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Genetic variation in South Korean natural populations of wild soybean (*Glycine soja*)

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Summary

Wild relatives are valuable genetic resources for crop improvement. Evaluating genetic variation in these species is not only important for their use in breeding programs, but will also provide information about evolution of crops. Seeds representing six natural populations were used to study the level of variation in the South Korean wild soybean. Electrophoretic assays of the seeds on horizontal slab gels were conducted to determine the genotypes of each natural plant at 35 loci in 17 isozymes and one protein. The results indicated a surprisingly high variation. The number of alleles at each locus was as high as four. Seventy two of the 94 reported alleles for the 35 loci were present in these populations. The average number of alleles per locus, 99% polymorphism and the expected heterozygosity in the total population were 2.1, 77.1% and 0.215, respectively. This amount of variation was not only higher than that reported for 857 soybean cultivars and wild soybean populations from other geographic regions, but also higher than the average for 123 self-fertilized plant species and 473 plant species of all mating systems. The high variation in the South Korean wild soybean as well as cultivated soybean indicated in this and other population genetic studies prompts us to propose that South Korea is one of the major soybean gene centers.

Introduction

Genetic variation is the basis of crop improvement. However, most modern cultivars of major crops are developed from a limited number of ancestral lines. For further improvement of the cultivars, new genes need to be introduced. Wild relatives can interbreed with the cultigens, and often serve as such gene sources. Wild soybean (*Glycine soja* Sieb. & Zucc.) is believed to be the progenitor of the cultivated soybean (*Glycine max* [L.] Merr.) (Hymowitz & Singh, 1987). These two species produce fertile offspring upon hybridization. Together they form the soybean gene pool. Evaluating genetic variation in wild soybean will not only help facilitate its use in breeding programs, but will also provide information about evolution of soybean (including *G. soja* and *G. max* in this paper).

Genetic variation in soybean has been examined in a number of studies with isozymes and other biochemical and morphological genetic markers (Broich & Palmer, 1981; Bult, 1989; Chiang, 1985; Hu & Wang, 1985; Hymowitz & Kaizuma, 1979, 1981). The results indicate that the amount of variation is comparable to or higher than that in other self-fertilized plant species (Bult, 1989; Chiang, 1985; Kiang et al., 1987). Wild soybean consistently shows a higher level of variation than domestic cultivars (Kiang & Gorman, 1983; Kiang et al., 1987). There seems to be a trend that South Korea and

southern Japan have more rare alleles and higher variation for soybean than other geographic regions (Broich & Palmer, 1981; Hymowitz & Kaizuma, 1979, 1981; Kiang & Gorman, 1983; Kiang et al., 1987).

The objective of this study was to characterize specifically the genetic (isozymic) variation in the South Korean wild soybean using natural populations, and to determine whether South Korea has a higher level of variation for wild soybean than other geographic regions.

Materials and methods

Wild soybean seeds were collected from six natural populations at the geographic locations A to F (Fig. 1, Table 1) in South Korea in early October, 1986 by Yun-Tzu Kiang. The distance from the most northern population (A) to the most southern population (F) was approximately 194 kilometers. Eighteen to 41 plants were sampled for each population (Table 1). The distance between plants within each population was at least 3 meters. Nine to 90 seeds were collected from each plant. The seeds were brought back to the U.S. and kept in a lab freezer at – 20° C before use.

For each natural plant, five original seeds were examined by electrophoresis. One additional seed was sown in the greenhouse. Six progeny seeds from this greenhouse plant also were examined. Results from the five original and six progeny seeds were compared. If any discrepancies were found, more original seeds as well as progeny seeds were examined until genotypes at all the isozyme loci were correctly identified for each natural plant.

For electrophoresis, each seed was cut into three pieces, one for half the number of the enzymes examined, one for the other half, and the third piece as a backup for a repeat of the assays. Electrophoresis was conducted on horizontal slab gels made of various concentrations of acrylamide and starch. Methods of electrophoresis previously were described by Bult et al. (1989).

We examined 35 loci in 17 enzymes and one protein, which were aconitase (ACO), alcohol dehydrogenase (ADH), beta-amylase (AM), acid phosphatase (AP), diaphorase (DIA), endopeptidase

Table 1. Geographic locations of six South Korean natural populations of wild soybean

Population	N[a]	Location	Latitude	Longitude
A	27	Wang Shium Ri, Bong Dam Myeon, Gyeon Gi Do	37°14′ N	126°56′ E
B	30	Gook-Kyeo River, Yeum Chi Myeon, A San Gun, Chung Ch'ong Nam Do	36°51′ N	126°56′ E
C	30	Worl Gae River, Dae Gyo Ri, Hong-Sun Gup, Chung Ch'ong Nam Do	36°34′ N	126°41′ E
D	18	Chang Am Ri, Jusam Myeon, Bo Lung Gun, Chung Ch'ong Nam Do	36°11′ N	126°34′ E
E	41	Saeg Chang River, Nam Gu Dong, Chonju City, Cholla Buk Do	35°49′ N	127°07′ E
F	26	Osu Ri, Cholla Buk Do	35°32′ N	127°20′ E

[a] Sample size (number of plants).

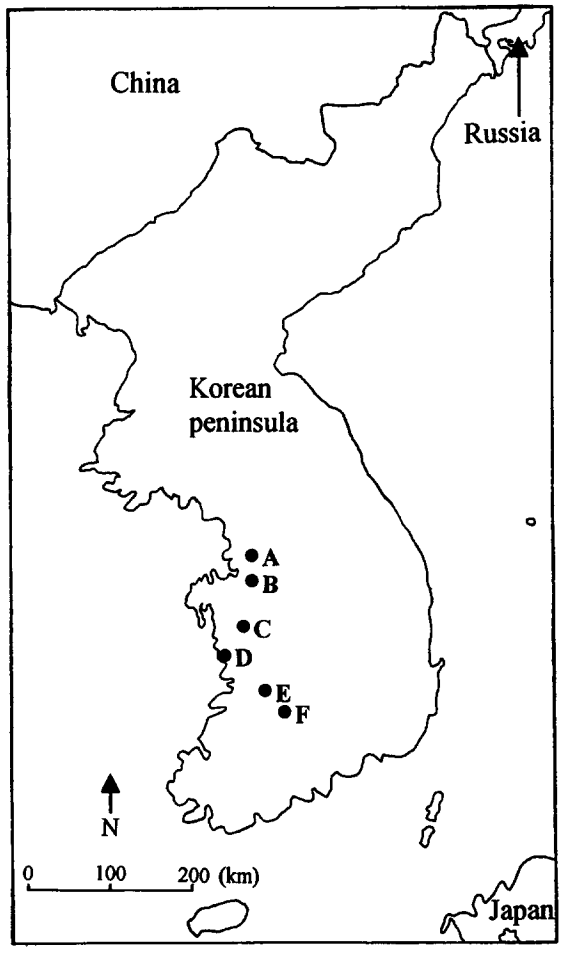

Fig. 1. Geographic locations of six South Korean natural populations of wild soybean.

(ENP), esterase (EST), urease (EU), fluorescent esterase (FLE), glutamate oxaloacetic transaminase (GOT), NADP-active isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), mannose-6-phosphate isomerase (MPI), 6-phosphogluconate dehydrogenase (PGD), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), shikimate dehydrogenase (SDH), and the Kunitz trypsin inhibitor (TI).

Data were analyzed with Biosys-1, a Fortran computer program (Swofford & Selander, 1981).

Results

New isozyme variants involving ADH, MPI, PGM and SDH were found. Their inheritance was studied (Yu & Kiang, 1993a). A variant with an even slower band 1 than the known slow type for LAP also was found. However, we failed to obtain the inheritance data due to unsuccessful hybridizations. For the convenience of this study, the *Lap1-c* allele symbol was assigned for this variant.

The 35 loci examined, the alleles present and their frequencies at these loci are given in Table 2. Of the 35 loci, 27 were polymorphic, and eight (*Aco4, Adh1, Adh2, Fle, Got, Pgd3, Pgi3, Pgm3*) monomorphic (Table 2). Although genotypes for some plants were not identified at the *Pgm3* locus due to overlapping bands, those that were determined showed no variation; *Pgm3* was considered monomorphic. For the 35 loci, 94 alleles have been reported in the literature (Bult, 1989; Chiang, 1985; Yu & Kiang, 1993a), and 72 were present in these six populations. The maximum number of alleles was four (*Ap* and *Mpi,* Table 2).

The mean number of alleles per locus, 99% polymorphism and the expected heterozygosity were 1.4, 37.2% and 0.134, respectively, averaged over the six populations, and 2.1, 77.1% and 0.215 in the total population (Table 3). The mean observed heterozygosity was 0.004 for these populations (Table 3). Population F had the highest variation by all measures, followed by population A. Population D had the lowest variation (Table 3).

Partitioning of the total gene diversity for the 27 polymorphic loci showed that G_{ST} (coefficient of gene differentiation) varied from 0.077 for *Aco5* and *Dia3* to 0.675 for *Pgm2* with the mean G_{ST} of 0.383, which means that 38.3% of the total gene diversity existed between populations (Table 4).

Nei's genetic distance (Table 5) and cluster analysis based on this distance (Fig. 2) indicated that D and E were the closest populations. The largest genetic distance occurred between B and C (Table 5). The most northern population (A) somehow clustered with the most southern population (F) (Fig. 2). Population C was the most distinct among the six, as indicated by the largest mean genetic distance (Table 5) and the distinct cluster it formed (Fig. 2). The overall mean genetic distance was 0.117 (Table 5).

Discussion

The most used measures of genetic variation are the number of alleles per locus (A), polymorphism (proportion of polymorphic loci in the total number of loci analyzed, P), and the expected heterozygosity (H). H also is called gene diversity (Nei, 1973). In this study, the variation in the total population (A = 2.1, P = 0.771, and H = 0.215) was not only higher than the average for 123 self-fertilized plant species (A = 1.69, P = 0.418, and H = 0.124), but also higher than the average for 473 plant species of all mating systems (A = 1.96, P = 0.505, and H = 0.149) (Hamrick & Godt, 1990). The higher variation also was expressed at the population level. The population means of A, P and H were 1.4, 0.372 and 0.134, respectively, for these six populations, while they are 1.31, 0.200 and 0.074 for 113 self-fertilized plant populations, and 1.53, 0.342 and 0.113 for 468 plant pop-

Table 2. Allele frequencies at the 35 loci examined

Locus	Allele[a]	Population							Locus	Allele[a]	Population						
		A	B	C	D	E	F	Mean			A	B	C	D	E	F	Mean
Aco1	*a*	0.00	0.00	0.00	0.22	0.00	0.23	0.08	*Idh2*	*a*	0.78	1.00	0.45	1.00	1.00	1.00	0.87
	b	1.00	1.00	1.00	0.78	1.00	0.77	0.92		*b*	0.22	0.00	0.55	0.00	0.00	0.00	0.13
Aco2	*a*	0.56	0.03	0.00	0.00	0.00	0.31	0.15	*Idh3*	*a*	1.00	0.50	0.53	1.00	1.00	1.00	0.84
	b	0.44	0.97	1.00	1.00	1.00	0.50	0.82		*b*	0.00	0.50	0.47	0.00	0.00	0.00	0.16
	c	0.00	0.00	0.00	0.00	0.00	0.19	0.03	*Idh4*	*a*	0.78	1.00	0.45	1.00	1.00	1.00	0.87
Aco3	*a*	1.00	1.00	1.00	0.56	0.62	0.35	0.75		*b*	0.22	0.00	0.55	0.00	0.00	0.00	0.13
	b	0.00	0.00	0.00	0.44	0.38	0.65	0.25	*Lap1*	*a*	0.00	0.47	0.03	0.00	0.35	0.00	0.14
Aco4	*b*	1.00	1.00	1.00	1.00	1.00	1.00	1.00		*b*	1.00	0.53	0.97	1.00	0.65	0.92	0.85
Aco5	a	1.00	1.00	1.00	1.00	0.93	0.89	0.97		*c*	0.00	0.00	0.00	0.00	0.00	0.08	0.01
	b	0.00	0.00	0.00	0.00	0.00	0.11	0.02	*Mpi*	*a*	0.41	0.00	0.00	0.78	0.43	0.00	0.27
	–	0.00	0.00	0.00	0.00	0.07	0.00	0.01		*b*	0.56	1.00	0.00	0.00	0.07	0.65	0.38
Adh1	+	1.00	1.00	1.00	1.00	1.00	1.00	1.00		*c*	0.04	0.00	0.97	0.22	0.50	0.35	0.34
Adh2	+	1.00	1.00	1.00	1.00	1.00	1.00	1.00		*e*	0.00	0.00	0.03	0.00	0.00	0.00	0.01
Adh3	+	0.33	0.53	1.00	1.00	0.93	0.65	0.74	*Pgd1*	*a*	0.41	0.93	0.00	0.00	0.00	0.12	0.24
	–	0.67	0.47	0.00	0.00	0.07	0.35	0.26		*b*	0.59	0.03	1.00	1.00	0.80	0.42	0.64
Am3[b]	*a*	0.00	0.00	0.00	0.00	0.38	0.27	0.11		*c*	0.00	0.03	0.00	0.00	0.20	0.46	0.12
	b	1.00	1.00	1.00	1.00	0.62	0.73	0.89	*Pgd2*	*a*	0.87	1.00	1.00	0.78	0.35	0.81	0.80
Ap	*a*	0.70	0.47	0.70	0.78	0.00	0.39	0.51		*b*	0.13	0.00	0.00	0.22	0.65	0.19	0.20
	b	0.00	0.50	0.00	0.00	0.00	0.00	0.08	*Pgd3*	*b*	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	c	0.04	0.03	0.30	0.22	1.00	0.61	0.37	*Pgi1*	*a*	0.07	0.47	0.00	0.00	0.00	0.00	0.09
	d	0.26	0.00	0.00	0.00	0.00	0.00	0.04		*b*	0.93	0.07	1.00	1.00	1.00	1.00	0.83
Dia1	+	0.48	0.47	0.43	0.44	0.00	0.00	0.30		–	0.00	0.46	0.00	0.00	0.00	0.00	0.08
	–	0.52	0.53	0.57	0.56	1.00	1.00	0.70	*Pgi2*	+	0.52	1.00	0.93	1.00	0.93	0.11	0.75
Dia2	*a*	0.26	0.47	0.00	0.78	0.52	0.00	0.34		–	0.48	0.00	0.07	0.00	0.07	0.89	0.25
	b	0.74	0.53	1.00	0.22	0.48	1.00	0.66	*Pgi3*	*b*	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dia3	*a*	0.07	0.03	0.00	0.00	0.15	0.19	0.07	*Pgm1*	*a*	0.78	1.00	1.00	1.00	1.00	1.00	0.96
	b	0.93	0.97	1.00	1.00	0.85	0.81	0.93		*b*	0.22	0.00	0.00	0.00	0.00	0.00	0.04
Enp	*a*	0.00	0.00	0.45	0.00	0.35	0.00	0.13	*Pgm2*	*b*	1.00	1.00	0.00	1.00	0.93	0.89	0.80
	b	1.00	1.00	0.55	1.00	0.65	1.00	0.87		*c*	0.00	0.00	0.82	0.00	0.07	0.11	0.17
Est1	*a*	0.00	0.00	0.42	0.00	0.07	0.19	0.11		*d*	0.00	0.00	0.18	0.00	0.00	0.00	0.03
	b	1.00	1.00	0.58	1.00	0.93	0.81	0.89	*Pgm3*	+	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Eu[c]	*a*	1.00	1.00	1.00	1.00	1.00	0.89	0.98	*Sdh*	*a*	1.00	1.00	1.00	0.78	1.00	1.00	0.96
	–	0.00	0.00	0.00	0.00	0.00	0.11	0.02		*b*	0.00	0.00	0.00	0.22	0.00	0.00	0.04
Fle	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	*Ti*	*a*	0.93	0.97	0.43	1.00	1.00	1.00	0.89
Got	*b*	1.00	1.00	1.00	1.00	1.00	1.00	1.00		*b*	0.07	0.03	0.57	0.00	0.00	0.00	0.11
Idh1	*a*	0.00	0.00	0.00	0.22	0.00	0.04	0.04									
	b	1.00	1.00	1.00	0.78	1.00	0.96	0.96									

[a] + and – denote the dominant and recessive (null) alleles, respectively.

[b] Same as *Sp1*.

[c] Considered one locus, although a separate locus was proposed for the null type (Kloth et al., 1987).

Table 3. Genetic variation of populations

Population	Mean no. alleles per locus	Polymorphism (99%)	Heterozygosity	
			Expected[a]	Observed
A	1.5	42.9%	0.158	0.001
B	1.4	31.4%	0.113	0.000
C	1.3	34.3%	0.129	0.016
D	1.3	25.7%	0.100	0.000
E	1.4	40.0%	0.133	0.005
F	1.5	48.6%	0.168	0.000
Mean	1.4	37.2%	0.134	0.004
Total population	2.1	77.1%	0.215	0.004

[a] Nei's unbiased estimate of the expected heterozygosity (Nei, 1978).

Table 4. Partitioning of the gene diversity (H_T) within (H_S) and between (D_{ST}) populations to obtain the coefficient of gene differentiation (G_{ST}) for the polymorphic loci (Nei, 1973)

Locus	H_T	H_S	D_{ST}	G_{ST}
Aco1	0.140	0.117	0.023	0.163
Aco2	0.307	0.196	0.111	0.361
Aco3	0.371	0.236	0.135	0.364
Aco5	0.061	0.056	0.005	0.077
Adh3	0.384	0.255	0.129	0.335
Am3	0.192	0.144	0.048	0.252
Ap	0.600	0.368	0.232	0.387
Dia1	0.423	0.330	0.093	0.220
Dia2	0.448	0.288	0.160	0.357
Dia3	0.138	0.127	0.011	0.077
Enp	0.232	0.159	0.073	0.316
Est1	0.201	0.155	0.046	0.229
Eu	0.038	0.034	0.004	0.098
Idh1	0.083	0.070	0.013	0.159
Idh2	0.224	0.140	0.084	0.375
Idh3	0.270	0.166	0.104	0.385
Idh4	0.224	0.140	0.084	0.375
Lap1	0.266	0.194	0.072	0.272
Mpi	0.664	0.325	0.339	0.511
Pgd1	0.515	0.253	0.262	0.509
Pgd2	0.318	0.223	0.095	0.298
Pgi1	0.293	0.116	0.177	0.604
Pgi2	0.376	0.161	0.215	0.573
Pgm1	0.071	0.058	0.013	0.192
Pgm2	0.328	0.107	0.221	0.675
Sdh	0.071	0.058	0.013	0.192
Ti	0.199	0.115	0.084	0.421
Mean	0.275	0.170	0.105	0.383

ulations of all mating systems (Hamrick & Godt, 1990).

In soybean, although basically the same enzymes were examined in this and other studies in our laboratory, the total numbers of loci used in analyses were different. The hypothesized loci without inheritance data also were included in the previous studies (Bult, 1989; Kiang et al., 1987). Since the number of loci controlling an enzyme is not known until its inheritance is studied, only the genetically studied loci were included in the present study. In order to compare with the previous studies, the data of this study were reanalyzed using the same total numbers of loci as in the previous studies (i.e. including more monomorphic loci) (see Table 6). The variation in these six natural populations was similar to that in the 66 accessions of the South Korean wild soybean (Table 6), reflecting the fact that all these wild soybeans were from South Korea and the consistent results of isozyme studies. These six populations had much higher variation than the 857 accessions of the cultivated soybean (Table 6), which was in agreement with previous findings that *G. soja* had higher variation than *G. max.* They also had higher variation than wild soybean populations from other geographic regions (Table 6). However, it should be noted that the sample sizes were smaller for other populations.

The genetic variation between populations (i.e. genetic differentiation of populations) are measured by Nei's gene differentiation (proportion of

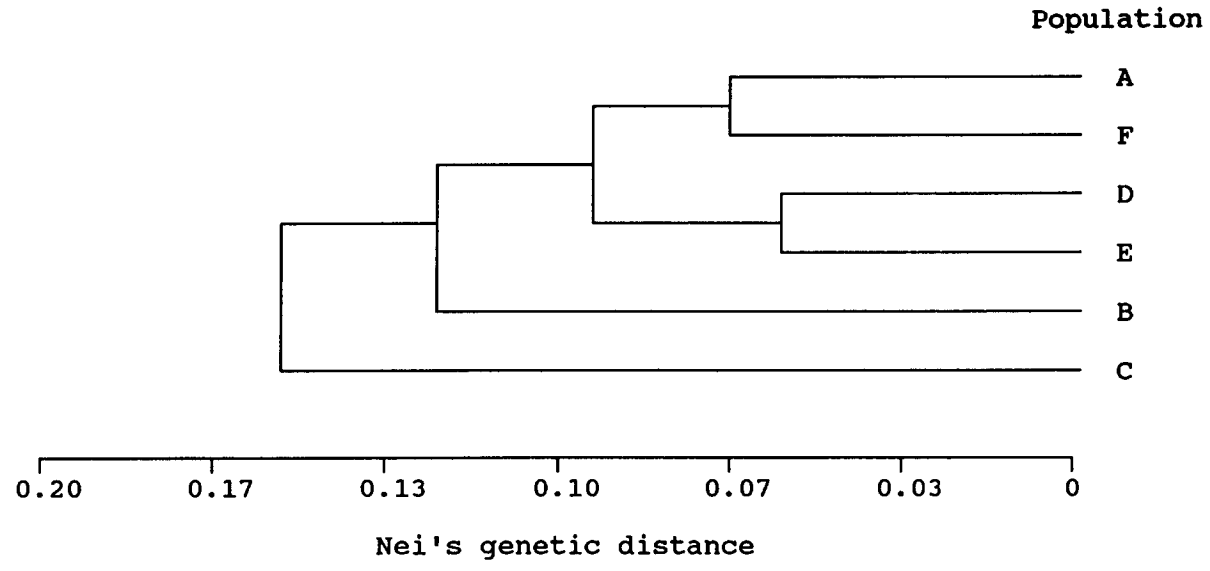

Fig. 2. Dendrogram obtained from cluster analysis based on the unbiased estimate of Nei's genetic distance using the average linkage between groups method (UPGMA).

the total gene diversity between populations, denoted by G_{ST}) (Nei, 1973). The G_{ST} of 0.383 in this study was between 0.510, the average for 78 self-fertilized plant species and 0.224, the average for 406 plant species of all mating systems (Hamrick & Godt, 1990). But, these six populations were very greatly differentiated according to the 0.25 F_{ST} criterion (F_{ST} is the fixation index, same as G_{ST}) (Hartl, 1988). The 0.383 G_{ST} value was higher than the 0.198 G_{ST} for four natural populations of wild soybean along the Kitakami River of Japan (Chiang, 1985). The mean value for Nei's genetic distance (Nei, 1972, 1978) also can be a measure of population differentiation. It was 0.117 for these six populations, again higher than 0.044 for the four populations along the Kitakami River of Japan, and also higher than 0.063 for seven local natural populations of wild soybean in Mishima City, Japan (Bult, 1989).

The high within- and between-population variation resulted in the high variation in the total population. Several unique isozyme variants and a leaf margin necrosis mutant were found in these populations (Yu & Kiang, 1993a, b). The number of alleles at single loci was as high as four. These six populations growing within a 200 km geographic range possessed 76.6% of the reported alleles for the 35 loci studied (72/94). The more rare alleles and higher variation in South Korea for wild soybean and the cultivated soybean than other regions based on this and other studies (Broich & Palmer, 1981; Hymowitz & Kaizuma, 1981; Kiang & Gorman, 1983; Kiang et al., 1987) prompt us to propose that South Korea is one of the world major soybean gene centers.

One explanation is that wild soybean may have become adapted to the South Korean natural habitats for a longer period of time than to other habitats, resulting in the accumulation of more variation, which then has been transferred to the cultivated soybean through gene flow. Wild soybean often grows adjacent to the soybean fields; there is no

Table 5. Unbiased estimate of Nei's genetic distance between populations (Nei, 1972, 1978)

Population	A	B	C	D	E	F	Mean
A	***	0.081	0.135	0.072	0.110	0.066	0.093
B		***	0.193	0.125	0.147	0.143	0.138
C			***	0.133	0.140	0.166	0.153
D				***	0.057	0.106	0.099
E					***	0.079	0.107
F						***	0.112
							0.117

reproductive barrier for hybridizations between the two species. The reverse gene flow may not be as important, since the hybrids are not as competitive as their wild counterpart in natural conditions (Oka, 1983). Domestication is a process of trial and error (Hymowitz, 1970). It probably was not a single event. The longer natural history of wild soybean also may have provided more opportunities for more ancestors to be domesticated so that the high variation in the South Korean cultivated soybean has arisen from diverse ancestors. In other words, South Korea is not only one of the areas where the first adaptations of wild soybean to the natural habitats occurred, but also one where the first domestications of soybean occurred. This is not surprising. Fukuda (1933) proposed that soybean was first domesticated in Northeast China (used to be called 'Manchuria'). Geographically the Korean peninsula is attached to Northeast China. It is possible that many independent domestication events occurred in this vast region. Similar studies of wild soybean populations from other geographic regions are needed to support our hypothesis of the Korean peninsula as one of the major gene centers.

Hymowitz and associates suggested that the cultivated soybean was first domesticated in the eastern half of North China (Hymowitz, 1970), and later reached the Korean peninsula, possibly from Northeast China (Hymowitz & Kaizuma, 1981; Hymowitz & Newell, 1981). Enormous variability can be packed in a small geographic area outside of the center of origin (Harlan, 1971). If this is the case for soybean, there must be physical peculiarities for the Korean geography to nurture high variation. However, examination of the Nei's genetic distance (Table 5) and dendrogram generated from it (Fig. 2) did not reveal any correlations between geographic distance and genetic distance (Spearman rank r = 0.007), indicating that geographic distance was not a factor in the genetic differentiation of these natural populations.

Recent advances in molecular biology have made it possible to detect variation at the DNA level. There have been a few studies of soybean variation using restriction fragment length polymorphisms. The mitochondrial DNA (Grabau et al., 1992; Sisson et al., 1978), chloroplast DNA (Close et al., 1989; Shoemaker et al., 1986), nuclear DNA encoding the 18s–25s and 5s ribosomal RNAs (Doyle, 1988; Doyle & Beachy, 1985), and genomic DNA (Apuya et al., 1988; Keim et al., 1989) were digested with type II restriction endonucleases, followed by probing with DNA clones or direct gel electrophoresis. These studies are useful in understanding the amount of variation, the parentage relationships of modern cultivars, and the evolutionary relationships of different taxa within the *Glycine* genus. However, it is difficult to compare the results of these studies with those of isozymes. Some organelle DNA sequences, especially the chloroplast

Table 6. Comparisons of these six populations with *G. max* and other wild soybean populations in terms of genetic variation

Populations[a]	Sample size[b]	No. Loci	No. alleles per locus		Polymorphism (99%)		H_{exp}[c]	
			Pop. mean	Total pop.	Pop. mean	Total pop.	Pop. mean	Total pop.
G. max	857a	46	–	1.43	–	0.457	–	0.140
China	21a	46	–	1.37	–	0.237	–	0.110
Japan	41a	46	–	1.58		0.467	–	0.168
South Korea-1	66a	46	–	1.93	–	0.578	–	0.149
USSR	20a	46	–	1.21	–	0.158	–	0.057
Mishima, Japan	111p	49	1.14	–	0.140	–	0.046	–
South Korea-2	172p	–[d]	1.29	1.83	0.266	0.587	0.094	0.164

[a] The *G. max* populations and the China, Japan, South Korea-1 and USSR wild soybean populations were adopted from Kiang et al. (1987); The Mishima, Japan wild soybean populations from Bult (1989); The South Korea-2 populations from this study.

[b] 'a' denotes accessions from the USDA Soybean Germplasm Collection; 'p' denotes natural plants.

[c] The expected heterozygosity.

[d] 49 for population means and 46 for the total population.

DNA, are highly conserved, and contain low variation (Close et al., 1989; Shoemaker et al., 1986). In the study with the genomic DNA, 15 of the 17 markers used revealed two alleles and two revealed three alleles in 58 cultivated and wild soybean lines, and the authors concluded that soybean had low molecular diversity (Keim et al., 1989). This seems contradictory with the results of our isozyme studies. But, the authors failed to cite what plant species were compared to. The conclusion of isozyme studies that soybean, especially wild soybean, contains high variation is based on the comparisons with other plant species, especially the self-fertilized plant species.

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