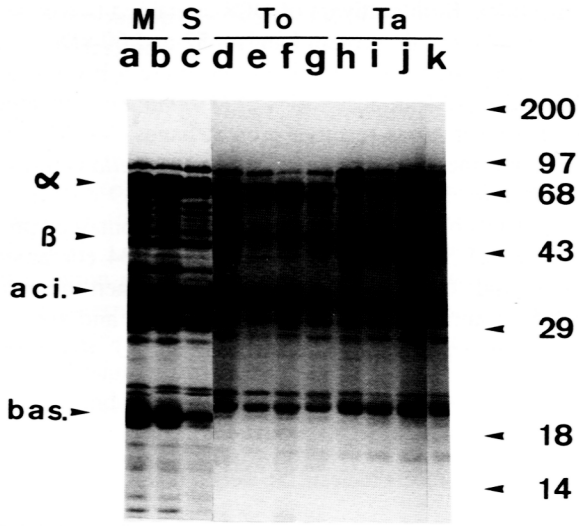

Figure 2. SDS-PAGE of protein extracts of the cultivated and wild soybean seeds. Electrophoresis (12.5% slab gel) was performed in the Laemmli system and stained for protein with Coomassie blue. The α and β-conglycinin and the acidic (aci.) and basic (bas.) polypeptides of glycinin are indicated. Proteins were prepared from (a) *G. max* Shi-shi and (b) W82; (c) *G. soja* S001; (d) *G. tomentella* To029, (e) To034, (f) To037, and (g) To039; (h) *G. tabacina* Ta004, (i) Ta016, (j) Ta019, and (k) Ta064. M (*G. max*), S (*G. soja*), To (*G. tomentella*), and Ta (*G. tabacina*) indicate the species of the accessions. The weight of molecular weight standards is given in kDa.

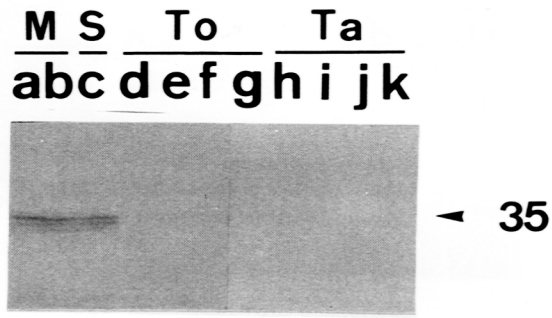

Figure 3. Immunostaining of soybean lectin cross-reactive polypeptides. SDS-PAGE was carried out as in Figure 2, and the separated polypeptides were transferred to nitrocellulose paper. The Western blot was then reacted with rabbit anti-lectin antiserum as the primary antibody. The catalog letters of the accessions used are the same as in Figure 2. The weight of molecular weight standards is given in kDa.

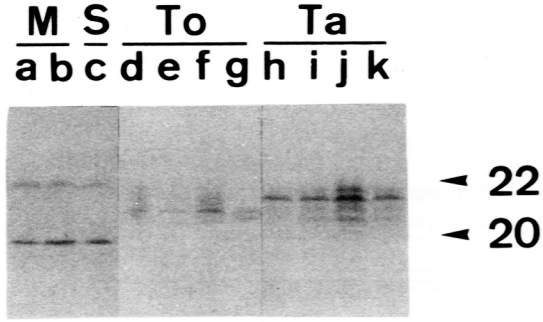

Figure 4. Immunostaining of soybean seed maturation protein GmPM1 cross-reactive polypeptides. SDS-PAGE was carried out as in Figure 2, and the separated polypeptides transferred to nitrocellulose paper. The Western blot was then reacted with rabbit anti-GmPM1 antiserum as the primary antibody. The catalog letters of the accessions used are the same as in Figure 2. The weight of molecular weight standards are given in kDa.

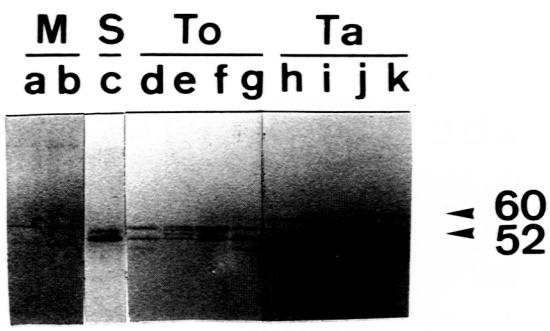

Figure 5. Immunostaining of soybean seed maturation protein GmPM2 cross-reactive polypeptides. SDS-PAGE was carried out as in Figure 2, and the separated polypeptides transferred to nitrocellulose paper. The Western blot was then reacted with mice anti-DC8 antiserum as the primary antibody. The catalog letters of the accessions used are the same as in Figure 2. The weight of molecular weight standards are given in kDa.

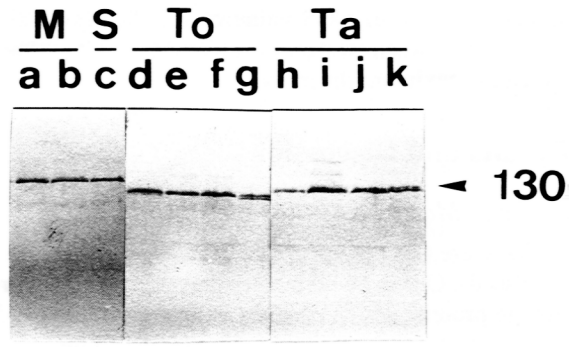

Figure 6. Immunostaining of 130-kDa soybean seed maturation protein cross-reactive polypeptides. SDS-PAGE was carried out as in Figure 2, and the separated polypeptides transferred to nitrocellulose paper. The Western blot was then reacted with mice anti-130-kDa antiserum as the primary antibody. The catalog letters of the accessions used are the same as in Figure 2. The weight of molecular weight standards are given in kDa.

tive phenotypes of *G. max* and *G. soja* were reported to be caused by the insertion of transposable elements in the gene coding region (Goldberg et al., 1983; Vodkin et al., 1983; Vodkin and Rhodes, 1986). Whether the null phenotypes of the perennial wild soybeans, *G. tomentella* and *G. tabacina,* are caused by insertion of transposable elements remains unknown.

Soybean Seed Maturation Protein Analysis

The soybean seeds synthesize *de novo* several maturation proteins during drying treatment or at the late maturation stage (Rosenberg and Rinne, 1986; 1988). These proteins, also called Lea proteins, are distinct from the known storage proteins in terms of protein molecular mass, expression pattern, etc. Their cDNA has been cloned, characterized, and sequenced in our lab (Hsing et al., 1990; Hsing and Wu, 1992; Hsing et al., 1992; Chen et al., 1992; Lee et al., 1992). These cDNA clones were designated pGmPM1 through 10. GmPM stands for *Glycine max* physiological maturation.

Several anti-soybean seed maturation protein sera were used for the study. The results of Western blotting are shown in Figures 4 to 6 and summarized in Table 2. When the anti-130-kDa soybean seed maturation protein antibody was used, the cross-reactive polypeptides in *G. max* and *G. soja* seeds were identical size. All four *G. tabacina* accessions contained a positive, 120-kDa polypeptide. Likewise, *G. tomentella* accessions contained 118 and 116 kDa polypeptides. Both cultivars of DC8 contained two cross-reactive polypeptide protein bands—52 and 60 kDa. The *G. soja* accession also contained two protein bands—56 and 58 kDa. All four accessions of *G. tabacina* contained the same three cross-reactive protein bands, 50, 52, and 60 kDa, but the four accessions of *G. tomentella* could be grouped into three types—To029 and To039 contained protein bands of 50, 57, and 60 kDa, To037 contained protein bands of 57, 59, and 60 kDa, and To034 contained protein bands of 57, 58, 59, and 60 kDa. When the anti-GmPM1/9 antibody was used, the number and size of cross-reactive polypeptides of *G. max* and *G. soja* were

Table 2. Summarization of Western blot analysis of seed maturation proteins. The numbers indicate the weight (kDa) of the cross-reactive polypeptide bands.

	Anti-130 kDa	Anti-DC8	Anti-GmPM1
Shi-shi	130	52, 60	20, 22
W82	130	52, 60	20, 22
S001	130	56, 58	20, 22
To029	118	50, 57, 60	21.5, 21.7
To034	118	57, 58, 59, 60	21.0, 21.7, 22
To037	118	57, 59, 60	21.0, 21.7, 21.8, 22.0
To039	116, 118	50, 57, 60	21.5, 21.7
Ta004	120	50, 52, 60	20.7, 21.5, 21.7, 22.0, 22.2
Ta016	120	50, 52, 60	20.7, 21.5, 21.7, 22.0, 22.2
Ta019	120	50, 52, 60	20.7, 21.5, 21.7, 22.0, 22.2
Ta064	120	50, 52, 60	20.7, 21.5, 21.7, 22.0, 22.2

the same—20 and 22 kDa. The four accessions of *G. tabacina*, however, contained five polypeptides—20.7, 21.5, 21.7, 22.0, and 22.2 kDa. The patterns for accessions of *G. tomentella* were even more complex—To029 and To039 contained two bands—21.5 and 21.7 kDa, To034 contained three bands—21.0, 21.7 and 22.0 kDa, and To037 contained four bands— 21.0, 21.7, 21.8, and 22.0 kDa. The immunoblot detection of soybean seed maturation proteins showed very complex patterns for the cultivars and wild soybean accessions.

Several soybean seed maturation proteins are coded by low-copy-number genes in a cultivated variety, Shi-shi, as indicated by hybrid select translation and Northern blot (Hsing and Wu, 1992). For instance, GmPM1 and GmPM9 are cDNA clones belonging to the same family. GmPM1 protein contains a sector of 23 amino acids that is deleted in GmPM9 protein, and the remainder have 95% homology (Chen et al., 1992; Lee et al., 1992). GmPM8 and GmPM10 belong to another gene family. The GmPM10 protein is 15 amino acids longer than GmPM8 protein, and the remainder has an extremely high homology (Hsing et al., unpublished data). The antibody immunized with GmPM1 fusion protein recognized two cross-reactive protein bands in cultivated soybean seeds (Figure 4). According to the present study, many wild soybean relatives have several copies of genes, and recognize several protein bands (confirmed by sequence data). Some of them have four or five gene products in one protein family, such as the GmPM1/9 protein family of *G. tomentella* or *G. tabacina*. It is suggested that seed maturation proteins mark the relationships between the member species of the *Glycine* taxon.

RFLP Analysis of Soybean Seed Maturation Protein Genes

cDNA clones of soybean seed maturation proteins were used for the investigation of DNA polymorphism. pGmPMs 1, 2, 4, and 6 were used as probes. All the hybridization patterns are single copy or low copy number gene patterns, and all the probe-enzyme combinations result in useful polymorphism. Representative hybridization data are shown in Figure 7—DNAs were digested with EcoRV and probed with pGmPM2 insert. Coefficients of genetic similarity between pairs of accessions were calculated from RFLP data (Table 3).

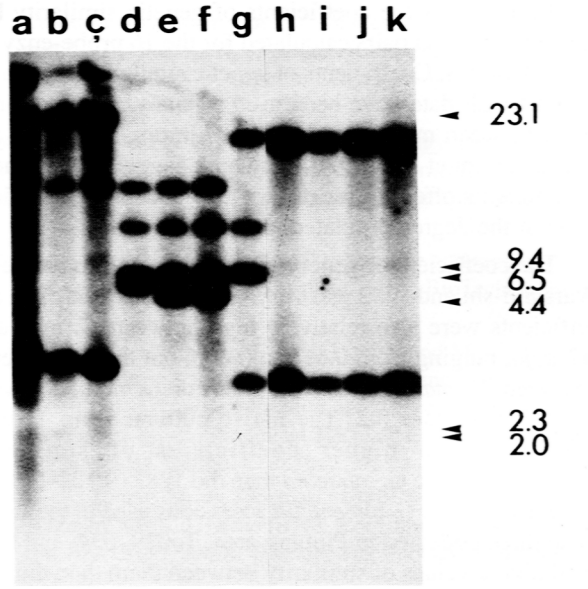

Figure 7. Autoradiographs of *Glycine* species DNAs hybridized with soybean seed maturation protein cDNA insert. This is EcoRV-digested DNA hybridized with pGmPM2 insert. DNAs were prepared from *G. max* Shi-shi (a), W82 (b); *G. soja* S001 (c); *G. tomentella* To029 (d), To037 (e), To034 (f), To039 (g); *G. tabacina* Ta004 (h), Ta016 (i), Ta019 (j), Ta064 (k). DNA size markers are shown to the right.

Glycine max and *G. soja* showed a highly conserved RFLP pattern (Figure 7). The three *G. tomentella* accessions (To029, 034, and 037) collected from the Pintung area had essentially identical patterns, and the patterns of the four *G. tabacina* accessions collected from Penghu area differed from those of *G. tomentella*, though they were identical among themselves. The *G. tomentella* accession To039 (collected in the Taitung area) had a pattern that shared two out of four fragments with the *G. tomentella* pattern, while another two fragments were similar to that of *G. tabacina*. This similarity in fragment pattern indicates a relationship between *G. tabacina* and the *G. tomentella* collected from the Taitung area. The variations of the hybridization patterns in our studies (Figure 7) are more obvious than in any of the previous studies (Doyle and Beachy, 1985; Doyle et al., 1990; Menancio et al., 1990).

Table 3. Coefficients of genetic similarity (GS × 100) between *Glycine* accession pairs used in the study.

	Shi-shi	W82	S001	To029	To037	To034	To039	Ta004	Ta016	Ta019	Ta064
W82	82.6										
S001	65.3	61.5									
To029	13.5	18.5	17.5								
To037	19.2	20.0	17.5	82.1							
To034	18.7	17.5	22.6	92.2	76.5						
To039	19.6	22.4	17.8	45.5	41.8	62.1					
Ta004	19.8	14.4	22.0	23.9	18.3	10.7	39.3				
Ta016	19.8	14.4	18.0	20.2	16.5	10.7	37.4	100.0			
Ta019	19.6	12.2	21.8	25.5	20.0	10.6	40.7	99.1	99.1		
Ta064	19.8	12.4	20.0	20.2	18.3	10.7	39.3	100.0	100.0	99.1	

Table 2 lists the coefficients of genetic similarity between accession pairs calculated for the 16 probe-enzyme combinations. Coefficients of genetic similarity calculated from RFLP data have been used to analyze the relationship between maize inbred lines (Ajmone-Marsan et al., 1992; Livini et al., 1992). They both suggested that these coefficients offer a powerful tool for accurate quantification of the degree of relatedness between lines.

The coefficient of genetic similarity between the cultivars Shi-shi and W82 was very high, as expected. The coefficients were also relatively high between *G. max* and *G. soja*, ranging from 0.615 to 0.653, but the coefficients between *G. max* and *G. tomentella* or *G. tabacina* were very low—under 0.2. The native cultivar from Taiwan (Shi-shi) had higher coefficients of similarity (0.196–0.198) to *G. tabacina* than did W82 (0.122–0.144). There were four *G. tomentella* accessions used in the study. The three collected in Pintung area, To029, 034, and 037, had higher values of similarity between them than did any of them to the one collected in the Taitung area (To039). It is worth noting that To039 had higher coefficients of similarity to *G. tabacina* (GS = 0.374 to 0.407) than did other *G. tomentella* (GS = 0.106 to 0.255). There were four *G. tabacina* accessions used in this study, collected from four islands in the Penghu area. The coefficients of similarity between them were extremely high, ranging from 0.991 to 1.00. These values were much higher than those of cultivars or of *G. tomentella* accessions.

Conclusion

All of the protein or DNA data collected in the present study indicate that *G. soja* is very close to the cultivated soybean, as the current model of the taxonomic relationships suggests. There were four accessions of *G. tomentella* used in the study. DNA data from the three collected in the Pintung region showed a very close relationship, but their seed maturation proteins varied. The one collected in the Taitung region, To039, varied from other *G. tomentella* accessions at the DNA and protein levels. It also showed some genetic similarity to *G. tabacina* (the Chinese name of *G. tabacina* is Penghu soybean). All the local *G. tabacina* accessions available now were collected from the Penghu region, although according to Tateishi and Ohashi (1992), some were found in the Taichung area. Since the protein and DNA data from the four *G. tabacina* accessions used in the study were identical, they are very closely related, even though they were collected from different islands. These results suggest that there is less genetic differentiation among the populations than within *G. max* or *G. tomentella*, and thus low genetic diversity within accessions from different collection regions. Whether the population genetic structure of the Penghu *G. tabacina* was influenced by founder effect, genetic drift, or bottleneck effect (Loveless and Hamrick, 1984) needs to be determined.

Evidence accumulated from cytogenetic and morphological studies and RFLP analysis supports the hypothesis that *G. soja* is the wild ancestor of the cultivated soybean, *G. max* (e.g. Ahmad et al., 1976; Hymowitz and Newell, 1980). But then, what is the probable ancestor of *G. soja*? Two wild perennials, *G. tomentella* and *G. tabacina*, have been suggested (Hymowitz and Singh, 1987)—especially *G. tomentella*, because of its successful hybridization with the cultivated soybean (Newell and Hymowitz, 1982) and the fertility of their progeny (Singh et al., 1990).

Table 4 indicates the growth habits and the geographic distributions of 11 *Glycine* species. The only annual wild soybean, *G. soja*, has been found in Taiwan, China, Japan, Korea, and the USSR. Taiwan is the southernmost of these areas. The two wild perennials that have been suggested to be the probable ancestor of *G. soja*—*G. tomentella* and *G. tabacina*—are found in Taiwan, southern China, the Philippines, South Pacific islands, and Australia. Taiwan is the northernmost of these areas. At present, the distribution of *G. tabacina* and *G. tomentella* overlaps that of *G. soja* only in Fujien province of China and in Taiwan. The collection and study of wild soybeans in Taiwan and the adjacent islands are important in developing an understanding of their evolution.

Table 4. The genus *Glycine* Willd., growth habbit, and geographic distribution (Hymowitz and Singh, 1987; Tindale and Craven, 1988).

Species	Growth habbit	Distribution
G. max (L.) Merr.	A	Cultigen
G. soja Sieb. & Zucc.	A	Taiwan, China, Japan, Korea, Russian
G. tomentella Hayata	P	Taiwan, South China, Philippines, Australia
G. tabacina (Labill.) Benth.	P	Taiwan, South China, South Pacific Islands, Australia
G. canescens F. J. Herm.	P	Australia
G. latrobeana (Meissn.) Benth.	P	Australia
G. latifolia (Benth.) Newell and Hymowitz	P	Australia
G. falcata Benth.	P	Australia
G. clandestina Wendl	P	Australia
G. hirticaulis	P	Australia
G. lactovirens	P	Australia
G. albicans	P	Australia

A, annual; P, perennial.

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由種子蛋白探討栽培大豆與台灣野生大豆間的關係

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我們利用栽培種大豆 (*Glycine max*) 及在台灣採集到的一年生野生大豆 (*Glycine soja*)、多年生野生大豆(*Glycine tomentellah* 與 *G. tabacina*) 做爲材料，由蛋白質及 DNA 的層次來探討它們在分類學上的關係。蛋白質層次的探討係利用電泳與西方墨點分析法，檢測這些材料種子中的兩種主要貯存蛋白、凝血蛋白與成熟蛋白。 DNA 層次的探討則以種子成熟蛋白的 cDNA 殖系做爲探針。進行各參試材料基因組 DNA 與限制酵素組合的限制酶片段長度多型性的分析。兩者的結果除可印證種間及種內遺傳的差異性與相似性之外，亦顯示大豆種子成熟蛋白是探討大豆親緣關係的有用標誌。同時由結果亦指出台灣確實具備大豆種原研究的豐富資源。

關鍵詞：遺傳相似度係數； *Glycine* 屬；膠片電泳；限制酶片段長度多型性；大豆種子凝血蛋白；大豆種子成熟蛋白；大豆種子貯存蛋白；西方墨點分析法。