5. BREEDING FOR CHEMICAL QUALITY OF SOYBEAN IN JAPAN

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Introduction

It is now generally known that there exist many regions all over the world where the chronic nutrition defficiency, especially the protein defficiency, is becoming a serious problem. Many children in these regions are subjected to the protein defficiency disease (Kwashiokor.) The breeding and production of soybean with high protein contents could be a promising clue to settle such an universal problem on the provisions and nutrition.

The amount of crude protein contents in soybean seed is the highest among the crop plants. It can attain to the degree of about 40–50% (protein factor 6.25), so soybean is also known as the "field beef". But its scanty contents of mentionine, which is one of the essential amino acids for humman nutrition are considered as the nutritional draw-back of soybean protein. The methionine contents of soybean scarcely attain to the degree of 1%. It is about 1/4–1/2 in comparison with that of animal protein.

Metz et al. (1964) had succeeded in discovering a high lysine mutant strain of corn, which constains about 1.7 times of lysine contents in the seed protein compared with that of ordinary strain, so in the same way, if possible, to develop soybean varieties or strains of high methionine contents must be very useful.

We have carried out, since 1968, the fundamental experiments on the breeding of soybean with high protein contents and high contents of sulfur-containing amino acids (especially with high methionine contents). The authors wish to describe the outline of some knowledges already obtained and expect the critical opinions, though our study is not yet completed and consequently there remain some questions unsolved.

In Japan there are so many researchers, besides us, who are engaging in researches on chemical constituent breeding of soybean, so it is now impossible to describe completely all of their works. The Ministry of Agriculture and Forestry established "The general researches on the high utilization technique of protein and exploitation of resources", in 1969, and promoted the researches on the breeding and cultivation of soybean with high protein contents. Therefore we have also taken part in this project to promote the studies of soybean with high protein contents, co-operating with the researchers of experiment institutes of Ministry of Agriculture and Forestry. In this work, the members engaged for the researches of breeding are as follows:

1) The first field crop laboratory of the first crop division in Hokkaido Agricultural Experiment Station (Studies on the simple testing methods of soybean protein).

2) The third experimental laboratory of the second cultivation division of Tohoku Agricultural Experiment Station (Studies on the breeding of soybean varieties with high protein).

3) Institute of Radiation Breeding, National Institute of Agricultural Sciences (Studies on the genetic effects of ionizing radiation to the protein contents of soybean).

4) The laboratory of statistic in National Institute of Agricultural Sciences and the laboratory of biometry in Agricultural Faculty of Tokyo University (Studies on the

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hereditary mechanism of the protein of soybean seed).

5) The laboratory of breeding in Agricultural Faculty of Iwate University (Studies on the gene resources for the total protein of soybean seed and for the sulfur-containing amino acid contents in its seed protein).

6) And furthermore, the authors (the breeding laboratory of the Agricultural Faculty of Iwate University) have been co-operating with the Bioassay Laboratory of National Food Research Institute (the Ministry of Agriculture and Forestry), separately from above described experimental system.

Thus, as so many studies, even only in the field of breeding of soybean with high contents of protein, have been carried out, the authors wish, as above mentioned, to describe mainly the outline of our own studies, and also keenly hope to be acknowledged by all the persons concerned.

Present Situation of Studies on the Breeding of Soybean Seed Protein

The studies on the protein contents and the studies on the amino acid contents in the seed protein shall be described separately. At first, as for the studies on the protein contents, many researches have been carried out from various points of view, so the authors wish to prefer the subjects of gene resources and inheritance which are closely related to our own studies.

As to the differences of seed protein contents among the various cultivated soybean, many varieties of soybean of all over the world have been investigated on their crude protein contents in seed by Hokkaido Agricultural Experiment Station, Tokachi Agricultural Experiment Station of Hokkaido (1962–1964) and by National Institute of Agricultural Sciences (1963). And as the results of these experiments, it has been manifested that the crude protein contents of the summer soyeban cultivated in the Kyûshû district of Japan is the highest in the world.

The agricultural experiment station of Saga prefecture has promoted the project on the breeding of soybean with high protein and high crop, and in the process of this breeding, Ichigo-wase, Shirosaya 1 and Kanagawa-wase (these are the varieties of early maturing types of summer soybean) were recognized distinctly as the excellent parents for the breeding of soybean with high protein contents. This experiment station succeeded to breed Higomusume from the cross with Shirosaya 1 as a parent (1965), and Saikai 20 from the cross with Kanagawa-wase as a parent (1964), and their crude protein contents were higher than that of their parents (Table 1).

Williams (1948) and Weber (1950) made crosses between Glycine soja (wild soybean) and G. max (cultivated soybean), and investigated its progenies on the segregation of crude protein contents, and then, they revealed the transgressive progenies

Table 1. Comparison of the crude protein contents between high protein varieties and their parents

Variety	Parentage	Year			
		1960	1962	1963	1964
Higomusume	Shin 3 ×Shirosaya 1	45.9% (F_7)	46.3% (F_9)	47.0% (F_{11})	46.4% (F_{12})
Shirosaya 1	——	44.4	46.1	45.7	43.7
Saikai 20	Kanagawawase ×Saikai 6		49.8 (F_4)	47.9 (F_5)	48.5 (F_6)
Kanagawawase	——		47.7	43.2	45.9

(after Saga Agr. Exp. Station)

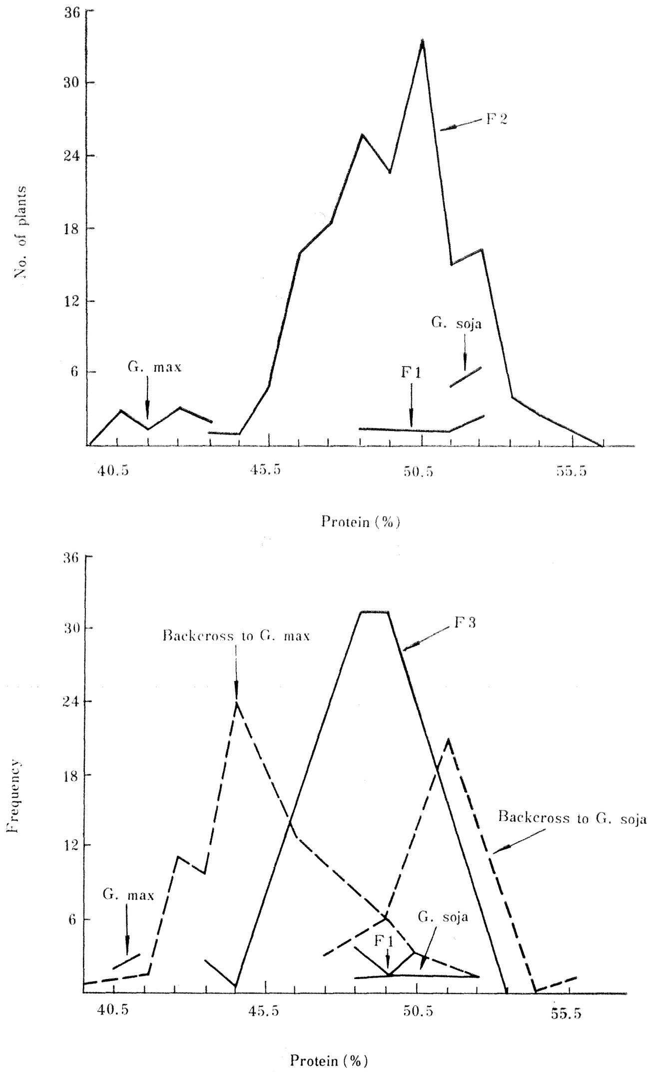

Fig. 1.

of high crude protein contents (Fig. 1). We carried out, in 1971, the pot cultures with 29 strains of G. soja collected from all over the country except Hokkaido, and with 7 varieties of G. max as the control in the same season at the Agricultural Faculty of Iwate University (Morioka city). And after the determination of crude protein contents of harvested seeds, it has been manifested that every strain of G. soja is superior to G. max in their protein contents, and that the high protein contents of G. soja must be a species specificity (Fig. 2). Furthermore, we are now carrying out the determination of crude protein contents of the F_2 individuals obtained from the cross between a strain of G. soja (T106-6) and a variety of G. max (Tairamame), and, the transgressive individuals have been revealed in this investigation as well as the cases of Williams and Weber (Fig. 3).

From these results, it seems that the wild soybean G. soja could be an excellent species as the parent of cross with G. max (cultivated soybean) raising its crude protein contents, and to be so, the quality of seed protein of G. soja must be superior, or

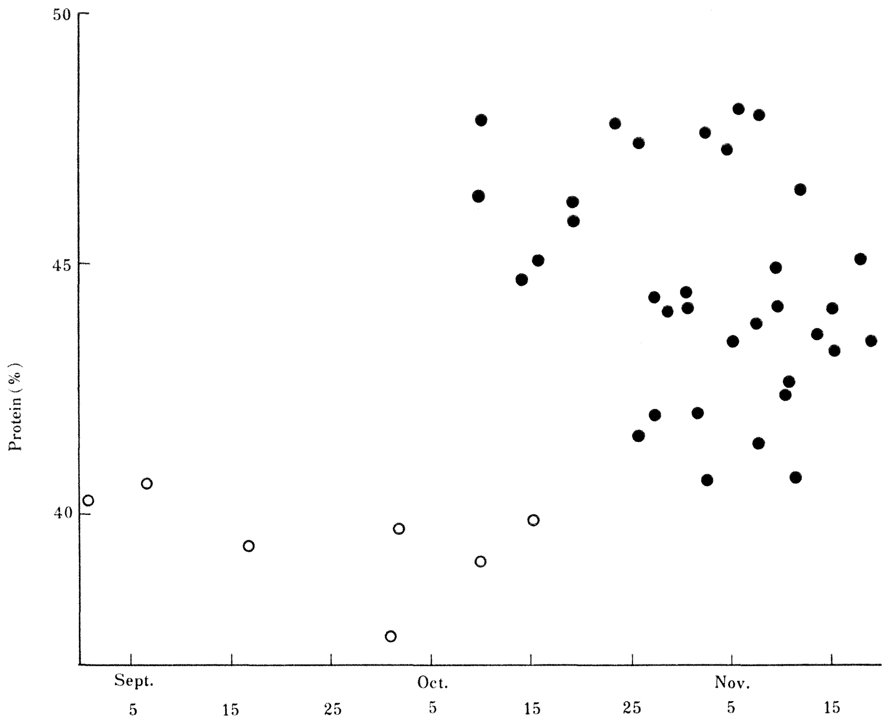
50
45
40
Protein (%)
Sept.
Oct.
Nov.
5
15
25
5
15
25
5
15

Fig. 2.

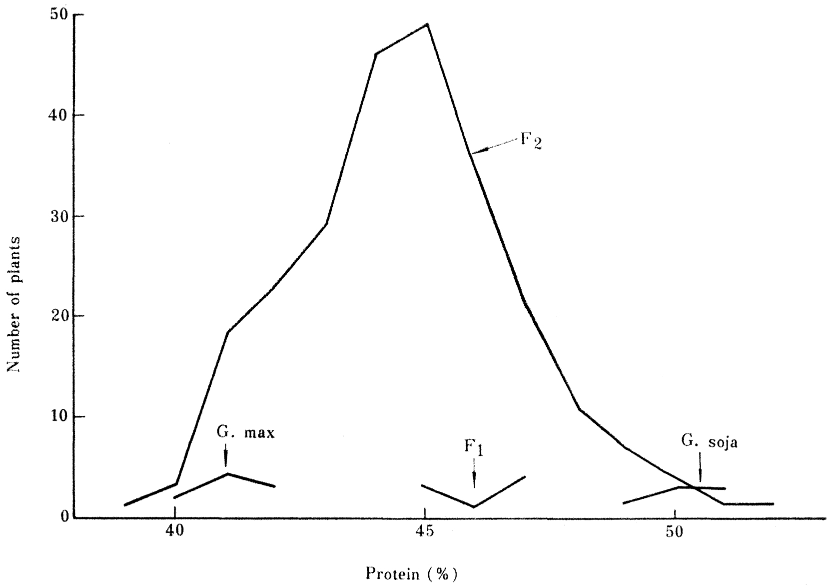
50
40
30
20
10
0
Number of plants
F_2
G. max
F_1
G. soja
40
45
50
Protein (%)

Fig. 3.

at least, as equal as to that of G. max. Gavrilyuk et al. (1970) examined the specificity of seed protein of several species of genus Glycine (containing G. soja) by the immunochemical electrophoresis method, and manifested that there exists, in G. soja, a component closely related to the 11S which is the principal component of seed protein of G. max. And consequently he reported that the theory that G. soja and G. max are the taxonomically closely related species was proved. We have also investigated the

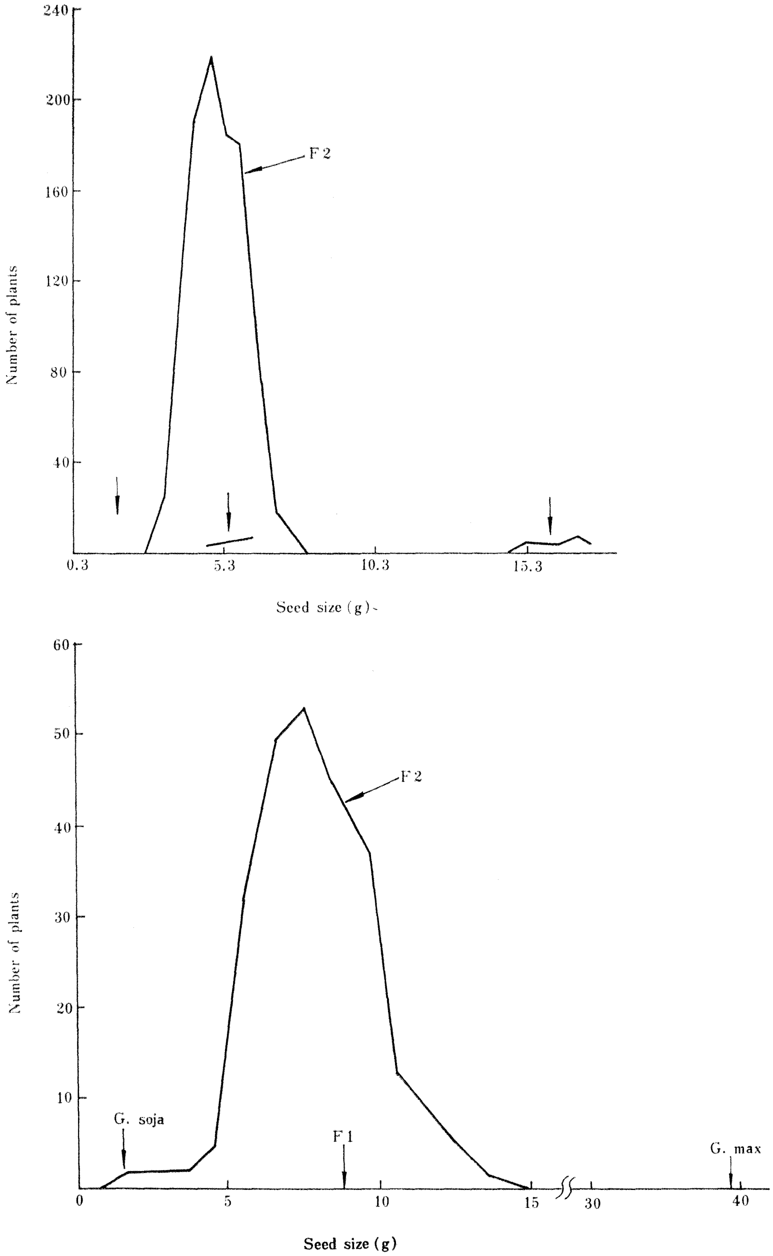

Fig. 4.

amino acid composition in seed protein of various species of genus Glycine, and could not find any significant difference between G. soja and G. max (it shall be detailed later). Therefore at present, we can not find any proof which can prove that the seed protein quality of G. soja is inferior to that of G. max. But the characteristics of interspecific hybrids, not only the crude protein contents of seed but also the other morphological characters, show the tendency to deviate positively toward the characters of G. soja. So it seems that the hybrids might be characterized by the disadvantageous specificities for the agronomically important characters. This is a rather serious problem (Fig. 4). Therefore, when the hybrid of G. max×G. soja is introduced to the breeding of commercial varieties, the backcrossing method must be also adopted.

Many researchers have studied on the inheritance of crude protein contents in seed, and as for their experimental results, some of the investigated matters came to accord with each other. That is, the direction of dominance was well coincided, the high protein seemed to be an incomplete dominance (Viljoen 1937; Williams 1948; Weber 1950; Leffel and Weiss 1958), but the differences between resiprocal crosses were recognized only by a few of the researchers. Many researchers have estimated the narrow sense heritabilities by the segregations in the generations subsequent to F_2, parent-offspring regression in F_2 and F_3 generations, and genetic gain obtained from the selection experiment. And their estimates indicated generally high value, so the selection effect in early generation seemed to be high when the genetic correlation with other characters were ignored (Table 2). But on the other hand, it is known that the crude protein content reveals the unfavourable genetic correlations with some other practically important characters. In Table 3, which indicates the experimental results of Saga

Table 2. Heritabilities for crude protein content

No.	Estimated value (%)	Estimation method	Researchers
1	70	Regression F_3 on F_2	Weber (1950)
2	39, 83	Variance F_3 lines	Johnson et al. (1955)
3	63	Selection differential	Johnson et al. (1955)
4	88. 1, 90. 0, 91. 9, 93. 2	Variance varieties	Kumamoto et al. (1964)
5	71. 3	Variance F_2 population	Unno et al. (1960)

Table 3. Genetic correlations between seed protein percentage and some agronomical characteristics concerning seed yield in summer soybean varieties grown in Kyushu district, Japan

Locations / Estimates	Kyushu district			
	Saga	Fukue	Miyakonojo	Kanoya
Genetic correlations of protein vs.				
1. No. days until flowering	0. 02	−0. 39		−0. 44*
2. No. days until maturing	−0. 52*	−0. 35		−0. 59**
3. Duration of maturing	−0. 28	0. 13		−0. 50*
4. Stem length	−0. 44*	−0. 36	−0. 27	−0. 59**
5. Pod number per plant	−0. 48*	−0. 43*	−0. 33	−0. 49*
6. 100 seed weight	−0. 05	−0. 37	0. 26	0. 30
7. Yield	−0. 59**	−0. 60**	−0. 38	−0. 53**

(Kumamoto et al, 1964)

Agricultural Experiment Station, an unfavourable negative correlation is clearly recognized between the crude protein contents and other characteristics closely related to the yield. As for the number of genes which can control the crude protein contents, Weber (1950) estimated it at 3. We have treated the ethyleneimine upon the soybean seeds (Raiden and Shirosaya 1 as original varieties), and have rather easily succeeded, up to now, to induce the mutant strains of which crude protein contents are considerably different from that of the original variety. Consequently, the controlling genes for the crude protein contents can be positively presumed to be a few of major genes (Table 4).

Table 4. Crude protein content of the mutant strains for protein, induced with ethyleneimine treatment

Original variety	Name of strains	Protein percentage		
		M_2 (1969)	M_3 (1970)	M_4 (1971)
Raiden	R2H 26-1	46.7	51.7	54.9 high
	R3H 9-4	42.3	39.0	46.7 low
	R3H 9-5	42.2	40.1	46.6 low
	Control	43.2	42.2	48.1
Shirosaya 1	S2H 63-6	50.5	56.3	58.1 high
	S3H 16-3	51.2	52.5	59.0 high
	S3H 34-4	43.0	47.6	51.0 low
	Control	47.3	50.3	54.8

Then, the authors wish to describe the amino acid composition which has the most close relation to the seed protein quality of soybean.

Kuiken (1948) examined 20 varieties of soybean in U.S., and analyzed on the quantities of 11 kinds of amino acids contained in the protein, and he reported that any significant varietal difference could not be found. Krober (1956) also examined 14 varieties of soybean in U.S., and investigated the varietal differences of methionine contents and the variation of menthionine contents based on years and locations. Then he described that there exists evidently the varietal difference of methionine contents caused by the genotypical difference, though it can be variable by the years and locations. And he emphasized that the breeding of high methionine variety is possible. Krober and Carter (1966) also examined several varieties, and reported that the quantity of methionine contents in the protein increases according to the increase of crude protein contents of seed. But we are obliged to consider that their opinions can not be accepted easily, because the numbers of varieties and strains subjected to their experiments seem to be not sufficient and some of their results wanted of the significant tendency.

The authors carried out, since 1968, some analyses on the amino acid contents in the seed protein of soybean in cooperation with the bioassy laboratory of National Food Research Institute of Ministry of Agriculture and Forestry. At first in 1968 and 1969, we investigated the varietal difference of the sulfurcontaining amino acid contents (methionine and cystine) of seed protein, and got the results shown in Table 5. That is, Kosodefuri and Gokuwase-Hayabusa (names of varieties) indicated rather high contents of methionine and cystine in both of investigated years, but Okutekurodaizu indicated, contrary, a rather low value of contents. Then the authors calculated the correlation coefficients between the crude protein contents, methionine contents and cystine contents from these data, and recognized that the correlation between the

Table 5. Varietal differences of the sulfur-containing amino acid content in soybean seed protein

No.	Variety name	Protein* (%)	Met. (g/16 gN)**		Cys. (g/16 gN)**	
			1968	1969	1968	1969
1	Kosodefuri	49.2	0.90	0.95	1.23	1.21
2	Laredo	34.8	0.93	0.92	1.15	1.17
3	Gokuwase-hayabusa	48.5	0.86	0.96	1.17	1.16
4	Gokuwase-edamame	47.1	0.90	0.91	1.25	1.06
5	Rikuu 20	42.3	0.95	0.94	1.11	0.99
6	Gokuwase-yaefusanari	39.1	0.91	0.85	1.18	0.96
7	Wasemidori	40.2	0.93	0.84	1.21	0.92
8	Waseshiratori	41.0	0.96	0.86	1.13	0.95
9	Wasemidori-osodefuri	40.1	0.93	0.87	1.13	0.94
10	Tokyo	44.2	1.01	0.90	1.01	0.95
11	Shirobana-osodefuri	40.6	0.90	0.89	1.10	0.93
12	Okuhara 1	44.2	0.76	0.86	1.10	1.06
13	Shirotsurunoko	42.7	0.93	0.84	1.01	1.00
14	Chusei	46.9	0.99	0.90	0.98	0.90
15	Yoshiokadairyu	41.8	0.87	0.80	1.13	0.96
16	Wasehikarikuro	41.3	0.88	0.87	1.05	0.96
17	Kisaya	43.9	0.88	0.85	1.03	0.98
18	Iwate 1	40.5	0.84	0.78	1.08	1.00
19	Wasebon	46.1	0.89	0.87	1.00	0.91
20	Ogura-azemame	42.3	0.86	0.83	0.96	1.01
21	Nakate	45.3	0.89	0.83	1.03	0.89
22	Hichigatsumame	42.3	0.91	0.82	0.98	0.91
23	Kanro	42.2	0.89	0.85	0.94	0.91
34	Shirohachikoku	44.3	0.82	0.82	0.95	0.94
25	Rikuu 15	42.0	0.89	0.84	0.89	0.85
26	Goyodaizu	41.2	0.81	0.78	0.97	0.91
27	Nioimame	43.2	0.86	0.77	0.88	0.89
28	Kurakake	41.8	0.91	0.84	0.88	0.73
29	Kojiirazu	41.9	0.87	0.75	0.93	0.79
30	Chamame	37.9	0.85	0.82	0.73	0.90
31	Edamame-zairai (Nagasawa)	41.0	0.84	0.81	0.76	0.84
32	Yukinoshita	41.4	0.79	0.79	0.85	0.78
33	Edamame-zairai (Wakamatsu)	42.6	0.83	0.74	0.82	0.73
34	Okutekurodaizu	43.9	0.70	0.64	0.65	0.59
35	Sango-wasedaizu	45.1		0.89		1.19

No.	Variety name	Protein* (%)	Met. (g/16 gN)**		Cys. (g/16 gN)**	
			1968	1969	1968	1969
36	Chusei-hadaka	46.6		0.90		1.10
37	Norin 1	44.2		0.86		1.06
38	Taichu-aokawamame	46.9		0.89		0.97
39	Hitashimame-Chohin 1	40.5		0.95		0.89
40	Shakujo	43.0		0.81		1.01
41	Norin 2	47.2		0.83		0.95
42	Akasaya	41.8		0.81		0.96
43	Iwateyagi 1	42.1		0.82		0.94
44	Goyodaizu (Iwate U.)	41.3		0.82		0.92
45	Tanbakuro	41.6		0.79		0.90
46	A 92	44.4		0.76		0.88
47	Green and Black	42.0		0.82		0.81
48	Ichibaarashi	40.9		0.78		0.83
49	Rikuu 27	43.8		0.80		0.79
50	Shirodaizu	46.5		0.75		0.81
51	Ginjiro	45.0		0.77		0.78
52	Kurakake	43.7		0.89		0.68
53	Tanokurodaizu	40.7		0.73		0.77
54	Tairamame	42.9		0.76		0.67

Note: * The values of from No. 1 to No. 34 varieties are the average of 1968 and 1969. The values of No. 35 variety and thereafter are those of 1969 determination.
** The g/16 gN unit corresponds to the percentage in the protein containing 16% nitrogen.

crude protein contents and sulfur-containing amino acid contents is not significant, and the characteristic values of these characters are independently variable, but on the contrary, the correlatoin coefficients between the methionine and cystine contents is significantly positive, and they are both variable in the same trend (Table 6).

The differences of amion acid contents among the species of genus Glycine are shown in Table 7. The difference in each subgenus was scarcely recognized, but the differences between the subgenera were rather significant. That is, subgenus soja is superior to subgenus Leptocyamus with the contents of gultamic acid, aspartic acid, alanine, tyrosine, arginine, leucine and isoleucine, but as for the histidine contents, subgenus Soja

Table 6. Correlation among protein percentage and two sulfur-containing amino acid contents in soybean seed protein

Characters	Methionine	Cystine
Protein	−0.043 0.141	0.096 0.103
Methionine		0.495** 0.681**

* Significant at 1% level.
The upper figures were obtained from 1968's data and the below ones were calculated from 1969's data.

Table 7. Interspecific differences for amino acid composition of seed protein in the genus Glycine (g/16 gN)

Species \ Amino acids	GLY	ALA	VAL	ILEU	LEU	ASP	GLU	LYS	ARG	HIS	PHE	TYR	PRO	TRY	MET	CYS	SER	THR
Subgenus soja																		
G. max																		
Shirosaya 1	4.2	4.8	5.5	5.1	7.4	11.5	17.1	7.5	6.9	2.7	4.5	3.2	4.9	1.2	0.7	0.8	5.1	3.9
Yamashiratama	4.2	4.6	5.7	4.9	7.1	11.7	17.9	7.3	7.4	2.8	4.5	3.0	4.7	1.1	0.8	0.9	5.4	3.8
Kitamishiro	4.3	4.9	5.6	4.8	7.6	11.4	17.8	7.3	7.0	2.6	4.6	3.1	4.9	0.9	0.7	0.9	5.3	3.8
Hakuho	4.2	5.5	5.5	4.9	7.2	11.5	17.3	7.7	8.4	2.8	4.6	3.0	4.9	1.0	0.8	1.0	5.1	3.9
Yoshiokatairyu	4.2	4.4	5.7	5.0	7.4	11.6	17.5	7.7	7.1	2.8	4.5	3.1	4.7	1.0	0.8	0.8	5.5	3.7
Tenbokuwase	4.3	4.7	5.4	4.8	7.0	12.2	17.9	7.6	7.2	2.8	4.6	3.1	4.8	1.1	0.8	1.0	5.4	3.8
Marukotsubu	4.3	5.0	5.3	4.9	7.0	11.9	17.1	7.2	6.7	2.7	4.6	3.0	4.8	1.1	0.8	0.9	5.3	3.9
Lincoln	4.3	4.6	5.5	5.0	7.4	12.0	17.3	7.4	6.9	2.8	4.6	3.3	5.0	1.2	0.8	0.8	5.2	3.8
G. gracilis																		
T 34	4.7	5.3	5.8	5.0	7.5	12.4	18.3	7.4	7.2	3.0	5.1	3.2	5.2	1.1	0.9	1.1	5.7	4.1
PI 134-589	4.8	5.5	5.9	5.2	7.8	12.6	19.7	7.7	7.4	3.2	5.0	3.3	5.0	1.2	1.0	1.1	5.2	4.2
Giken	4.5	5.1	5.8	4.9	7.3	12.1	17.7	7.5	7.0	3.0	4.8	3.2	5.0	1.1	1.0	1.1	5.2	4.0
PI 135-590	4.3	5.1	5.3	4.8	6.9	11.7	18.3	7.3	6.9	2.9	4.7	3.0	4.8	1.1	0.9	0.9	4.8	3.7
G. soja																		
T 133	4.2	4.3	5.6	4.6	6.8	11.5	16.9	7.1	7.2	2.8	4.7	3.1	4.9	1.0	0.8	0.9	5.2	3.7
T 106-2	4.7	5.4	5.6	4.9	7.4	12.8	19.7	7.7	7.5	2.8	4.8	3.2	5.2	1.2	0.9	1.2	5.5	4.0
T 106-6	4.4	4.9	5.4	4.7	7.0	11.8	17.7	7.1	7.0	3.0	4.5	3.0	5.0	1.0	0.8	1.0	5.2	3.9
Nakatsutsumi 2	4.3	4.4	5.4	4.5	6.9	10.7	17.8	6.6	7.1	3.0	4.6	2.8	4.8	1.0	0.8	1.0	5.7	3.8
Nakatsutsumi 3	4.1	4.4	5.2	4.3	6.7	10.9	17.8	6.5	6.9	3.0	4.3	2.8	4.8	1.0	0.8	0.9	5.3	3.9
Futsuryu	4.3	4.3	5.3	4.7	6.7	10.7	17.2	7.4	6.5	3.0	4.3	2.9	4.4	1.0	0.8	1.0	5.4	3.8
Tairyu	4.1	4.2	5.4	4.5	6.5	10.6	17.3	6.6	7.1	3.1	4.3	2.8	4.6	1.1	0.8	0.9	5.5	3.9
Subgenus Leptocyamus																		
G. tabacina																		
Giken	4.2	3.7	5.4	4.0	6.2	9.9	16.5	6.6	7.2	3.5	4.3	2.7	4.7	1.0	0.7	0.7	5.4	3.9
Hutton	4.5	4.3	5.6	4.3	6.4	9.8	16.3	7.1	5.7	3.4	4.3	2.7	4.9	1.0	0.7	0.6	5.5	4.1
Formosa 4	4.1	4.3	5.0	4.0	6.0	8.8	15.9	7.3	5.7	3.3	4.5	2.8	4.5	1.1	0.7	0.7	5.3	3.9
G. tomentella																		
Eskdale	4.3	4.2	5.2	3.8	6.2	9.1	16.3	7.6	5.4	3.4	4.2	2.7	4.5	1.0	0.7	1.0	5.6	3.9
Inverelle	4.2	4.1	5.5	3.9	6.1	9.2	15.8	7.6	5.6	3.4	4.4	2.9	5.0	0.9	0.7	0.7	5.3	3.8
G. clandestina	4.3	4.6	5.5	4.1	6.4	8.7	17.6	8.4	6.3	3.5	4.7	3.3	4.4	0.9	0.7	0.9	5.8	3.9

is inferior to subgenus Leptocyamus. Furthermore we analyzed the sulfur-containing amino acid contents with 47 strains of G. soja collected from all over the country, but no significant species specificity, not the same as the crude protein contents, was recognized, and their values did not differ much from that of ordinary cultivated soybean (Table 7). Consequently, it is presumable that the gene resources which can greatly

Table 8. Increment of variances for sulfur-containing amino acid content in the M_2 populations treated with ethleneimine (EI).

(a) Methionine (g/16 gN)

Original variety	EI treatment hours	Materials in M_2		Mean	Variance ($\times 10^{-3}$)	Coefficient of variation
		No. lines	No. plants			
Raiden	0 (control)	9	9	0.89	1 245	4.0%
	2	71	224	0.93	2.568	5.5
	3	21	73	0.89	2.853	6.0
	4	21	82	0.89	1.179	3.9
Shirosaya 1	0 (control)	11	11	0.89	0.516	2.6
	2	62	221	0.92	3.114**	6.1
	3	39	122	0.93	3.310*	6.2
	4	33	112	0.95	3.248*	6.0

(b) Cystine (g/16 gN)

Original variety	EI treatment hours	Materials in M_2		Mean	Variance ($\times 10^{-3}$)	Coefficient of variation
		N.o lines	No. plants			
Raiden	0 (control)	11	11	0.96	4.167	6.7%
	2	71	224	0.91	9.597	10.8
	3	21	74	0.85	11.717*	12.7
	4	21	82	0.87	5.886	8.8
Shirosaya 1	0 (control)	11	11	0.89	3.629	6.8
	2	62	212	0.88	6.819	9.4
	3	39	122	0.90	7.387	9.5
	4	33	115	0.88	3.690	6.9

(c) Methionine+Cystine (g/16 gN)

Original variety	EI treatment hours	Materials in M_2		Mean	Variance ($\times 10^{-3}$)	Coefficient of variation
		No. lines	No. plants			
Raiden	0 (control)	11	11	1.77	8.000	5.1%
	2	71	223	1.84	12.752	6.1
	3	21	73	1.74	21.267	8.4
	4	21	82	1.76	8.140	5.1
Shirosaya 1	0 (control)	11	11	1.83	6.150	4.3
	2	62	212	1.80	12.145	6.1
	3	39	121	1.84	12.804	6.1
	4	33	112	1.83	9.522	5.3

*, ** = significant at 5 and 1% level, respectively.

increase the sulfur-containing amino acid contents of cultivated soybean could not be found among the related wild species of soybean.

The authors, in 1968, tried to induce the mutant strains related with the crude protein contents and sulfur-containing amino acid contents by the ethyleneimine treatment on Rainden and Shirosaya 1, the well known excellent varieties of soybean. The seeds set on M_2 plants were measured individually on their crude protein contents and sulfur-containing amino acid contents, and these measured values were arranged and examined according to the differences of used original varieties and treated times, and the means and variances of each treated group were compared with that of non-treated control group (Table 8). In the groups which were treated for 2 and 3 hours, the values of variances of every examined original variety and of every kind of examined amino acids increased clearly more than that of non-treated control group. And the increase of variance of the group treated for 3 hours is larger than that of 2 hour treatment corresponding to the intensity of treatment (length of treated hours). But all the variances of every group treated for 4 hours decreased. This is probably due to the high mortality caused by the too strong intensity of treatment of M_1 generation. But in anyway, it may be considered that some genetic variations were induced on the sulfur-containing amino acids of the treated groups, because the variances were varied in accordance with the intensity of ethyleneimine treatment.

We carried out some selections on the methionine contents with the generations subsequent to M_2. The strains of M_4 generation of which the mentionine contents were obviously increased or decreased in comparison with that of original variety are indicated in Table 9, and the M_5 generation is destined to come out from these strains in 1972.

The number of mutant strains that we keep now is seen in Table 10. All of these

Table 9. M_4 mutant Strains for methionine content in soybean seed protein

Original variety	Name of strain	M_2 (1969)		M_4 (1971)	
		Protein (%)	Methionine (g/16 gN)	Protein (%)	Methionine (g/16 gN)
Raiden	R2H 20-3	43.5	1.03	47.9	0.98 high
	R2H 21-5	41.7	1.03	47.0	0.97 high
	R3H 2-5	43.6	0.81	47.0	0.80 low
	R3H 5-7	41.2	0.80	48.6	0.80 low
	R3H 10-1	49.3	0.77	50.0	0.81 low
	Control	43.2	0.86	48.0	0.88
Shirosaya 1	S3H 3-4	47.5	0.96	53.9	0.96 high
	S3H 12-6	51.5	0.79	55.4	0.81 low
	S2H 3-3	49.1	0.81	52.9	0.80 low
	Control	46.2	0.90	54.7	0.84

Table 10. List of the mutant strains obtained with EI treatment for seed protein percentage and sulfur-containing amino acid content in protein

Original variety	Materials in M_2		M_4 lines					
			Protein		Methionine		Cystine	
	No. line	No. plant	high	low	high	low	high	low
Raiden	114	380	4	4	3	3	3	4
Shirosaya 1	134	458	3	4	4	4	3	2

are the mutant stains concerned to the seed protein contents and sulfur-containing amino acid contents of protein. The observation and investigation on the agronomically important characters and the seed constitutent analysis shall be carried out this year.

The Subjects for the Future Study

As we have got, step by step, the prospect of possibility to select out the mutant strains of sulfur-containing amino acid contents, we hope to clarify the mechanism of protein changes which induces such variation.

Fukushima and Koshiyama (1967) reported that there are two main globulin components which constitute the seed protein of soybean, that is, 11S and 7S, and their amino acid compositions are different from each other. It is said that the 7S component scarcely contains any sulfur-containing amino acid, so the total sulfur-containing amino acid contents are variable according to the change of constitutional ratio between 11S and 7S. But this conception is not always accepted without any reservation. At present the mechanism of the variation of sulfur-containing amino acid contents which can be recognized on the varieties of soybean remains unsolved.

We are intending this year, to separate the seed protein into some fractions with Sephadex G-200, and investigate some principal fractions, preliminarily if possible, to detect the variation of sulfur-containing amino acid contents in the protein.

Refferences

1. Fukui, J. and N. Kaizuma (1971): Morphological and ecological differenciation of various characters of the Japanese wild soybean, *Glycine soja*. Jour. Fac. Agr. Iwate Univ., 10(4), 195–208 (Japanese).
2. Fukui, J., Hi. Taira, N. Kaizuma and Ha. Taira (1972): Subgeneric and specific differences in the content and amino acid composition of the seed protein in the genus *Glycine*. Japan J. Breed., 22(4), 1–6 (Japanese).
3. Fukushima, D. and I. Koshiyama (1967): 7S and 11S components of soybean globulins. Food Industry, 10(16), 104–118 (Japanese).
4. Gavrilyuk, I. P., S. T. Satbaldina and V. G. Konarev (1970): Absence of the 11S component of the gloublins of wild species of soybean. (translated from Doklady Akademii Nauk SSSR, 190(5), 1229–1231, 1970)
5. Hokkaido National Agr. Exp. Station (1962–1964): Reports on the soybean breeding experiments (mimeographed, Japanese).
6. Hokkaido Prefectural Agr. Exp. Station (1963–1964): Reports on the soybean breeding experiments (mimeographed, Japanese).
7. Krober, O. A. (1956): Methionine content of soybeans as influenced by location and season. Jour. Agr. Food Chem., 4, 254–257.
8. Krober, O. A. and J. L. Cartter (1966): Relation of methionine content to protein levels in soybeans. Cereal Chem., 43, 320–325.
9. Kumamoto, T., S. Yoshitomi and T. Momoshima (1964): On the heritabilities for protein content of summer soybean seed and its genetic correlations with other agronomic characters (Japanese). (Kyushu Agr. Res., 26, 89–90.
10. Leffel, R. C. and M. G. Weiss (1958): Agron. J. 50, 528–534. (cited from "The Soybean" edited by A. G. Norman and published by Academic Press, New York, 1967).
11. Metz, E. T. and L. S. Bates (1964): Mutant gene that changes protein composition and increases lysine content of maize endosperm., Science 45, 279–280.
12. National Institute of Agricultural Sciences, Tokyo (1963): The basic experiments on the breeding of high protein soybean (mimeographed, Japanese).
13. Saga Prefectural Agr. Exp. Station (1968): Studies on the breeding of high protein soybean varieties (cited from "Soybean Breeding", Chap. 5, 98–147, edited

by J. Fukui and published by Latice, Tokyo).
14. Vilijoen, N. J. (1937): An investigation into the composition of the soybean South Africa. Union of South Africa Dept. Agr. and For. Sci., 169, 1–68, (cited from Weber: 1950).
15. Weber, C. R. (1950): Inheritance and interrelation of some agronomic and chemical characters in an interspecific cross, *Glycine max* × *G. ussuriensis*. Iowa Agr. Exp. Sta. Res. Bull., 374, 767–816.
16. Williams, L. F. (19648): Inheritance in a species cross in soybeans. Genetics, 33, 131–132.