Korean J. Crop Sci. 44(3) : 243~247 (1999)

RFLPs of Mitochondrial DNA in Korean Wild Soybeans

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ABSTRACT

Mitochondrial DNA restriction fragment length polymorphisms are convenient markers for identifying cytoplasmic variation among plants. We have collected 212 wild soybeans (*Glycine soja* Sieb. et Zucc) from all over Korea, and classified mitochondrial genome types based on hybridization patterns in DNA gel-blot analyses using two mitochondrial DNA clones, *cox2* and *atp6*, as probes. Korean wild soybean was classified with eight-mtDNA types, and some of the mtDNAs showed geographical clines among the regions. The diversity index of the mtDNA was much higher in the western and southern regions than in the eastern and northern regions of Korea, respectively. Dissemination and distributive characteristics of wild soybeans in Korea were discussed.

Keywords : Korean wild soybean, mitochondrial genome, geographical differentiation.

Wild soybeans, *Glycine soja* Sieb. et Zucc, are thought to be a direct progenitor of the domesticated soybean, *G. max* (L.) Merr, and have much more genetic variation than cultivated soybeans (Hymowitz & Newell, 1981). *G. soja* and *G. max* constitute the subgenus *Soja*. These two species can be crossed to produce easily fertile offspring, and together may form a common gene pool (Newell & Hymowitz, 1982). Some wild soybeans have been used as a genetic resource for ciltivar improvement.

Discovering sources of diversity is an important component in plant breeding studies, especially for a crop like soybean that has such a narrow germplasm base. Several molecular approaches have been utilized to search for diversity among soybean species, including RFLPs within the nuclear DNA (Keim et al., 1989; Skorupska et al., 1993), within the chloroplast DNA (cpDNA; Close et al., 1989; Kanazawa et al., 1998), and within the mitochondrial DNA (mtDNA; Moeykens et al., 1995; Tozuka et al., 1998). They reported that the domesticated soybean and the wild soybean could be recognized polymorphically by their chloroplast and mitochondrial genomes. Close et al. (1989) defined six soybean cytoplasmic groups within the subgenus *Soja*. Grabau & Davis (1992) roughly classified the mtDNAs of wild soybeans by using the 2.3-kb *Hind*III mtDNA fragment as probe. Grabau et al. (1992) also classified the mtDNAs of 138 cultivated soybeans into four groups by using cloned *Hind*III mtDNA fragment from a cultivar William 82. Palmer (1990), and Shimamoto et al. (1998) showed that mtDNAs had many variations with or between closely related species, and Tozuka et al. (1998) reported that the distribution of mtDNAs had geographic clines among the regions of Japan.

The aim of the present study is to characterize specifically the mtDNA of Korean wild soybeans by using RFLPs. By partitioning the collection sites into five regions, we are to reveal the geographic distribution of the various types with respect to mtDNA, and to consider the differentiation among the regions in the mtDNAs of Korean wild soybeans.

MATERIALS AND METHODS

Plant materials

We used 212 wild soybean plants for RFLP analyses, collected from 102 natural habitats in Korea. The collection sites were classified into five regional groups: North-West (Kyonggi-do and Chungchong-do), South-West (Cholla-do), North-East (Kangwon-do), South-East (Kyongsang-do) and South (Cheju-do).

Sourthern-blot analysis

Total DNA for RFLP analyses was isolated according to the methods of Doyle & Doyle (1987) from juvenile leaves of 5 to 8 week old plants based on one plant. The DNAs were digested with restriction endonucleases, *Bam*HI and *Hind*III, and fractionated by electrophoresis in a 0.8% agarose gel. The DNAs were transferred to nylon membranes (Hybond N$^+$; Amersham) and allowed to hybridize with probes labeling by ECL and detection system (Amersham). Hybridization was carried out according to the supplier's instructions. Membranes were washed twice with 6 M urea, 0.4 % SDS and 0.5 × SSC for 20 minutes at 42℃, and twice with 2 × SSC for 5 minutes at room temperature. The membranes were used to expose X-ray films for 1 to 4 hours. Two clones

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Received 15 June., 1999.

of mtDNA fragments were used as probes; a 0.7-kb *Nco*-*Hind*III fragment that contained *cox*2 (a gene encoding subunit 2 of cytochrome oxidase) from soybean (Tozuka et al., 1998) and a 0.7-kb *Sty* I fragment that contained *atp*6 (a gene encoding mitochondrial ATPase subunit 6) from *Oenothera* (Schuster & Brennicke, 1987). These two gene probes have been previously shown to detect polymorphic fragments, even when DNAs were digested with different enzymes (Grabau et al., 1989, 1992; Hirata et al., 1996; Tozuka et al., 1998).

Data analysis

Mitochondrial genomes were classified as combinations of types 'I' to 'V', and types 'a' to 'i', based on hybridization patterns detected by *cox*2 and *atp*6 probes, and restriction endonucleases (*Hind*III or *Bam*HI), respectively (Tozuka et al., 1998). The H statistics for evaluating the diversity was calculated by $1-\Sigma pi^2$, where *pi* is proportion of *i* th genome type (Shimamoto et al., 1998).

RESULTS

*Hind*III or *Bam*HI digests of the DNAs of Korean wild soybeans were probed with the mitochondrial clone containing *cox*2 exon. Three types of hybridization patterns, 1.6, 3.5 and 5.5 kb, were detected in *Hind*III digest (Table 1, for photograph on the mtDNA types, see Tozuka et al., 1998), and in the *Bam*HI digest, two polymorphic patterns, having 5.8 and 8.1 kb, were detected (Table 2).

Four hybridization patterns were detected when DNAs were digested with the *Bam*HI and hybridized with the mitochondrial clone probe containing the partial *atp*6 exon (Table 3). Most (97%) of the plants examined had a profile with above two fragments, suggesting that two copies of *atp*6 or an *atp*6 homolog existed in the mitochondrial genome of the Korean wild soybean. Grabau et al. (1988) actually detected two copies of *atp*6 in mtDNA isolated from a soybean cultivar William 82.

The geographic distribution of the mtDNAs and the extent of diversity were shown in Table 1, 2, and 3. With regard to the fragments hybridized with *cox*2 as a probe, the 3.5-kb *Hind*III fragment was found in more than 75% of the plants examined and observed predominantly in every region. Plants with the 5.8-kb *Hind*III fragment were frequently observed in the northwestern (20.4%) and southeastern (26.3%) regions, but were not observed in the southern region. Wild soybeans with the 1.6-kb *Hind*III fragment showed high frequency in the southern region (35.7%) compared with the other regions. These two mtDNA types, 5.8-kb and 1.6-kb *Hind*III fragments, had geographical clines in the opposite direction (Table 1). In *Bam*HI digest, the 8.1-kb *Bam*HI fragment was

Table 1. Frequency distribution of RFLPs probed by mitochondria *cox*2 in the *Hind*III-digested DNAs of Korean wild soybean.

Region	Number of plants	Fragment hybridized (kb)			H value
		1.6 (I)†	3.5 (IV)	5.8 (V)	
North-West	49	-	39	10	0.325
South-West	42	6	34	2	0.321
North-East	36	2	31	3	0.249
South-East	57	1	41	15	0.414
South	28	10	18	-	0.459
Total	212	19	163	30	0.381

†Mitochondrial genome types were designed according to the methods of Tozuka et al. (1998).

Table 2. Frequency distribution of RFLPs probed by mitochondria *cox*2 in the *Bam*HI-digested DNAs of Korean wild soybean.

Region	Number of plants	Fragment hybridized (kb)		H value
		5.8 (I)	8.1 (IV, V)	
North-West	49	-	49	0.000
South-West	42	6	35	0.245
North-East	36	2	34	0.106
South-East	57	1	56	0.035
South	28	10	19	0.459
Total	212	19	193	0.164

found in more than 90% of plants examined and predominant in every region. The 5.8-kb *Bam*HI fragment was scarce and mainly distributed in the southern region (Table 2). With regard to the fragments hybridized with *atp*6, the 2.4- and 5.0-kb *Bam*HI fragments were predominantly observed in about 60% of the plants tested, and in particular, was frequently distributed in the northeastern region (88.9%), while it showed low frequency in the northwestern region (34.7%). Plants with the 2.9- and 5.0-kb fragments were found in every region collected, and were presented in the highest in the northwestern region (65.3%). Plants with 5.0-, and 5.2- and 12.0-kb *Bam*HI fragments were found only in the southern and northeastern regions with extremely low frequency (Table 3).

We employed three combinations of mtDNA probes and restriction endonucleases : *cox*2 and *Bam*HI; *cox*2 and *Hind*III; *atp*6 and *Bam*HI, and classified the mtDNAs of Korean wild soybeans. In our classification, the mtDNAs were designated as types 'I' to 'V', and types 'a' to 'i', based on the combined profiles of the *cox*2 and *atp*6 probes, respectively (Tozuka et al., 1998). Table 4 presented the frequencies of eight-mtDNA types classified by the three combinations of mtDNA probes and restriction endonucleases, namely, Ⅰc, Ⅳa, Ⅳb, Ⅳc, Ⅳi, Va, Vb, and Vc. Two mtDNA groups, types Ⅳa and Ⅳb, were 77% in Korean wild soybeans, and type Ⅳa contained members from all geographical regions. The geographical clines of the eight-mtDNA types were shown also in Table 4. Most of the observed types showed a marked difference in frequency among different geographical regions. Wild soybeans belonging to type Ⅰc were predominant in the southern region, while it was not observed in the northwestern region. Type Ⅳa was found in every region, but it was rare in both the northwestern and southern regions compared with the others. Type Ⅳb was frequently observed in the northwestern region, while it not observed in the northeastern region. Mitochondrial partial genome types of Ⅳc, Ⅳi, and Vc were found only in wild soybeans originating from the southern, northeastern, and southeastern regions, respectively. Type Vb, which is distributed mainly in Korea (Kanazawa et al., 1998), was frequently found in the northwestern and southeastern regions, while it not observed in the southern region.

We evaluated the diversity of mtDNAs in each region of Korea (Table 4). The diversity index (H) showed that the southern region holds the greatest diversity (0.712) of the mtDNAs. It was one unique type, Ⅳc, and retained a moderate diversity. Although there were five different mtDNA types, the northeastern region showed the lowest diversity (0.378) in every region.

Table 3. Frequency distribution of RFLPs probed by mitochondria *atp*6 in the *Bam*HI-digested DNAs of Korean wild soybean.

Region	Number of plants	Fragment hybridized (kb)				H value
		2.4, 5.0 (a)	2.9, 5.0 (b)	5.0 (c)	5.2, 12.0 (i)	
North-West	49	17	32	-	-	0.453
South-West	42	28	14	-	-	0.444
North-East	36	32	1	-	3	0.206
South-East	57	37	20	1	-	0.468
South	28	19	3	6	-	0.482
Total	212	132	70	7	3	0.503

Table 4. Frequency in mitochondrial genome types of wild soybeans in the various regions of Korea.

Region	Number of plants	Fragment hybridized (kb)								H value
		Ⅰc†	Ⅳa	Ⅳb	Ⅳc	Ⅳi	Va	Vb	Vc	
North-West	49	-	0.33	0.47	-	-	0.02	0.18	-	0.637
South-West	42	0.14	0.51	0.31	-	-	0.02	0.02	-	0.623
North-East	36	0.06	0.78	-	-	0.08	0.06	0.02	-	0.378
South-East	57	0.02	0.58	0.14	-	-	0.05	0.21	0.02	0.597
South	28	0.36	0.32	0.11	0.21	-	-	-	-	0.712
Overall	212	0.09	0.50	0.22	0.04	0.02	0.03	0.10	0.01	0.680

†Mitochodrial genome types were classified based upon a combination of mtDNA probes and restriction endonucleases (Tozuka et al., 1998).

DISCUSSION

Mitochondria (mt) and chloroplast (cp) in many high plants are cytoplasmically inherited. Restriction endonucleases digestion has compared mtDNAs of a large number of plant species. The comparisons have proved particularly useful to determine the diversification in cytoplasmic genome among different regions and different populations.

In this study, the observed number of RFLPs varied with both the mtDNA clones and the enzymes used. We have identified eight-mtDNA types of wild soybeans grown in Korea when DNAs were probed with *cox*2 and *atp*6. The number of mtDNA types was slightly lower in Korean wild soybeans than in either Japanese or Chinese ones. In a similar manner, Japanese (1,097 collections from 349 sites) and Chinese (753 collections from 215 sites) wild soybeans were classified within eighteen and fourteen cytoplasmic groups, respectively (Shimamoto et al., 1998; Tozuka et al., 1998). However, our result was not sufficient to evaluate the degree of mtDNA type of the Korean wild soybean because we utilized only 212 samples collected from 102 sites for the classification of samples. The cytoplasmic richness in a germplasm collection is usually influenced by the samples of a particular size, especially for accessions from collection sites that have many wild relatives. It is possible that additional samples would reveal the added degree of mtDNA type. In previous studies, Abe et al. (1992), and Yu & Kiang (1993) reported that wild soybean accessions from Korea has more rare alleles and higher variation for isozymes than those from China, Japan and Taiwan, indicating the possibility that the Korean wild soybean has higher cytoplasmic variation than wild soybeans in other countries.

Two mtDNA types, IVa and IVb, which are widely distributed in Korea, Japan, and China, were found in more than 70% of the plants examined, and were similar with Chinese wild soybeans (77.5%) in having its frequency. Geologically the Korean peninsula is attached to northeastern China, proposed as the primary gene center of soybeans (Hymowitz & Kaizuma, 1981). Enormous variability can be packed into a small geographical area outside of the center of origin (Harlan, 1971). We found that the index of the diversity of mtDNA was higher in the western region (0.630) as well as in the southern region (0.644) closest to China than in the eastern region (0.487), supporting a possibility that the great number of Korean wild soybeans originated from China. On the other hand, Type I c was widely distributed in Korea and Japan but it was not found in China (Shimamoto et al., 1998; Tozuka et al., 1998). Recently, Tozuka et al. (1998) showed that Type I c, which was higher frequency in the southern region than in the different regions of Korea, was predominantly found in 47% of Japanese wild soybeans, and at extremely high frequencies (about 70%) in southern Japan, especially in the Kyushu district (73%), that lie closest to the southern regions of Korea. The region has a simple genetic diversity. Therefore, this result suggests that wild soybeans have been partly disseminated from the southern region of Korea into the southern region of Japan through gene flow.

Considering previous studies and the present results, we assume that Korean wild soybeans partly formed their own gene pool which have been accumulated independently and gradually, although it may possibly consist of a mixture of the plants from the gene center.

Some Korean wild soybeans possessed mtDNA types that were identified with those wildely detected in cultivated soybeans. Thus, a predominant mtDNA type in cultivated soybeans, namely, IVb (Hirata et al., 1996), was observed in the wild soybeans of all regions except the northeastern region. The wild soybean has become better adapted to the Korean natural habitats for a long period of time than to other habitats, and commonly grows adjacent to domesticated soybean fields, which then gene flow often arises by hybridization between wild and closely related domesticated species (Broich & Palmer, 1981). This observation indicates a possibility of gene flow between a cultivated species and its wild progenitors, and this gene flow plays an important role in the evolution of crops.

Our studies currently in progress on the diversity of isozymes and cytoplasmic DNA of cultivated soybeans will provide useful information to solve clues about the process of domestication of soybeans in Korea. The results will be presented in further papers.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Y. Shimamoto (Hokkaido University, Japan) and Dr. Chang-Hwan Cho (Dankook University, Korea) for supports.

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