Korean J. Crop Sci. 45(2): 139~142(2000)

Genotypic and Geographical Variations of β-amylase Isozyme in Soybean Land Races by Isoelectric Focusing (IEF)

Mun Sup Yoon*†, Jong Woong Ahn, Jung Hoon Kang*, Hyung Jin Baek*, Nam Kyu Park* and Young Deok Rho*****

**National Seed Management Office, RDA, Suwon 441-707, Korea*
***International Technical Cooperation Center, RDA, Suwon 441-707, Korea*
****Dept. of Agronomy, Kyung Hee Univ., Suwon 449-701, Korea*

ABSTRACT : The experiment was carried out to study the variations and geographical distribution of β-amylase isozyme by isoelectric focusing (IEF) within Korean, Chinese and Japanese soybean land races. In pH 3~10 gel of IEF, the amylase of soybean accessions was separated into low pI group isozymes (Sp_1^b) and high pI group isozymes (Sp_1^a). In pH4~6.5 gel, isoelectric points were at 5.07, 5.15, 5.25, 5.40, and 5.94, and h, j, and k bands also were found. The distribution of Sp_1^a allele (high pI type) was 29.3% in soybean accessions from Korea, 10.1% in those from China, and 6.9% in Japanese accessions. The percentage of Sp_1^a allele was the highest in soybean accessions from Kyungsang province (35%) in Korea, then central China (32%) in China, and Honshu (10%) in Japan.

Keywords : soybean (*Glycine max* L.), land races, isoelectric focusing, β-amylase isozymes.

The soybean, *Glycine max* (L.) Merrill, is the most important grain legume in the world in terms of total production and international trade (Golbitz, 1995). Soybean was cultivated in the eastern half of north China as early as the 11th century B.C. Domestication probably occurred even earlier in the same region. The eastern half of north China and Manchuria be considered primary and secondary gene centers, respectively (Hymowitz, 1970). Soybean grows only under cultivation, while *G. soja* grows wild in China, Japan, Korea, Russia, and Taiwan. *Glycine max* and *G. soja* form the primary gene pool for the cultivated soybean (Harlan & de Wet, 1971).

For centuries, soybean has been an indispensable component in East Asian nutrition and cuisine. In this region, many different types of foods were developed from the soybean. Five important soybean-based foods are soy paste, soy sauce, soy curd, soymilk, and tempeh. In addition, in Asia, cooked and salted immature green beans and sprouted soybeans are consumed in great quantities (Hymowitz & Newell, 1981).

Isozyme and seed protein were used as genetic markers to study the distinction of variety, genetic linkage, plant breeding, tissue culture, and evolution (Kiang & Gorman, 1983). The dissemination path of the crop from its origin was studied using historical records, linguistic comparison, and archaeological evidence, as well as, certain morphological traits such as, flower color, plant height, and seed size. However, the electrophoresis patterns of protein soybean, soybean trypsin inhibitor and β-amylase, have been used as genetic markers for evolutionary study. Hymowitz & Kaizuma (1981) defined three gene centers containing seven germplasm pools and proposed pathways of dissemination of cultivated soybean from northeast China based upon a combination of electrophoretic data, and available historical, agronomic and biogeographical literature. Park & Kwon (1993) reported the frequency of the Amy3*1 of cultivar and wild types of soybean plant in Korea.

This study aimed to investigate the variations and geographical distribution of β-amylase among Korean, Chinese and Japanese soybean by IEF.

MATERIALS AND METHODS

Materials

The materials used in this study were from the genetic stocks of the Genetic Resources Division, National Seed Management Office, Rural Development Administration. Korean accessions were collected in 1985, and Chinese and Japanese accessions were introduced from USDA. A total of 1,152 soybean accessions containing 771 Korean accessions, 237 Chinese accessions, and 144 Japanese accessions were used for β-amylase isozyme analysis by IEF. In the regional distribution of accessions, Korean accessions was comprised by 78 Kyunggi, 47 Kangwon, 43 Chungchong, 249 Chulla, and 354 Kyungsang. Chinese accessions contained 195 northeast China, 28 central China, 5 south China, and 9 others. Japanese accessions was composed of 72 Hokkaido,

†Corresponding author: (Phone) +82-331-299-2793 (E-mail) msyoon@seed.go.kr <Received April 22, 2000>

60 Honshu, 2 Kyushu, and 10 others.

Extraction

The 100 mg of dry soybean seed milled finely was homogenized with 4% polyvinyl pyrrolidone solution. The homogenates were centrifuged at 12,000 rpm for 30 min. at 4°C, and the supernatant were subjected to polyacrylamide gel electrophoresis.

Electrophoresis

Electrophoresis procedure followed the methods described by Pharmacia LKB Biotechnology (1992) with some modifications, which was Multiphor electrophoresis system with polyacrylamide gel IEF. Electrophoresis gel used 5% ampholine PGA plates (245×110×1 mm) of pH 3~10 and pH 4~6.5, which were purchased from Pharmacia Biotech Co. After prefocusing for 30 min. at 100 volts, gel was electrophoresized for 3 hrs at 1.5 watts, 4°C with NaOH for cathode buffer, and acetic acid for anode buffer.

Staining

Gel was incubated at 37°C in 1% soluble starch containing 0.2% $CaCl_2$ for 40 min. After incubation, the gel was immersed in 5% acetic acid for 3 min., and developed with KI-iodine solution.

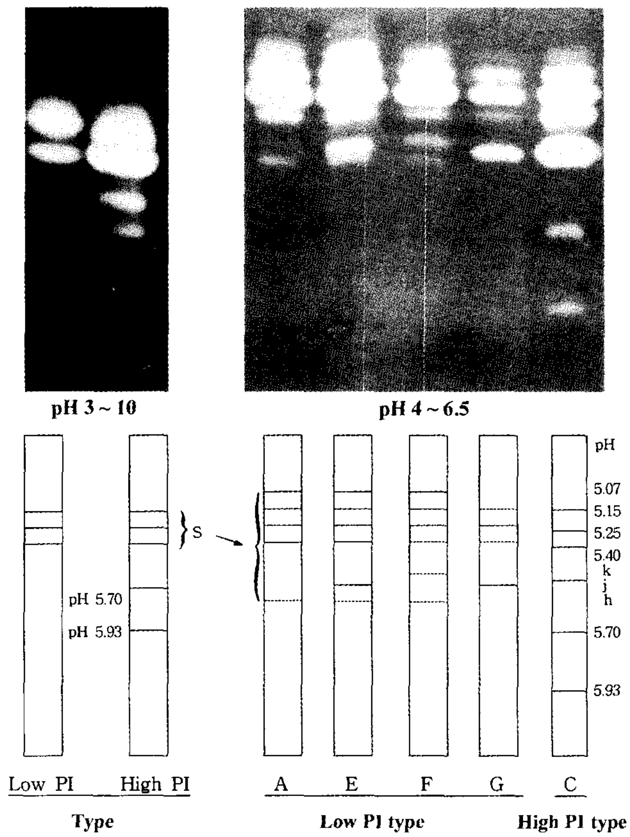

Fig. 1. Amylase isozyme phenotypic groups and types identified by isoelectric focusing in pH 3~10 and pH 4~6.5 gel in Korean, Chinese, and Japanese soybean germplasm.

RESULTS AND DISCUSSION

Genotypic variation of β-amylase isozyme

β-amylase in soybean seeds can be separated into two (Sp_1^a and Sp_1^b) variant bands, which are controlled by two codominant alleles (Hildebrand & Hymowitz, 1980). By gel IEF, Sp_1^a variant band consisted of high pI type isozymes with low mobility, and the Sp_1^b band was composed of low pI type isozymes with high mobility (Nakamura & Futsuhara, 1985). Mikami & Morita (1982) detected seven isozymes (pI 5.07, 5.15, 5.25, 5.40, 5.55, 5.70, and 5.93) of the soybean enzyme using IEF.

Fig. 1 shows the results of electrophoresis in pH 3~10 gel. The low pI type isozymes, and high pI type isozymes having pH 5.70 and 5.93 were detected. However, it was difficult to distinguish "S" part of high pI type. As separated in pH4~6.5 (Fig. 1), however, electrophoresis pattern corresponded with the result of Nakamura & Futsuhara (1985), that low pI type had pH 5.07, 5.15, 5.25, and 5.40, and high pI type had pH5.15, 5.25, 5.40, 5.70, and 5.93. Also, this study discovered new bands such as, h, j, and k bands. The three bands found in low pI type isozymes (Sp_1^b) were characterized with four types such as, A type containing only h band, E type containing both h and j bands, F type containing both h and k bands, and G type containing only j band. High pI type isozymes (Sp_1^a) had only j band.

Geographical distribution of β-amylase isozyme variation

Table 1 gives the distribution of isozyme variations. High pI type (Sp_1^a) was revealed in 29.3% of Korean accessions, 10.1% of Chineses accessions, and 6.9% of Japanese accessions. Hymowitz & Kaizuma (1981) reported that Sp_1^a of accessions from northeast China and U.S.S.R. appeared 10%, respectively. It also appeared in accessions from central and south China (8.5%), from Korea (19.9%), and from Japan (5.5%). Sp_1^a proportion of this study, however, showed higher frequency than that of study by Hymowitz & Kaizuma (1981) and Zhao *et al.* (1991). The shift of the allelic proportion may happen according to genotype of their holdings. The accessions from Korea of Sp_1^a revealed the highest frequency over all regions.

The regional distribution of high pI type isozymes (Sp_1^a)

Table 1. Distribution of β-amylase isozyme variations of Korean, Chinese, and Japanese soybean germplasm by isoelectric focusing in pH 4~6.5 gel.

Country (n)/Locality (n)	Low pI type (Sp_1^b)	High pI type (Sp_1^a)
	--------------------%--------------------	
Korea (771)	70.7	29.3
Kyunggi (78)	84.6	15.4
Kangwon (47)	76.5	23.4
Chungchong (43)	69.8	30.2
Chulla (249)	73.4	26.5
Kyungsang (354)	64.9	35.0
China (237)	89.9	10.1
Northeast China (195)	93.8	6.2
Central China (28)	67.9	32.1
South China (5)	80.0	20.0
Others (9)	77.8	22.2
Japan (144)	93.1	6.9
Hokkaido (72)	95.9	1.4
Honshu (60)	90.0	10.0
Kyushu (2)	100.0	0.0
Others (10)	90.0	10.0

Table 2. Distribution of low pI types of amylase isozyme of Korean, Chinese, and Japanese soybean germplasm by isoelectric focusing in gel of pH 4~6.5.

Country (n)/ Locality (n)	Low pI type			
	A	E	F	G
	--------------------%--------------------			
Korea (545)	85.0	13.6	2.7	1.3
Kyunggi (66)	100.0	0.0	0.0	0.0
Kangwon (36)	86.1	13.9	0.0	0.0
Chungchong (30)	90.0	6.7	0.0	3.3
Chulla (183)	78.2	16.3	4.9	0.5
Kyungsang (230)	85.4	12.2	1.2	2.2
China (213)	86.7	10.9	0.5	1.9
Northeast China (183)	89.6	8.2	0.6	1.7
Central China (19)	63.2	31.5	0.0	5.3
South China (4)	100.0	0.0	0.0	0.0
Others (7)	71.5	28.5	0.0	0.0
Japan (134)	81.3	16.4	0.7	1.5
Hokkaido (69)	82.6	16.0	0.0	1.4
Honshu (54)	77.8	18.5	1.9	1.9
Kyushu (2)	100.0	0.0	0.0	0.0
Others (9)	88.9	11.1	0.0	0.0

of Korean accessions appeared in highest frequency in the accessions from Kyungsang (35.0%), followed by Chungchong (30.2%), Chulla (26.5%), Kangwon (23.4%), and Kyunggi (15.4%). These results were similar pattern to the report by Hymowitz & Kaizuma (1981) except accessions from Kangwon. High pI type isozymes (Sp_1^a) of Chinese accessions appeared highest frequency in the accessions from central China (32.1%) located near the Korean peninsula. Second high frequency of Sp_1^a were south China (20%). Also, frequency of high pI type isozymes in Japanese accessions was 10% of accessions from Honshu and 1.4% from Hokkaido. These results showed much more proportion than the previous result (Hymowitz & Kaizuma, 1981) for Chinese accessions and a little higher proportion for Japanese accessions. Vavilov (1951) first determined that the primary center of diversity for soybean was northeast, central and south China, and Korea. The Japanese soybeans were probably introduced from either Korea or central China (Hymowitz & Kaizuma, 1981).

On the other hand, Hymowitz & Kaizuma (1981) suggested soybeans of southern Korea that lie closest to Kyushu Island, Japan have extremely high frequencies for Ti^b and Sp_1^a, and most probably the buildup of the Ti^b and Sp_1^a alleles in the Korean soybean population is due to the modern introduction of Japanese cultivars having the $Ti^bTi^bSp_1^aSp_1^a$ genotype. In this study, it is difficult to compare the relationship between accessions from southern part of Korean and those from Kyushu because the number of accessions from Kyushu was only 2. However, the southern ones Sp_1^a over all regions has higher proportion than those of the northern. It is possible to be happened with a geographically different ecotype. Zhao *et al.* (1991) reported that the frequency of Ti^a and Sp_1^b was higher as the cultivated history increased, and may have some correlation with some economic, resistance, and adaptation characters.

Table 2 given the distribution by country and region for four variants of the low pI type isozymes, Sp_1^b. A type by country was 86.7% in the accessions from China. E type accessions was 16.4% in Japan, and G type was 1.9% in Korea. The regional variation was most diverse in Chulla and Kyungsang of Korean accessions, central China of Chinese accessions, and Honshu of Japanese accessions. A relatively similar proportion of polymorphic collections were observed in accessions from Korea, China, and Japan.

REFERENCES

Golbitz, P. (ed.). 1995. '95-'96 Soya Bluebook Plus. Soyatech, Inc., Bar Harbor, ME.

Harlan, J. R. and J. M. de Wet. 1971. Toward a rational classification of cultivated plants. *Taxon* 20:509-517.

Hildebrand, D. F. and T. Hymowitz. 1980. The *Sp1* locus in soybean codes for β-amylase. *Crop Sci.* 20:165-168.

Hymowitz, T. 1970. On the domestication of the soybean. *Econ. Bot.* 24:408-421.

Hymowitz, T. and N. Kaizuma. 1981. Soybean seed protein electrophoresis profiles from 15 Asian countries or regions: Hypotheses on paths of dissemination of soybeans China. *Econ. Bot.* 35:10-23.

Hymowitz, T. and C. A. Newell. 1975. A wild relative of the soybean. *Illinois Res.* 17(4):18-19.

Kiang, Y. T. and M. B. Gorman. 1983. Soybean. In S.D. Tanksley and T.J. Orton (ed.) Isozymes in plant genetics and breeding, Part B. Elsevier Science Publishing Co., New York. p. 295-328.

Mikami, B. S. A. and Y. Morita. 1982. Distribution and properties of soybean β-amylase isozyme. *Agric. Biol. Chem.* 46(4):943-953.

Nakamura, I. and Y. Futsuhara. 1985. Relationship among three electrophoretic characteristics of β-amylase in soybean and wild soybean seeds. *Japan. J. Breed.* 35:153-159.

Park, K. S. and S. H. Kwon. 1993. Variation of Beta-amylase in Korean land races and wild soybeans. *Korean J. Breed.* 25(2): 151-155.

Pharmacia LKB Biotechnology. 1992. Multiphor II electrophoresis system. Polacco, J. C., A. L.

Vavilov, N. I. 1951. The origin, variation, immunity and breeding of cultivated plants.(Translated by K.S. Chester.). *Chron. Bot.* 13:1-364.

Zhao, S., S. Zhou, M. Hu, X. Meng, A. Li, and S. Wang. 1991. Genotypes of SBTi-A_2 and Sp1 of cultivated soybean in Northeast China. Soybean Genet. *Newsletter* 18:111-112.