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Genetic Diversity of Soybean Landraces in Korea

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ABSTRACT

To evaluate the genetic diversity and structure of the South Korean soybean population, 233 landraces collected in various regions of the country were surveyed for 15 allozyme loci and one protein locus. The South Korean population was fixed or nearly fixed at seven of the 16 loci tested. The number of alleles per locus was 2.06 and Nei's gene diversity was 0.194. These values were lower than the values for the same 16 loci previously reported for the Japanese and Chinese populations. The differences among eight regional groups were not so marked, with only 7.2% of the total variation arising from regional differentiation. Three southern regional groups (Chollabuk-do, Chollanam-do and Kyongsangnam-do) exhibited a relatively high variability because of frequent occurrence of alleles characteristic of the Japanese population. A marked difference was found in allelic frequencies at the *Dial* locus between large-seeded landraces and small-seeded ones, suggesting that the latter, which are used mainly for bean sprouts, had been established independently of the former, which are used mostly for soysauce and cooking with rice. Not only the region but also the usage as food materials should therefore be taken into consideration in designing an efficient collection and preservation method for the Korean soybean landraces.

Keywords : soybean, *Glycine max*, landraces, isozyme, seed protein, genetic diversity.

Soybean [*Glycine max* (L.) Merr.] is one of the world's premier crops, which is cultivated as a source of dietary vegetable oil and is also a nutritionally excellent and inexpensive source of protein for both humans and animals. Soybean is considered to have been domesticated in China from its wild relative, *G. soja* Sieb. et Zucc. and thereafter has been disseminated to various regions of Asia (Hymowitz, 1970). In Korea, soybean cultivation can be traced back to about 2,500 years ago (Kwon, 1972). Consequently, various landraces with different morphological and agronomic traits have been established, and are very useful as genetic resources for improvement of modern cultivars (Kwon, 1972). However, most of such indigenous soybeans are being rapidly replaced by newly released improved varieties. This will doubtless cause a loss of genetic diversity in the Korean soybean population.

Allozyme markers are often used to measure the level of variation within and among plant populations. Studies of allozyme markers have collectively made an important contribution to our understanding of the degree of genetic diversity, geographical patterns of variation and genetic structure for both cultivated and wild soybean populations (Abe et al., 1992a; Brown et al., 1990; Fujita et al., 1997; Griffin & Palmer, 1995; Hirata et al., 1996, 1999; Kiang et al., 1992; Kiang & Gorman, 1983; Perry et al., 1991, Yu & Kiang, 1993). The techniques for assaying isozyme variability in soybean are well defined (Cardy & Beversdorf, 1984; Hedges & Palmer, 1989; Kiang & Gorman, 1983), and more than 40 loci encoding isozyme and protein variants are available for genetic studies (Palmer & Kilen, 1987; Rennie & Palmer, 1987). Genetic structure of the Korean wild and cultivated soybean populations have also been studied based on variations at isozyme and seed protein loci (Abe et al., 1992a; Griffen & Palmer, 1995; Hirata et al., 1999; Hymowitz & Kaizuma, 1981; Kiang & Gorman, 1983; Kiang et al., 1987; Perry et al., 1991; Yu & Kiang, 1993). There is a discrepancy in the amount of variation in the Korean cultivated soybean population. Kiang et al. (1987), and Yu & Kiang (1993) reported that both cultivated and wild soybean populations in Korea were rich in rare alleles and possessed higher variation than the other populations from different geographic regions. Yu & Kiang (1993) proposed that the Korean peninsula is one of the major gene centers for both cultivated and wild soybeans. On the contrary, Griffen & Palmer (1995), and Hirata et al. (1999) suggested a low diversity in the Korean cultivated soybean population compared with the Japanese and Chinese populations. In addition to the inconsistent results between the studies cited above, most of the earlier studies treated the Korean accessions tested only as a whole population, and had not provided any detailed insight on the genetic structure, such as geographic patterns of variation and differentiation between cultivar groups, of the Korean population. In this paper, we evaluate the genetic diversity of the South Korean cultivated

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soybean population and characterize its structure, by analyzing variations at 15 isozyme and one seed protein loci for unnamed landraces collected in various regions of the country.

MATERIALS AND METHODS

Plant materials

Two hundred and thirty-three landraces of the South Korean cultivated soybeans were screened in this study. The landraces were provided by Professor J. H. Kim, Kyongsang University, Jinju, and the Gene Bank of Rural Development Administration (RDA), Suwon. Based on their collection sites, the landraces were classified into eight regional groups according to the administrative districts of South Korea (Fig. 1): Kyonggi-do (KG), Chungchongnam-do (CN), Chungchongbuk-do (CB), Kangwon-do (KW), Chollabuk-do (CLB), Chollanam-do (CLN), Kyongsangbuk-do (KB) and Kyongsangnam-do (KN). In addition, the landraces were grouped into five classes of seed size: very small (< 10.0 g: VS), small (10.1 g ~ 20.0 g: S), middle (20.1 g ~ 30.0 g: M), large (30.1 g ~ 40.0 g: L), very large (> 40.1 g: VL), based on their one-hundred seed weight, and six classes of seed-coat colors: yellow, pale green, green, brown, black, and saddle pattern.

Fig. 1. Eight regional groups of South Korea and the number of landrace collected.

Electrophoresis procedures

Preparation of starch gels, electrophoresis procedures, and enzyme activity staining methods followed those of Abe et al. (1992a). Two different buffer systems, 0.065 M histidine / 0.007 M citrate (pH 6.5; D buffer of Cardy & Beversdorf (1984)) and 0.4 M tris (hydroxymethyl) aminomethane / 0.132 M histidine hydrochloride (pH 7.0), were used to separate 15 isozymes of nine enzymes. The enzymes assayed were: aconitase (*Aco1* to *Aco5*), acid phosphates (*Ap*), diaphorase (*Dia1*), isocitrate dehydrogenase (*Idh1* and *Idh2*), endopeptidase (*Enp*), esterase (*Est1*), leucine aminopepidase (*Lap1*), mannose-6-phosphate isomerase (*Mpi*) and phosphoglucomutase (*Pgm1* and *Pgm2*). We also examined one protein locus, the Kunitz trypsin inhibitor (*Ti*). The trypsin inhibitor analysis was analyzed according to the Kollipara et al. (1991). For both isozyme and seed protein analyses, at least three seeds per landrace were individually assayed. When heterogeneity was detected within a landrace, three additional seeds were examined.

Genetic diversity analyses

Standard statistics for characterizing genetic variability, the number of alleles per locus (A), the proportion of loci polymorphic at the 95% level (P) and Nei's (1973) gene diversity (H) were computed for the regional groups of landraces and the total population. H was calculated for each locus in a population by $H = 1 - \sum pi^2$ where pi was the frequency of the i-th allele in the population. The mean H value in a population was obtained by averaging H values over all loci. The degree of genetic differentiation between regional groups, seed size classes, and seed-coat color classes was evaluated by Nei's (1973) coefficient of gene differentiation (G_{ST}). Nei's (1987) standard genetic distance was calculated to evaluate the genetic relationships between regional groups.

RESULTS AND DISCUSSION

Observed variation

The alleles observed in the 233 landraces tested and their frequencies for eight regional groups are given in Table 1. A total of 35 alleles, all of which except *Idh2-c* are genetically determined, were found in the 16 loci tested. The allelic designation followed Griffin & Palmer (1995) for *Aco1, Aco2, Aco3, Aco4,* and *Enp*, Kiang & Bult (1991) for *Aco5*, Bult & Kiang (1989) for *Est1*, and Palmer & Kilen (1987) for the other loci. The number of alleles per loci (A) in the South Korean population was 2.06, being slightly lower than the value (2.19) reported by Hirata et al. (1999) who tested 158 Korean accessions for the same 16 loci used in this study. Two rare alleles,

Table 1. Allelic frequencies for 16 loci in regional groups of the Korean soybean landraces.

Locus	Allele	Collection site								Total (233)
		KG† (44)‡	CN (17)	CB (10)	KW (24)	CLB (24)	CLN (18)	KB (53)	KN (43)	
Aco1	*a*	1.000	1.000	1.000	0.847	0.917	1.000	0.991	1.000	0.969
	b	0.000	0.000	0.000	0.153	0.083	0.000	0.009	0.000	0.031
Aco2	*a*	0.121	0.019	0.000	0.090	0.222	0.056	0.038	0.085	0.088
	b	0.879	0.941	1.000	0.914	0.778	0.944	0.962	0.915	0.912
Aco3	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Aco4	*a*	0.000	0.000	0.000	0.000	0.125	0.111	0.031	0.046	0.037
	b	1.000	1.000	1.000	1.000	0.875	0.889	0.969	0.954	0.963
Aco5	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ap	*a*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.002
	b	1.000	1.000	1.000	0.973	1.000	1.000	1.000	0.988	0.991
	c	0.000	0.000	0.000	0.027	0.000	0.000	0.000	0.000	0.007
Dia1	*a*	0.685	0.471	0.584	0.514	0.583	0.418	0.515	0.387	0.523
	b	0.315	0.529	0.416	0.486	0.417	0.582	0.485	0.613	0.477
Enp	*a*	0.405	0.402	0.634	0.499	0.473	0.426	0.289	0.442	0.413
	b	0.595	0.598	0.366	0.501	0.528	0.574	0.711	0.558	0.587
Est1	*a*	0.038	0.176	0.000	0.028	0.139	0.167	0.075	0.054	0.082
	b	0.962	0.824	1.000	0.972	0.861	0.883	0.925	0.946	0.918
Idh1	*a*	0.841	0.705	0.800	0.944	0.638	0.602	0.830	0.807	0.792
	b	0.159	0.295	0.200	0.056	0.362	0.398	0.170	0.193	0.208
Idh2	*a*	0.940	0.765	1.000	1.000	0.958	0.870	0.755	0.902	0.879
	b	0.060	0.235	0.000	0.000	0.042	0.130	0.226	0.098	0.112
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.009
Lap1	*a*	0.011	0.000	0.000	0.000	0.000	0.056	0.012	0.039	0.016
	b	0.989	1.000	1.000	1.000	1.000	0.944	0.988	0.961	0.984
Mpi	*a*	0.008	0.000	0.000	0.014	0.000	0.000	0.012	0.000	0.006
	b	0.621	0.588	1.000	0.632	0.639	0.573	0.824	0.562	0.665
	c	0.371	0.412	0.000	0.354	0.361	0.427	0.164	0.438	0.329
Pgm1	*a*	0.300	0.118	0.350	0.292	0.264	0.352	0.163	0.365	0.273
	b	0.700	0.882	0.650	0.708	0.736	0.648	0.837	0.635	0.731
Pgm2	*b*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ti	*a*	0.830	0.765	0.950	0.875	0.771	0.806	0.962	0.826	0.856
	b	0.170	0.235	0.050	0.083	0.229	0.194	0.038	0.174	0.139
	c	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.004
A¶		1.688	1.563	1.313	1.750	1.688	1.688	1.875	1.750	2.063
P		0.500	0.563	0.250	0.500	0.625	0.688	0.500	0.563	0.563
H		0.174	0.200	0.114	0.171	0.230	0.227	0.157	0.196	0.194

† KG = Kyonggi-do; CN = Chungchongnam-do; CB = Chungchongbuk-do; KW = Kangwon-do; CLB = Chollabuk-do; CLN = Chollanam-do; KB = Kyongsangbuk-do; KN = Kyongsangnam-do. ‡ The number of accessions tested.

¶ A = Mean number of alleles per locus. P = Proportion of loci polymorphic in which the frequency of common allele is less than 0.95. H = Average gene diversity (Nei, 1973).

Enp-υ and *ti*, which were observed in the Korean soybean population by Hirata et al. (1999), and Hymowitz & Kaizuma (1981), respectively, were not detected in the landraces screened in this study.

Of the 16 loci, three (*Aco3, Aco5*, and *Pgm2*) were monomorphic. The South Korean population possessed *Aco3-a, Aco5-a*, and *Pgm2-b*. In addition, *Aco1, Aco4, Ap*, and *Lap1* were weakly polymorphic, with the frequencies of the most common alleles (*Aco1-a, Aco4-b, Ap-b*, and *Lap1-b*) in the total population being greater than 95%. On the other hand, *Dia1, Enp, Idh1, Mpi*, and *Pgm1* exhibited high H values of 0.353 to 0.499, and *Aco2, Est1, Idh2*, and *Ti* showed moderate values of 0.153 to 0.259 (Table 3). The Korean population, as a whole, had *Aco2-b, Est1-b, Idh1-a, Idh2-a, Mpi-b, Pgm1-b*, and *Ti-a* in high frequencies. The results obtained in this study were thus in good accordance with those obtained by Hirata et al. (1999) for all of the 16 loci and Griffin & Palmer (1995) for nine of the 16 loci (*Aco1, Aco2, Aco3, Aco4, Ap, Dia1, Enp, Idh1* and *Idh2*).

Comparison of diversity between the Korean population and the Japanese and Chinese populations

The proportions of polymorphic loci (P) and the mean gene diversity index over the 16 loci (H) in the total population were 0.56 and 0.194, respectively. Table 2 compares the diversity statistics of the Korean population and the Japanese and Chinese populations obtained in this study and previous studies. Hirata et al. (1999) surveyed variations at the same 16 loci used in this study and found that P and H were lower in the Korean population (0.56 for P and 0.209 for H) than in the Japanese population (0.69 for P and 0.248 for H) and the Chinese population (0.75 for P and 0.249 for H) (Table 2). The diversity statistics obtained in this study were thus almost the same as, but slightly lower than the results obtained for the 158 accessions introduced from the Korean peninsula and maintained in the Gene Bank of the National Institute of Agrobiological Resources, Tukuba, Japan. These results may indicate that the Korean soybean population is more uniform than the Japanese and Chinese populations. This result is also supported by that of Griffin & Palmer (1995) who estimated a lower diversity for 13 loci, including nine of the 16 loci tested in this study, in the Korean population compared with the Japanese and Chinese populations. Diversity statistics were re-calculated for the results of Perry et al. (1991) for six loci and those of Hymowitz & Kaizuma (1981) for two loci, respectively (Table 2). Both results indicated that the H values in the Korean population were almost the same as or slightly lower than those of the Japanese population, although the former had more alleles than the latter. Low gene diversity in the Chinese population for *Ti* and *Sp1* (Hymowitz & Kaizuma, 1981) is ascribed to the fact that the Chinese population exclusively had *Ti-a*. On the contrary, the results obtained in this study and those cited above are inconsistent with the findings of Kiang et al. (1987), and Yu & Kiang (1993). Based on the variation at 35 isozyme and seed-protein loci, Yu & Kiang (1993) reported that the Korean wild soybean population possessed a higher variation than those from different geographic regions. They assumed that the Korean peninsula is one of the world's major soybean gene centers, and the high diversity found in the Korean cultivated soybean population (Kiang & Gorman, 1983; Kiang et al., 1987) probably resulted from gene flow from wild soybeans sympatric to the cultivars. The inconsistent results between these studies might partly be caused by the different kinds of loci tested, because the degree of diversity is often dependent on the loci tested. In addition, a sampling bias may be another cause of the inconsistent results. A further study of landraces from North Korea is also needed to gain the final picture of the genetic diversity of the Korean soybean population. Molecular markers, such as AFLPs (Amplified Fragment Length Polymorphisms)

Table 2. Comparison of genetic diversity among the Korean, Japanese and Chinese cultivated soybean populations.

	Region	A†	P†	H¶	n§	Locus assayed	Reference
1	South-Korea	2.06	0.56	0.194	233	*Aco1, Aco2, Aco3, Aco4, Aco5, Ap, Dia1, Enp, Est1, Idh1, Idh2, Lap1, Mpi, Pgm1, Pgm2, Ti* (16)	This study
2	Korea	2.19	0.56	0.209	158	*Aco1, Aco2, Aco3, Aco4, Aco5, Ap, Dia1, Enp, Est1, Idh1, Idh2, Lap1, Mpi, Pgm1, Pgm2, Ti* (16)	Hirata et al. (1999)
	Japan	2.31	0.69	0.248	781		
	China	2.13	0.75	0.249	94		
3	Korea	2.00	0.53	0.176	178	*Aco1, Aco2, Aco3, Aco4, Aco5, Ap, Dia1, Enp, Idh1, Idh2, Lap1, Me, Sod1, Sod2, Sp1* (13)	Griffin & Palmer (1995)
	Japan	2.00	0.59	0.196	133		
	China	1.94	0.65	0.199	96		
4	Korea	2.14	0.29	0.137	783	*Lap1, Lap2, Pgd1, Pgi1, Pgi2, Sod1, Sod2* (7)	Perry et al. (1991)
	Japan	1.86	0.49	0.156	80		
	China	2.14	0.29	0.140	779		
5	Korea	3.00	1.00	0.268	417	*Sp1, Ti* (2)	Hymowitz & Kaizuma (1979, 1981)
	Japan	2.50	1.00	0.297	477		
	China	2.00	1.00	0.099	903		
6	Korea	1.46	0.37	0.142	73	The loci assayed were not presented (46)	Kiang et al. (1987)
	Japan	1.37	0.33	0.105	73		
	China	1.41	0.35	0.126	85		

† A = Mean number of alleles per locus. ‡ P = Proportion of loci polymorphic. ¶ H = Average gene diversity.
§ n = The number of accessions tested.

and SSRs (Simple Sequence Repeats), may be powerful tools for such a germplasm study because of their highly polymorphic nature.

Regional difference in allelic frequency and genetic diversity

To evaluate geographic patterns of variation in the South Korean soybean population, we compared allelic frequencies among the eight regional groups of landraces (Table 1). Values of Nei's coefficient of gene differentiation (G_{ST}), which represent the proportion of variation due to regional differentiation in the total variation, are presented in Table 3. G_{ST} ranged from 1.9% for *Ap* to 10.2% for *Aco1* with an average of 7.2%. This suggests that only 7.2% of the total variation observed in the Korean population arose from regional differentiation.

A similarity in allelic constitutions among regions was also revealed by Nei's standard genetic distance between regional groups (Table 4). The distance ranged from 0.006 for Chollanam-do vs. Kyongsangnamdo to 0.039 for Chungchongnam-do vs. Chungchongbuk-do, with an average of 0.18. Of the eight regional groups, however, Chungchongbuk-do had relatively high distances of 0.019 to 0.039 from the other groups, with the average distance being 0.027. The Chungchongbuk-do population was monomorphic in 11 of the 16 loci, and was lacking *Mpi-c* which was observed with frequencies of 0.164 to 0.438 in the other groups. This might be a factor causing the large genetic distances between Chungchongbuk-do and the other regional groups.

Some alleles also showed a trend in their geographical distribution. Rare or uncommon alleles, *Aco4-a, Est1-a,* and *Ti-b,* were observed in relatively high frequencies in southern regional groups such as the Chollabuk-do, Chollanam-do, Kyongsangnam-do and

Table 3. Proportion of the total gene diversity (G_{ST}) found among the regions, seed-size and seed-coat color classes for the 13 polymorphic loci.

Locus	H_T†	Region	Seed-size	Seed-color¶
Aco1	0.058	0.102	0.016	0.014
Aco2	0.153	0.051	0.033	0.038
Aco4	0.075	0.062	0.045	0.070
Ap	0.010	0.019	0.006	0.006
Dia1	0.499	0.033	0.502	0.119
Enp	0.494	0.034	0.031	0.065
Est1	0.154	0.052	0.059	0.259
Idh1	0.353	0.064	0.084	0.012
Idh2	0.182	0.086	0.020	0.043
Lap1	0.029	0.027	0.019	0.018
Mpi	0.438	0.094	0.088	0.018
Pgm1	0.399	0.036	0.039	0.019
Ti	0.259	0.041	0.030	0.039
Mean†	0.239	0.072	0.075	0.055

† H_T = Average total gene diversity. † The means were calculated by averaging H_S and H_T across the 13 loci and then calculating new values for D_{ST} and G_{ST}. ¶ G_{ST} among seed-color classes was calculated based on the data for five classes except for saddle patterns which have obscure seed-coat color.

Table 4. Nei's genetic distances between regional populations of soybean accessions.

Region	KG†	CN	CB	KW	CLB	CLN	KB	KN	Mean
Kyonggi (KG)	-	0.016	0.022	0.009	0.010	0.017	0.016	0.008	0.014
Chungnam (CN)		-	0.039	0.021	0.014	0.010	0.016	0.013	0.018
Chungbuk (CB)			-	0.019	0.027	0.033	0.023	0.028	0.027
Kangwon (KW)				-	0.017	0.022	0.019	0.009	0.016
Chonbuk (CLB)					-	0.010	0.023	0.013	0.016
Chonnam (CLN)						-	0.033	0.006	0.017
Kyongbuk (KB)							-	0.019	0.019
Kyongnam (KN)								-	0.014
Total									0.018

† KG = Kyonggi-do; CN = Chungchongnam-do; CB = Chungchongbuk-do; KW = Kangwon-do; CLB = Chollabuk-do; CLN = Chollanam-do; KB = Kyongsangbuk-do; KN = Kyongsangnam-do.

Chungchongnam-do populations, whereas the frequencies of these alleles, except for *Ti-b* in the Kyonggi-do population, were less than 10% in the northern groups. Of the three alleles, *Est1-a* and *Ti-b* are characteristic to the summer-maturing cultivars (short-season cropping type) in the Kyushu district of Japan (Hirata et al., 1996; Hymowitz & Kaizuma, 1981). Hirata et al. (1996) found that the summer-maturing cultivars in the Kyushu district were markedly different from the autumn-maturing cultivars (full-season cropping type) in having *Dia1-b, Enp-b, Est1-a* and *Ti-b* in high frequencies, whereas *Dia1-a, Enp-a, Est1-b* and *Ti-a* were predominant in the autumn group. For the diagnostic loci, *Dia1, Enp, Est1* and *Ti*, the summer cultivars had a combination of *Dia1-b, Enp-b, Est1-a*, and *Ti-b* in a high frequency (43%) (Hirata et al., 1996). This combination, however, was not observed in the Korean landraces tested in this study (data not presented). Instead, the Korean landraces had, with high frequencies, combinations of *Dia1-a, Enp-a, Est1-b*, and *Ti-a* (18%), *Dia1-a, Enp-b, Est1-b*, and *Ti-a* (23%), *Dia1-b, Enp-b, Est1-b*, and *Ti-a* (25%), and *Dia1-b, Enp-a, Est1-b*, and *Ti-a* (14%), the former two of which were predominant in the autumn maturing cultivars in Kyushu (Hirata et al., 1996). Hirata et al. (1996) assumed that the summer maturing cultivars in the Kyushu district had become established after the soybean had been disseminated into Japan. Our results support this hypothesis, in particular for the summer-maturing cultivars with the unique combination of *Dia1-b, Enp-b, Est1-a* and *Ti-b*.

The genetic diversity was the lowest in the Chungchongbuk-do population (Table 1). The genetic poverty in this population may be partly due to its small sample size. On the other hand, three southern regional group such as the Chollanam-do, Chollabuk-do and Kyongsangnam-do populations, had higher H values than any other regional group. High genetic diversity in these groups may be partly due to the presence of alleles characteristic of the Japanese soybean population as mentioned above. Presently, in the regions where the soybean has been intensely cultivated as one of the major food crops as well as farm income crops, the landraces are being replaced by improved cultivars with high productivity. Thus, we recommend that the southern region of South Korea be considered as a primary area for the collection and preservation for the Korean soybean landraces.

Differentiation between landraces with different seed characteristics

In Korea, soybean seeds are processed as daily meal into soysauce, bean curd, bean sprouts, soybean paste, and Kochu-jang (a kind of fermented soybeans), and are also used for cooking with rice and as baked powder for coating rice cakes and other candies (Kwon, 1972). For each of these foods, different cultivars with different seed characteristics are used. For bean sprouts, for example, small-seeded soybeans are preferred, while yellow and relatively

Table 5. Allelic frequencies at five polymorphic loci, by seed size classes, in the Korean soybean landraces.

Allele	Seed size class (g)†				
	VL (40)†	L (67)	M (79)	S (35)	VS (12)
Dia1-a	0.925	0.659	0.475	0.071	0.000
Enp -a	0.421	0.416	0.487	0.277	0.273
Idh1-a	0.816	0.877	0.793	0.682	0.515
Mpi-b	0.812	0.669	0.686	0.571	0.349
Pgm1-a	0.192	0.253	0.247	0.447	0.242

†Classified based on the weight of 100 seeds: VS (<10), S (11-20), M (21-30), L (31-40), VL (41<).
†The number of accessions classified into the class.

Table 6. Allelic frequencies at five polymorphic loci, by seed-coat color classes, in the Korean soybean landraces.

Allele	Seed-coat color class					
	Black (89)†	Yellow (61)	Green (42)	Pale green (16)	Brown (16)	Saddle pattern (9)
Dia1-a	0.640	0.270	0.454	0.750	0.656	0.000
Est1-a	0.065	0.033	0.070	0.459	0.000	0.000

†The number of accessions classified into the class.

large-seeded ones are mostly used for soysauce, and colored ones are mostly for cooking with rice.

Allelic frequencies were then compared between landraces with different seed sizes and seed-coat colors (Table 5 and Table 6). G_{ST} values among the five classes of seed size and the six classes of seed-coat color are also presented in Table 3. A high G_{ST} value (0.502) was observed at *Dia1* between the classes of seed size. There was no landrace that had *Dia1-a* in the small-seed class of less than 10 g per one hundred seeds, whereas the frequency of the allele increased with increasing of seed size class, and reached 0.925 in the large-seed class of more than 40 g per one hundred seeds. A relatively high G_{ST} value was also observed in *Est1* and *Dia1* for the classes of seed-coat color. The *Est1-a* allele was found in a high frequency of 0.459 in the landraces with a pale-green seed coat, and *Dia1-a* in a low frequency of 0.270 in the landraces with that of yellow.

A similar association between *Dia1* and seed size of soybean was also found in the Japanese population (Hirata et al., 1999), and the Chinese population (unpublished data). Accordingly, the association appears to be not specific to South Korea, but rather it is a widely distributed phenomenon in cultivated soybeans of East Asia. Two possible factors may account for the observed association: 1) linkage of *Dia1* with the genes controlling the seed size and 2) coexistence of the cultivars with different seed sizes that had originated independently in different regions. Abe et al. (1992b) analyzed the genetic basis of the association between allozyme markers and seed size observed in the landraces native to Hokkaido (northern Japan), and found that the association of *Dia1* with seed size was broken down in a F_2 population of the cross between landraces with different seed sizes. The observed association may therefore be not ascribed to the tight linkage of the marker with the gene for seed size. The landraces with different seed sizes in South Korea may rather have been established independently in different regions, although the possible role of the linkage should be determined in more detail. Not only the region but also the usage as food materials should be taken into consideration in designing an efficient collection and preservation method for the Korean soybean landraces.

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