韓作誌(Korean J. Crop Sci.) 35(2) : 171~175(1990)

Trypsin Inhibitor Variants in Korean Land Races and Wild Soybeans

Shin Han Kwon*, Mi Ryung Chae, Kyung Sook Park** and Hi Sup Song*****

한국 재래종 및 야생종 대두의 Trypsion Inhibitor 변이

권신한* · 채미령** · 박경숙** · 송희섭***

ABSTRACT : One thousand and seven hundreds and six soybean land reces and 103 wild soybeans of Korea, and 167 cultivars and 71 wild soybeans of exotic were analyzed using polyacrylamide gel electrophoresis to study the distribution of trypsin inhibitor phenotypes. The four ti/ti lines were observed only in Korean native land races. The Ti*a allele of cultivar was found to be the highest in Chinese soybean (0.9888) than in Korean soybean(0.8347) and Japanese soybean(0.5954).

The heterozygosity of typsin inhibitor in Korean lines occured in relatively high ratio. The percentages of heterozygosity of the Korean land race and wild soybean are 3.6% (N=61) and 9.7%(N=10), respectively.

The trypsin inhibitor proteins(Ti) have been proposed as one of the major antinutritional factors present in raw mature soybean, *Glycine max*(L.) Merrill. These proteins, which may make up to 6% of the total protein in soybean seed, perhaps account for 30% to 50% of the growth inhibition when raw soybeans are fed to monogastric animals.

Rackis et al.[15] reported that the molecular weight of soybean trypsin inhibitor is 21,100 daltons. It is four alleles that the three alleles (Ti-a, Ti-b and Ti-c) are located in a single locus and codominant to the null allele ti.[5,10,13,14] Hymowitz and Hadley[4] assigned symbol Ti^1(Ti-a) to the gene controlling the most commonly occurring electrophoretic form of R_f 0.79/10% (R_f=mobility relative to the dye front in a 10% polyacrylamide gel system), Ti^2(Ti-b) to the allele controlling the electrophoretic form found at R_f 0.75/10% and Ti^3(Ti-c) to the allele controlling the electrophoretic form found at R_f 0.83/10%.

Linkage test by Orf and Hymowitz[9,10,11,13] revealed that neither the Ti and Amy3(Sp1) loci nor ti and Le loci are linked. Hildebrand et al.[3] found that the acid phosphatase locus is linked to the Kunitz trypsin inhibitor locus with a crossover frequency of 16.2%. Kiang and Chiang[8] reported that the leucine aminopeptidase locus is found to be linked to the Kunitz trypsin inhibitor locus with a recombination frequency of 15.3±0.9%.

Because the trypsin inhibitor may be one of the reasons for the poor nutritional value of raw soybean meal, the objet of this research was to detect the absence of trypsin inhibitor lines in the land races and wild soybeans of Korea. And the gene frequencies of trypsin inhibitor of Korean land races and wild soybean are examined to compare with those from China and Japan.

METERIALS AND METHODS

The cultivated(*Glycine max*) and wild soybeans (*Glycine soja*) used in this study were obtained

*Department of Agronomy, Kyung Hee University, Suwon 449-701, Korea
**Department of Biology, Sung-Shin Women's University, Seoul 136-742, Korea
***Korea Atomic Energy Res. Inst., Seoul 302-353, Korea

This paper was supported by nondirected research fund, Korea Research Foundation, 1989.
<Received Feb. 10, 1990>

from Korea, Japan, China, Taiwan, Vietnam, USSR and USA : 1,706 land races and 103 wild soybeans of Korea, and 167 cultivars and 71 wild soybeans of other countries. Korean land races and wild soybean seeds used in this study were provided by Department of Agronomy, Kyung-Hee University, Korea and USDA Regional Soybean Laboratory, University of Illinois, USA, respectively.

The procedure for extracting soybean trypsin inhibitor and determining the electrophoretic banding patterns was as follows; 100 mg of soybean is filled with 1 ml deionizing water during a day. The soybean seed was homogenized and centrifuged at 3,000 rpm for 10 min. Electrophoresis was performed in 7%, 1 mm thick polyacrylamide gel. The electrophoresis was carried out 1 mA/cm for 2 hours at 4℃ with Tris -HCl(pH 8.8) buffer for gel and Boric acid-HaOH buffer for electrode. After electrophoresis, the gel plate was stained with Coomassie Brilliant Blue R 250.

RESULTS AND DISCUSSION

Soybean trypsin inhibitor phenotypes and the allele frequencies of cultivars and wilds are presented in table 1. The distribution Ti a/a is the common phenotype in both the *Glycine max* and *Glycine soja*. The frequency of the Ti*a allele is found to be higher in wild soybean (0.9223) than in Korean land races(0.8347). The Ti c/c homozygote is observed only in the Korean land races, but the freqnency of Ti*c was 0.0173 in the land races and 0.0194 in wild soybeans. The reason for the above observation is that Ti*c allele revealed as heterozygote. Four ti/ti types are observed only in Korean native soybean collections, and the frequency of ti* is 0.0023. The lines lack trypsin inhibitor, which might posses little or none of the nutritionally deleterious protein.

Soybean is a highly self-pollinated crop. however, heterozygosity of trypsin inhibitor appeared in relatively high ratio. The percentages of heterozygosity in Korean land races and wild soybeans are 3.6%(N=61) and 9.7%(N=10), respectively. This result indicates that the natural outcrossing has happened more frequently in the wild soybean than the in the cultivars.

Table 2 presents the distribution of trypsin inhibitor phenotypes in *Glycine max* collected from several different countries. Four ti/ti lines are observed only in the Korean collections. The Ti

Table 1. Soybean trypsin inhibitor phenotypes and gene frequencies of Korean native soybean cultivar and wild.

Cultivar	N	Ti a/a	Ti b/b	Ti c/c	Ti a/b	Ti b/c	Ti c/a	ti	Gene frequencies
Land races	1,706	1399 (82.0%)	226 (13.2%)	16 (1.0%)	34 (2.0%)	11 (0.6%)	16 (1.0%)	4 (0.2%)	Ti*a= 0.8347±0.00004 Ti*b= 0.1457±0.00004 Ti*c= 0.0173±0.00004 ti* = 0.0023±0.00004
Wilds	103	90 (87.4%)	3 (2.9%)	0	6 (5.8%)	0	4 (3.9%)	0	Ti*a= 0.9223±0.00225 Ti*b= 0.0583±0.00225 Ti*c= 0.0194±0.00225 ti* = 0.0000±0.00225

Table 2. Soybean trypsin inhibitor phenotypes of *Glycine max* in Korea and several other countries.

Countries	N	Ti a/a	Ti b/b	Ti c/c	Ti a/b	Ti b/c	Ti c/a	ti
China	29	25 (86.2%)	4 (13.8%)	0	0	0	0	0
Korea	1706	1399 (82.0%)	226 (13.2%)	16 (1.0%)	34 (2.0%)	11 (0.6%)	16 (1.0%)	4 (0.2%)
Japan	24	16 (66.7%)	7 (29.2%)	0	1 (4.1%)	0	0	0
Vietnam	14	8 (57.1%)	6 (42.9%)	0	0	0	0	0
USSR	86	64 (74.4%)	18 (20.9%)	0	4 (4.7%)	0	0	0

Table 3. Soybean trypsin inhibitor phenotypes and gene frequencies of *Glycine soja* in Korea and several other countries.

Countries	N	Ti a/a	Ti a/b	Ti b/b	Ti c/a	Ti c/c	Ti b/c	ti/ti	Gene frequencies
China	31	24 (77.4%)	0	7 (22.6%)	0	0	0	0	Ti*a= 0.7742±0.1748
Korea	103	90 (87.4%)	6 (5.8%)	3 (2.9%)	4 (3.9%)	0	0	0	Ti*a= 0.9223±0.0023
Japan	24	20 (83.4%)	2 (8.3%)	2 (8.3%)	0	0	0	0	Ti*a= 0.8750±0.1094
USSR	14	12 (85.7%)	2 (14.3%)	0	0	0	0	0	Ti*a= 0.9286±0.0487

b/b lines showed that a much higher percentage in Japanese soybean than in Korean and Chinese soybean. Hymowitz and Kaizuma[7] also had showed the same result.

Table 3 presents soybean trypsin inhibitor phenotypes and gene frequencies in *Glycine soja*. The frequency of Ti*a allele is found to be highest in Korea (0.9223) among the world collections (Japan ; 0.8750, China ; 0.774). None of Ti c/c and ti/ti types are found in *Glycine soja*.

Frequencies of the trypsin inhibitor allele reported in the East Asian lines are listed in Table 4. Ti*a (0.8347) had a higher gene fre-

Table 4. The frequency of trypsin inhibitor of Asian origin soybean.

Countries	N	Ti*a	Ti*b	Ti*c	ti*	Authors
China						
Northeast	803	0.9888	0.0112	-	-	Hymowitz et al., 1978
	628	0.9108	0.0892	-	-	Hymowitz, 1973
South & Central	126	0.9206	0.0794	-	-	Hymowitz, 1973
	153	1.0000	-	-	-	Clark et al., 1970
Korea	1706	0.8347	0.1457	0.0173	0.0023	Presentstudy
	417	0.8777	0.1151	0.0024	0.0048	Hymowitz et al., 1978
	350	0.7029	0.2943	0.0029	-	Hymowitz, 1973
	57	0.8421	0.1579	-	-	Choi et al., 1987
	22	0.9091	0.0909	-	-	Clark et al., 1970
Japan	477	0.5954	0.3920	0.0126	-	Hymowitz et al., 1978
	459	0.6318	0.3660	0.0014	-	Hymowitz, 1973
	348	0.5747	0.4080	-	-	Hymowitz & Kaizuma, 1979
	42	0.7143	0.2857	-	-	Clark et al., 1970

quency than the Ti*b(0.1457) in Korean land races. The frequency of the Ti*a allele is highest in Northeast China (Ti*a=0.9888, N=803) and lowest in Japan (Ti*a=0.5954, N=477). The genetic differences in frequencies of Ti alleles appear geographical cline. The Ti c/c type are observed in the population collected in Korea and Japan, and ti/ti type (N=4) observed only in the Korean collections. These data may suggest that Ti*c and ti alleles were originated from Korea. Hymowitz et al.[6] also suggested that Ti*c allele was introduced from Korea to Japan or probably arisen as a spontaneous mutation in the Tohoku District.

摘 要

1. Polyacrylamide gel electrophoresis를 이용하여 國內外 재래종과 야생종 大豆 系統들의 trypsin inhibitor의 變異를 규명하기 위하여 본 試驗이 시도되었으며 1706계통의 한국산 재래종과 103계통의 한국 야생종콩, 그리고 167계통의 외래 재배종과 71계통의 외국 야생종 大豆가 供試되었다.
2. Trypsin inhibitor를 함유하지 않은 ti/ti형과 Ti c/c형은 한국 재래종에서만 發見되었으며, Ti*c형을 Hymowitz도 일본 대두품종에서 報告한 바 있으나 그도 이 계통은 한국 渡來種일 가능성이 크다고 보고 한 바 있다.
3. 韓國起源의 콩에서 trypsin inhibitor에 관한 異形接合形의 출현빈도가 외국 기원 콩 계통에서 보다 비교적 높았으며, 재래종에서 3.6% (N=61)와 야생종에서 9.7%(N=10)이었으며 종합적으로 보아 중국, 일본 등의 大豆에 비해 한국 起源의 콩이 가장 큰 變異를 나타내고 있음을 確認하였다.

REFERENCES

1. Choi, Y.J., D.C.Lee and J.J.Kim. 1987. Genetic relationship among soybean varieties grown in highland Area. Res. Rept. RDA (Agri. Institutional Cooperation). 253-261.
2. Clark, R.W., D.W. Mies and T.Hymowitz. 1970. Distribution of a trypsin inhibitor variant in seed proteins of soybean varieties. Crop Sci. 10 : 486-487.
3. Hildebrand, D.F., J.H.Orf and T.Hymowitz. 1980. Inheritance of an acid phosphotase and its linkage with the Kunitz tripsin inhibitor seed protein of soybean. Crop Sci. 20 : 83-85.
4. Hymowitz, T. and H.H.Hadley. 1972. Inheritance of a trypsin inhibitor variante in seed protein of soybeans. Crop Sci. 12 : 197-198.
5. _____. 1973. Electrophoretic analysis of SBTI-A_2 in the USDA soybean germ plasm collection. Crop Sci. 13 : 420-421.
6. _____.J.H. Orf, N.Kaizuma and H. Skorupska. 1978. Screening the USDA soybean germplasm collection for kunitz trypsin inhibitor variants. Soybean Genet. Newsl. 5 : 19-22.
7. _____ and N. Kaizuma. 1979. Dissemination of soybean (*Glycine max*) : Seed protein electrophoresis profiles among Japanese cultivars. Economic Bot. 33 : 311-319.
8. Kiang, Y.T. and Y.C.Chiang. 1986. Genetic linkage of a leucine aminopeptidase locus with the Kunitz trypsin inhibitor locus in soybeans. J. Hered. 77 : 128-129.
9. Orf, J.H. and T.Hymowitz. 1976. The gene symbol Sp_t^a and Sp_t^b assigned to Larsen and Caldwell's seed protein band A and B. Soybean Genet. Newsl. 3 : 27-28.
10. _____ and _____. 1977a. Inheritance of a second trypsin inhibitor variant in seed protein of soybeans. Crop Sci. 17 : 811-813.
11. _____ and _____. 1977b. Lindage tests between Sp_t and Ti seed proteins. Soybean Genet. Newsl. 4 : 26-29.
12. _____ and _____. 1978. Soybean linkage tests between two seed proteins and other characters. Soybean Genet. Newsl. 5 : 22-24.
13. _____ and _____. 1979. Soybean linkage test between Ti and Le seed proteins. Soybean Genet. Newsl. 6 : 32.

14. Palmer, R.G., R.D. Shoemaker and Rennie. 1987. Approved soybean gene symbols. Soybean Genet. Newsl. 14 : 41-58.
15. Rackis, J.J., H.A.Sasame, R.K.Mann, R. L.Anderson and A.K. Smith. 1962. Soybean trypsin inhibitors : isolation, purification and physical properties. Arch. Biochem. Biophys. 98 : 471-478.