Genetic Analysis of Subunits of Two Major Storage Proteins (β -Conglycinin and Glycinin) in Soybean Seeds

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Eight hundred and fifty soybean accessions were screened for genotypes with electrophoretic variations of the β -conglycinin and glycinin subunits. We detected two abnormal SDS-gel electrophoretic forms of β -conglycinin α -subunit, the slow electrophoretic mobility type designated as α -slow-type and the low α -subunit content type designated as α -low-type. Each of these two forms was controlled by a codominant allele at a single locus. The gene symbol Cgy_2^a was assigned to the common type and Cgy_2^b to the α -slow-type. We found a new electrophoretic form of β -conglycinin β -subunit designated as β' -subunit which migrates slightly faster than the β -subunit on SDS-gels. Beta-prime (β') subunit was controlled by a single dominant gene designated as Cgy_3 . The homozygous recessive $cgy_3 cgy_3$ lacked the β' -subunit. We observed two variations of the glycinin group-I acidic subunits (A_1 , A_2 and A_3) by alkaline urea electrophoresis, i.e., the concomitantly slow and fast electrophoretic mobility types designated as A(I)-slow-type and A(I)-fast-type, respectively. The concomitantly slow and fast migrating A_1 , A_2 and A_3 subunits, respectively, behaved in the F_2 seeds as a single block composed of the three electrophoretic bands. Each of these electrophoretic forms was inherited as if controlled by a codominant allele at a single locus. The gene symbols, Gy_1^a , Gy_1^b and Gy_1^c were assigned to the alleles for the common electrophoretic type, the A(I)-slow-type and the A(I)-fast-type, respectively. Close linkage was found between Cgy_2 and Cgy_3 . The gene Cgy_1 controlling the β -conglycinin α' -subunit was independent of Gy_1 and Gy_4 which controls the A_5 subunit and its associated basic subunit.

KEY WORDS : *Glycine max*, storage protein subunits, β -conglycinin, glycinin, inheritance, genetic linkage.

Introduction

Soybean seeds contain 35~40% protein on a dry weight basis, about 70% of which consists of two major storage globulins (DERBYSHIRE *et al.* 1976), β -conglycinin (M.W.=150,000) and glycinin (M.W.=350,000). Beta-conglycinin which is a glycoprotein containing mannose and glucosamine is composed of six isomers with varying proportions of three kinds of subunits (α , α' and β) (THANH and SHIBASAKI 1976, 1977). On the other hand, glycinin is an oligomeric protein composed of several acidic and basic subunits, where specific pairs of acidic and basic subunits are linked through disulfide bonds (KITAMURA *et al.* 1976. MOREIRA *et al.* 1979, IYENGER and RAVESTEIN 1981).

Some electrophoretic variations of storage proteins in pea and common bean were reported (CASEY 1979, HALL *et al.* 1977, BROWN *et al.* 1981 a), and the inheritance of the variations and linkage among the genes of these storage proteins have been partially revealed (MATTA and GATEHOUSE 1982, MAHMOUD and GATEHOUSE 1984, BROWN *et al.* 1981 b). As for the storage proteins in soybean, some electrophoretic variations

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of the glycinin subunits on polyacrylamide gels containing urea (KITAMURA *et al.* 1980, MORI *et al.* 1981) and two mutant strains of the β -conglycinin subunits in SDS-gels (KITAMURA and KAIZUMA 1981) were reported. Recently, the primary structures of soybean storage proteins have been revealed by amino acid sequencing and by sequencing cDNAs.

However, as yet, only a few studies have been performed on the inheritance and linkage for β -conglycinin (KITAMURA *et al.* 1984, DAVIES *et al.* 1985) and glycinin (HARADA *et al.* 1983) subunit genes.

In this study, we attempted to identify genotypes showing electrophoretic variations of the β -conglycinin and glycinin subunits and analyzed the inheritance of the variations. Furthermore, we determined whether there are linkages among the genes coding these storage protein subunits.

Materials and Methods

Soybean accessions preserved in our laboratory were used for the screening of electrophoretic variations. Part of the soybean accessions (shown in Table 1) was grown and harvested at the Iwate University Experimental Farm or in a greenhouse in 1982 and 1983. Crosses among the electrophoretic variants shown in Table 2 were made in the summer of 1983. The F_2 seeds were obtained by selfing of F_1 plants grown in the greenhouse. The F_3 seeds from the cross between Keburi and Moshidou

Table 1. Electrophoretic variations and their gene symbols of storage protein subunits

Accessions	Variations			
	β -conglycinin		Glycinin	
	subunit(s)-type	gene symbol	subunit(s)-type	gene symbol
1. Raiden	α -common-type	Cgy_2^a	A(I)-common, A_4 -type	Gy_1^a , gy_4
2. Moshidou Gong 503	α -, β -low-type	—	A(I)-fast, A_5 -type	Gy_1^c , Gy_4
3. Keburi	α' -less-type	cgy_1	A(I)-common, A_5 -type	Gy_1^a , Gy_4
4. Iwate No. 1	β' -type	Cgy_3	A(I)-common, A_5 -type	Gy_1^a , Gy_4
5. Tohzan Kei BL 51	β' -type	Cgy_3	A(I)-common, A_4 -type	Gy_1^a , gy_4
6. Mikuriya Shiro	β' -type	Cgy_3	A(I)-common, A_5 -type	Gy_1^a , Gy_4
7. Zairai Aki Daizu	α -slow-type	Cgy_2^b	A(I)-common, A_5 -type	Gy_1^a , Gy_4
8. Oh-oka Zairai	α -slow-type	Cgy_2^b	A(I)-common, A_5 -type	Gy_1^a , Gy_4
9. PI 54608-1	α -slow-type	Cgy_2^b	A(I)-common, A_5 -type	Gy_1^a , Gy_4
10. PI 90763	α -low-type	—	A(I)-slow, A_5 -type	Gy_1^b , Gy_4
11. PI 253651-A	α -low-type	—	A(I)-slow, A_5 -type	Gy_1^b , Gy_4

Table 2. Cross combinations and marker genes

A. Keburi (cgy_1 , Cgy_2^a) $\times$ PI 54608-1 (Cgy_1 , Cgy_2^b)
B. Iwate No. 1 (Cgy_2^a , Cgy_3) $\times$ Zairai Aki Daizu (Cgy_2^b , cgy_3)
C. Tohzan Kei BL 51 (Cgy_2^a , Cgy_3 , gy_4) $\times$ Oh-oka Zairai (Cgy_2^b , cgy_3 , Gy_4)
D. Mikuriya Shiro (Cgy_3 , Gy_1^a) $\times$ PI 253651-A (cgy_3 , Gy_1^b)
E. PI 54608-1 (Cgy_2^b , Gy_1^a) $\times$ PI 90763 (Cgy_2^a , Gy_1^b)
F. Raiden (Cgy_2^a , gy_4) $\times$ PI 54608-1 (Cgy_2^b , Gy_4)
G. Keburi (cgy_1 , Gy_1^a) $\times$ Moshidou Gong 503 (Cgy_1 , Gy_1^c)

Gong 503 were obtained by selfing of F_2 plants grown at the farm in 1984.

In screening for the genotypes with electrophoretic variations of the major subunits of the β -conglycinin and glycinin proteins, the seed proteins were analyzed by sodium dodecyl sulfate (SDS) slab polyacrylamide gel electrophoresis (PAGE) according to the method of LAEMMLI (1970) and slab alkaline urea (AU) system PAGE according to the method of KITAMURA and SHIBASAKI (1975). It is easy to identify the major subunits of the β -conglycinin and glycinin proteins because the major electrophoretic bands of these subunits can be stained very strongly with Coomassie Brilliant Blue on the gels. To determine whether the variations detected are genuine or not, the isolated soybean globulin fraction was analyzed by two dimensional (2-D) electrophoresis based on the DAVIS (1964) disc polyacrylamide gel system followed by the SDS- or AU-slab polyacrylamide gel system, as described previously (KITAMURA *et al.* 1980). The soybean globulin fraction was prepared from seed meal by isoelectric precipitation at pH 4.5 in the buffer solution (0.05 M Tris-HCl).

Individual F_2 and F_3 seeds were crushed in coin envelopes with a hammer except for the cross between Keburi and Moshidou Gong 503, in which the distal portions (about 10 mg) of the F_2 seeds were cut off with a razor blade and crushed. The seed proteins were analyzed by the SDS- and AU-slab PAGE.

Results and Discussion

Electrophoretic variations of β -conglycinin

Figure 1 shows the electrophoretic variations of the β -conglycinin subunits on the SDS-gel: lane 1 corresponds to Raiden (common-type), lane 2 and 3 to Moshidou Gong 503 and Keburi, respectively (KITAMURA and KAIZUMA 1981), lane 4 corresponds to the variant having an extra β' -subunit band which migrates slightly faster than the β -subunit, lane 5 corresponds to the variant having the subunit α_s with a slow electrophoretic mobility designated as α -slow-type, and lane 6 to the variant having a low content of α -subunit designated as α -low-type. Of 850 accessions screened, four were found to belong to the β' -subunit, seven to the α -slow-type, and ten to the α -low-type.

Quantitative variations of the β -conglycinin β -subunit were observed, whereas no qualitative variations were detected. Genetic analysis of the β -subunit

Fig. 1. SDS slab PAGE of β -conglycinin subunit bands from the six soybean accessions. 1: Raiden, 2: Moshidou Gong 503, 3: Keburi, 4: Mikuriya Shiro, 5: Oh-oka Zairai, 6: PI 90763. Soybean flour (10 mg) was suspended in 1 ml of 0.05 M Tris-HCl (pH 8.0) containing 0.2% SDS, 6 M urea and 0.2 M 2-mercaptoethanol. Protein samples (about 10 μ l) were electrophoresed with a constant voltage of about 125 V for 5 hr. The gel (7.5%) was stained with Coomassie Brilliant Blue R-250. Cgy: β -conglycinin. Gy: glycinin.

gene(s) was very difficult because the quantitative variations of this subunit were associated with changes of the environmental conditions.

Two possible explanations for the difference in the electrophoretic mobility of the α -subunit on the SDS-gel can be proposed, i.e., 1) a difference in the number of amino acid residues and 2) a difference in the amino acid substitutions. A mutation which alters a signal peptidase recognition site may cause a difference in the number of amino acid residues because the α -polypeptide chain is synthesized as a precursor with a slightly larger molecular weight (TUMER *et al.* 1981). Amino acid substitutions in the α -polypeptide chain may also lead to differences in the electrophoretic mobility because such substitutions in certain polypeptides were found to change the electrophoretic mobility on the SDS-gel (De JONG *et al.* 1978, NOEL *et al.* 1979).

Electrophoretic variations of glycinin

SCALLON *et al.* (1985) divided the five glycinin subunits into group-I ($A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a}) and group-II (A_3B_4 , $A_5A_4B_3$) based on the molecular size and degree of sequence homology. According to their designation, the A_1 , A_2 and A_3 subunit (polypeptides) were treated as group-I acidic subunits since the group including the A_1 , A_2 and A_3 polypeptides and that including the A_4 and A_5 polypeptides correspond to the polypeptides referred to as group-I (A_{1a} , A_{1b} , A_2) and group-II (A_3 , A_4), respectively.

Figure 2 shows the electrophoretic variations of the glycinin acidic subunits on the

Fig. 2. AU slab PAGE of glycinin acidic subunits from the five soybean accessions. 1: Radiden, 2: Keburi, 3: PI 90763, 4: Moshidou Gong 503, 5: Keburi. Soybean flour (10mg) was suspended in 1ml of 0.05M Tris-HCl (pH 8.0) containing 8M urea and 0.2M 2-mercaptoethanol. Protein samples (about 10 μ l) were electrophoresed with a constant current of about 15mA for 6hr.

AU-gel: lane 1 and 2 correspond to the A_4 and A_5 glycinin type respectively (KITAMURA *et al.* 1980, HARADA *et al.* 1983), lane 3 and 4 indicate electrophoretic variations in which the A_1 , A_2 and A_3 subunits migrate concomitantly slower and faster than the normal group-I acidic subunits, respectively. The slow and fast-migrating group-I acidic subunits showed the same electrophoretic mobility as that of the normal group-I acidic subunits on the SDS-gel. We designated the concomitantly slow and fast electrophoretic mobility types as A(I)-slow-type and A(I)-fast-type, respectively.

CASEY *et al.* (1979, 1981) reported that there were considerable genetic variations in the type and number of legumin acidic subunits, and the amount of the total sulfur-containing amino acids of the purified legumin variants ranged from 1.09 to 1.75 mol%. It is suggested that the amount of total sulfur-containing

amino acids varied among the four subunit types detected in this investigation.

Two dimensional electrophoresis

Two dimensional PAGE (disc PAGE $\times$ SDS- or AU-slab PAGE) was performed to determine whether the electrophoretic variants of the β -conglycinin and glycinin subunits which were detected were genuine or not. It is possible to separate β -conglycinin and glycinin by disc gel electrophoresis because glycinin migrates over a greater distance than β -conglycinin, and few other soybean proteins migrate to the positions corresponding to the two proteins (HILL and BREIDENBACH 1974).

Fig. 3. 2-D PAGE of the soybean globulin fractions (A : Mikuriya Shiro and B : Moshidou Gong 503) using the disc PAGE in the first dimension and the SDS slab PAGE (A) or the AU slab PAGE (B) in the second dimension. Protein sample (20 μ l, ca. 0.1% in 35mM potassium phosphate buffer, pH containing 0.4M NaCl and 0.2M 2-mercaptoethanol) was electrophoresed with a constant current of 1mA per tube for 2hr in the first dimension. After incubation of the disc gel in an excess amount of the sample buffer, the gel was layered on the second dimensional gel. Electrophoretic conditions in the second dimension were the same as those outlined in Fig. 1 and 2, except for the concentration of acrylamide (7.5%~15%) on the SDS slab PAGE. C : control lane (globulin fraction).

Figure 3 shows that the globulin fractions from the variants separated in the first dimension gave banding patterns in the second dimension similar to those of the control lane: the β' -subunit band behaved as the β -conglycinin subunit (A), and the fast-migrating group-I acidic subunits behaved as the glycinin subunits (B). Similar results were obtained using the soybean globulin fractions from the α -slow-type and A(I)-slow-type. The globulin fractions from the accessions (shown in Table 1) grown in 1982 gave banding patterns of β -conglycinin and glycinin subunits identical with those of the accessions grown in 1983: the subunit patterns were consistent, regardless

of the environments in which the plants were grown.

These results demonstrate that the β' -subunit is a genuine subunit of β -conglycinin, and that the group-I acidic subunits of the A(I)-fast- and A(I)-slow-type soybean are also genuine glycinin acidic subunits.

Inheritance of β -conglycinin

The F_1 seeds from crosses between α -common-type and α -slow-type (Table 2 A, B, C, E and F) contained the α - and α_s -subunit bands. In the F_2 seeds a 1:2:1

Fig. 4. (A) SDS slab PAGE of the F_2 progeny for the β -conglycinin subunit patterns. 1: common-type from the cross F, 2: F_1 -type from the cross F, 3: α -slow-type from the cross E, 4: F_1 -type from the cross E, 5: α -low-type from the cross E, 6: β -type from the cross D, 7: F_1 -type from the cross D, 8: α -low-type from the cross D.

(B) AU slab PAGE of the F_2 progeny for the glycinin acidic subunit patterns. 1: A(I)-common-type from the cross G, 2: F_1 -type from the cross G, 3: A(I)-fast-type from the cross G, 4: A(I)-common-type from the cross D, 5: F_1 -type from the cross D, 6: A(I)-slow-type from the cross D. Cross combinations are shown in Table 2, Electrophoretic conditions were the same as those outlined in Fig. 1 and 2.

Table 3. Observed and expected F_2 segregation from five crosses for the electrophoretic bands of the β -conglycinin α -subunit

Cross		Genotype						χ^2 (1:2:1)	P
		Cgy_2^a	Cgy_2^a	Cgy_2^a	Cgy_2^b	Cgy_2^b	Cgy_2^b		
A.	Obs.	77	101	55				8.28	0.01
	Exp.	58.25	116.5	58.25					
B.	Obs.	27	81	36				3.38	0.1
	Exp.	36	72	36					
C.	Obs.	34	70	40				0.61	0.7
	Exp.	36	72	36					
E.	Obs.	11	21	11				0.03	0.98
	Exp.	10.75	21.5	10.75					
F.	Obs.	19	50	20				1.48	0.3
	Exp.	23	46	23					

ratio of the α -common-type : F_1 -type : α -slow-type patterns was obtained (Fig. 4, Table 3). From these results, we conclude that the α -subunit bands are controlled by codominant alleles as a single locus. The gene symbols Cgy_2^a and Cgy_2^b were assigned to the α -common-type and the α -slow-type, respectively.

Electrophoretic analysis of the cross between PI 54608-1 (α -slow-type) and PI 90763 (α -low-type) showed no recombinant genotypes in the F_2 segregation with respect to the subunit type and content : each of the F_2 seeds with α_s - and α -subunits showed a normal content and a low content of the subunits, respectively. This implies that the factor(s) lowering the α -subunit content would be strongly associated with the α -subunit structural gene. In other words, the control of the α -subunit content would be of the *cis*-regulation type rather than of the *trans*-regulation type as observed in the control of zein (maize storage protein) accumulation (SOAVE and SALAMINI 1982).

Table 4. Observed and expected F_2 segregation from three crosses for the electrophoretic band of the β -conglycinin β' -subunit

Cross		Genotype		χ^2 (3 : 1)	P
		Cgy_3 —	cgy_3 cgy_3		
B.	Obs.	108	36	0	1.0
	Exp.	108	36		
C.	Obs.	104	40	0.59	0.3
	Exp.	108	36		
D.	Obs.	143	37	1.89	0.1
	Exp.	135	45		

The F_1 seeds from the crosses between β' -type and common-type (β' -less) (Table 2 B, C and D) contained the β' -polypeptide (Fig. 4), and the F_2 seeds segregated in a ratio of 3 : 1 (present : absent) (Table 4). These results indicate that the presence of the β' -subunit is controlled by an allele designated as Cgy_3 at a single locus. This allele is dominant to the allele for the lack of the subunit, designated as cgy_3 . No β' -subunit was detected in the homozygous recessive cgy_3cgy_3 .

Inheritance of glycinin

The F_1 seeds from the three crosses between A(I)-common-type $\times$ A(I)-slow- and A(I)-fast-type (Table 2 D, E and G) showed patterns overlapping with all the glycinin group-I acidic subunits of the two patterns. No recombinant types of the glycinin group-I acidic subunits were obtained in the F_2 seeds from the three crosses. Only three banding patterns segregating in a 1 : 2 : 1 ratio of one parent : F_1 : the other parent patterns were observed (Fig. 4, Table 5). Furthermore the inheritance of the glycinin group-I acidic subunits from the cross between the A(I)-common-type and the A(I)-fast-type (Table 2 G) was investigated using F_3 seeds produced by the F_2 seeds with known phenotypes by AU-slab PAGE analysis of a part of cotyledons (Table 5). Segregation of the F_3 seeds followed the expected 1 : 2 : 1, 1 : 0, and 0 : 1 ratios for heterozygous, homozygous dominant, and homozygous recessive genotypes of the F_2 seeds, respectively (Table 6).

Table 5. Observed and expected F_2 segregation from three crosses for the electrophoretic banding patterns of the glycinin group-I acidic subunits

Cross		Genotype			χ^2	P
		$Gy_I^a \text{ } Gy_I^a$	$Gy_I^a \text{ } Gy_I^b$	$Gy_I^b \text{ } Gy_I^b$		
D.	Obs.	43	92	45	0.13	0.9
	Exp.	45	90	45		
E.	Obs.	12	18	13	1.19	0.3
	Exp.	10.75	21.5	10.75		
		$Gy_I^a \text{ } Gy_I^a$	$Gy_I^a \text{ } Gy_I^c$	$Gy_I^c \text{ } Gy_I^c$		
G.	Obs.	21	53	30	1.60	0.3
	Exp.	26	52	26		

Table 6. F_3 segregation data from the cross of Keburi $\times$ Moshidou Gong 503 for the electrophoretic banding patterns of the glycinin group-I acidic subunits

F_2 parents		F_3 seeds		
Phenotype	No. of plants	$Gy_I^a Gy_I^a$	$Gy_I^a Gy_I^c$	$Gy_I^c Gy_I^c$
$Gy_I^a Gy_I^a$	21	252	3 ¹⁾	0
$Gy_I^a Gy_I^c$	53	169 ²⁾	301 ²⁾	152 ²⁾
$Gy_I^c Gy_I^c$	30	0	5 ¹⁾	358

¹⁾ Contamination or outcrossing²⁾ χ^2 for $1Gy_I^a Gy_I^a : 2Gy_I^a Gy_I^c : 1Gy_I^c Gy_I^c = 1.51$ $P > 0.3$

From the above results, it is concluded that the genes controlling the A_1 , A_2 and A_3 subunits of glycinin are tightly linked and inherited like a single gene. The gene symbols Gy_I^a , Gy_I^b and Gy_I^c were assigned to the alleles for the common, the slow and the fast electrophoretic forms of the group-I acidic subunits, respectively. Thus, the gene symbol Gy_I refers to a complex of the genes controlling the A_1 , A_2 and A_3 subunits and probably the basic subunits specifically associated with A_1 , A_2 and A_3 because each of the glycinin polypeptides was translated as a precursor consisting of a signal sequence, an acidic and a basic polypeptide. A similar linkage was reported for some other legume storage protein (e.g., phaseolin and legumin) genes (BROWN *et al.* 1981 b, MATTA and GATEHOUSE 1982).

Linkage relationships among storage protein genes

The data on the segregation of the five loci were paired for testing the linkage relationships. Linkage was found between Cgy_2 and Cgy_3 . The chi-square value for independence was 310.3 which indicates a probability as low as 0.001 (Table 7), and 288 F_2 progenies from the two crosses between α -slow-type and β' -type (Table 2 B and C) contained no recombinant types of α - and β' -subunits, suggesting that the two loci are closely linked. A similar linkage was reported by DAVIES *et al.* (1985). In preliminary investigations, in the cross between Keburi and Moshidou Gong 503 we reported that there was a significant correlation between the α - and β -subunit contents, suggesting that the two genes controlling the α - and β -subunit products are closely linked together (TSUKADA *et al.* 1984). From these results, we propose

Table 7. F₂ segregation data from seven gene pairs of the dihybrid for good-of-fit to the 9:3:3:1 segregation ratio

Cross	Gene pair	Observed frequencies of genotype ¹⁾				Total	χ^2	P
		A-B-	A-bb	aaB-	aabb			
A	<i>Cgy</i> ₁ ³⁾ - <i>Cgy</i> ₂	144	44	34	11	233	4.30	0.2~0.3
G	<i>Cgy</i> ₁ - <i>Gy</i> ₁	58	23	16	7	104	1.30	0.7~0.8
B and C ²⁾	<i>Cgy</i> ₂ - <i>Cgy</i> ₃	212	0	0	76	288	310.32	0~0.001
E	<i>Cgy</i> ₂ - <i>Gy</i> ₁	23	9	7	4	43	0.95	0.8~0.9
C and F ²⁾	<i>Cgy</i> ₂ - <i>Gy</i> ₄ ⁴⁾	134	40	42	17	233	0.86	0.8~0.9
D	<i>Cgy</i> ₃ - <i>Gy</i> ₁	102	39	33	6	180	3.30	0.3~0.5
C	<i>Cgy</i> ₃ - <i>Gy</i> ₄	77	28	27	12	144	1.24	0.5~0.8

¹⁾ A and B represent the first and second loci of each gene pair.

²⁾ The data combined.

³⁾ *Cgy*₁: gene symbol of the β -conglycinin α' -subunit.

⁴⁾ *Gy*₄: gene symbol of the glycinin A₅ subunit and its associated basic subunit.

that these three genes which control the α -, β - and β' -subunit products should be located on the same chromosome.

Chi-square tests of all the other 6 dihybrid segregations showed a good agreement with the expected 9:3:3:1 ratio (Table 7): the *Cgy*₁ locus controlling the β -conglycinin α' -subunit product (KITAMURA *et al.* 1984) was found to be independent of the *Cgy*₂ and *Gy*₁ loci, while the *Cgy*₂ and *Cgy*₃ were independent of the *Gy*₁ and *Gy*₄ loci controlling the group-I acidic (A₁, A₂ and A₃) and the glycinin A₅ subunits, respectively. It is suggested that the *Gy*₄ locus is independent of the *Cgy*₁ locus (KITAMURA *et al.* 1984) and the *Gy*₁ locus (TOYOKAWA *et al.* unpublished data). These findings indicate that four soybean storage protein genes, *Cgy*₁, *Cgy*₂, *Gy*₁ and *Gy*₄, are located separately on different chromosomes or the same chromosome at a distance of over 50 centimorgan, each.

Regarding the relationships among the known linkage groups (2, 5 and 9) and the storage protein genes detected in the present study, the following conclusion was drawn. The *Ti* locus controlling the Kunitz trypsin inhibitor electrophoretic bands in the linkage group 9 (HILDEBRAND *et al.* 1980) segregated independently of the *Cgy*₁, *Cgy*₂, *Gy*₁ and *Gy*₄ loci, while the *L*₁ locus controlling the pod color in the linkage group 5 and the *P* locus controlling the pubescence in the linkage group 2 segregated independently of the *Cgy*₁ and *Gy*₁ loci (unpublished data).

These linkage data on the storage protein genes, together with their utilization as genetic markers, may contribute to the improvement of the nutritional quality of seeds along with detailed information on the linkage maps of soybean genes.

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ダイズ種子中の主要貯蔵タンパク質 (β -コングリシニンとグリシニン) サブユニットの遺伝分析

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ダイズ種子貯蔵タンパク質には、 β -コングリシニンとグリシニンの2つの主要成分が含まれ、その食品化学的および物理化学的性質が広く研究されている。しかし、他の豆類の貯蔵タンパク質に比べ、ダイズの貯蔵タンパク質サブユニットの遺伝様式や連鎖関係に関しては、あまり知られていない。

本研究は、この2主要貯蔵タンパク質サブユニット変異を探索し、見いだされた変異の遺伝様式とそれらの遺伝子座間の連鎖関係を明らかにしようとした。

ダイズ 850 品種・系統を、 β -コングリシニンとグリシニンの変異を探索するために、それぞれ SDS-ゲル電気泳動とアルカリ側尿素系電気泳動で分析した。その結果、 β -コングリシニンの α サブユニットに2つの変異が見いだされた。すなわち、SDS-ゲル電気泳動で電気泳動移動度(以下、単に移動度)が従来の α サブユニットの移動度よりもわずかに遅いバンドを示すタイプ(α -slow-type)と、 α サブユニット生産量が低下したタイプ(α -low-type)である。これらの変異は、単一座の共優性対立遺伝子によることが示され、従来の α サブユニットの生産を支配する遺伝子およびそれよりも移動度の遅い α サブユニットの生産を支配する遺伝子をそれぞれ Cgy_2^a と Cgy_2^b の遺伝子記号で表わした。また、 β -コングリシニンは3つの主要サブユニット(α , α' および β) から成るとされていたが、 β サブユニットよりも移動度が速いもう一本の主要バンドを有する変異体が見いだされ、この変異バンドを β' サブユニットと命名した。この β' サブユニットの存在は、単一優性遺伝子(Cgy_3)に支配され、この遺伝子の劣性ホモ(cgy_3cgy_3)により β' サブユニットの欠失を生じることが示された。

アルカリ側尿素系電気泳動でのグリシニン酸性サブユニットの変異に関しては、 A_1 , A_2 および A_3 サブユニットの移動度が同時に遅いタイプ($A(I)$ -slow-type)と速いタイプ($A(I)$ -fast-type)とが見いだされた。 F_2 種子において、この遅く移動および速く移動する A_1 , A_2 および A_3 サブユニットは、各々一つのブロックとして挙動した。これらの変異性は、あたかも単一座の共優性対立遺伝子に支配されているように考えられ、従来の移動度を示す A_1 , A_2 および A_3 サブユニットの生産を支配する遺伝子、 $A(I)$ -slow-type の生産を支配する遺伝子および $A(I)$ -fast-type の生産を支配する遺伝子をそれぞれ Gy_1^a , Gy_1^b および Gy_1^c の遺伝子記号で表わした。

これらの遺伝子座や既知のダイズ種子貯蔵タンパク質遺伝子座間の連鎖関係を分析した結果、 Cgy_2 座と Cgy_3 座との間に組換え型種子が得られなかったことから、これら2座が強く連鎖しているものと考えられた。また、ほかの遺伝子座間の組合せでは独立に遺伝することが示された。