

Fundamental Studies on Soybean (*Glycine max* (L.) Merrill) Seed Protein Improvement

By NORIHIKO KAIZUMA

Faculty of Agriculture, Iwate University

It is said that world protein production should be doubled by the end of this century to meet under- or mal-nourished nutrition problems prevailing in developing countries. Soybeans, as one of the most promising plant protein sources, are expected to be better utilized in many peoples over the world. Discovery of high lysine corn⁸⁾ and barley²⁾ encouraged further studies on nutritional improvement of various crops including soybeans. Main research objectives for genetic improvement of soybean protein are (1) further increase of protein percentage in seed, (2) increase in relative contents of sulfur-containing amino acids in unit weight protein, and (3) genetic removal of anti-nutritional factors, e.g., trypsin inhibitors, or unfavorable substances, e.g., beany-taste and flatulence-causing factors. In addition, it is a matter of course that high yielding ability should be pursued in soybean breeding programs because soybeans are remarkably low yielder compared with such cereals as rice and wheats.

In the present paper, studies on soybean protein breeding carried out by the author since 1968 will be briefly presented. Details of most of the studies are shown in a reference No. 5.

Some features of soybean seed protein

It is well known that, concerning essential amino acid balance, legume seed protein is generally deficient in sulfur-containing amino acids (methionine and cystine) in contrast with cereal seed protein which is short in lysine content. More than doubling of sulfur-con-

taining amino acid content is required for improving the quality of soybean protein to a level equivalent to animal protein.

Comparison of sulfur-containing amino acid content as well as protein percentage among various leguminous species is given in Table 1. It shows that soybeans have relatively higher protein percentage and their protein contains relatively higher sulfur-containing amino acids among all species tested. Consequently, soybeans can be regarded as one of the most well-developed pulse crops so far as their protein content and quality are concerned. However, as shown in Table 1, wild soybeans (*Glycine soja* Sieb. and Zucc.), which is likely a putative ancestor of cultivated soybeans, indicated higher protein percentage with almost similar sulfur-containing amino acid content compared with cultivated soybeans. This was reconfirmed by further similar determinations conducted with a number of wild soybean strains collected from all over Japan⁴⁾. Therefore, wild soybeans are considered to be a useful gene source for upgrading protein content of soybeans without undesirable effects on protein quality.

Potentiality of soybean seed protein improvement in quantity and quality

The author had conducted a number of hybridizations between soybeans and wild soybeans. As a result, it was clearly demonstrated as previously reported by Weber¹⁾ and Hartwig³⁾ that transgressive segregants for protein percentage could be easily selected out in F_2 and subsequent generations. Statistical

Table 1. Generic and specific differences for protein percentage and sulfur-containing amino acid content among various leguminous species

Species name	No. strains tested	Protein (%)	S-amino acid content (g/16 gN)
1. <i>Cercis chinensis</i>	1	40.2	1.69
2. <i>Cytisus scoparius</i>	1	34.4	1.89
3. <i>Lupinus luteus</i>	2	44.0	1.71
4. <i>Rhynchosia volubilis</i>	1	20.8	1.75
5. <i>Dumbaria vilosa</i>	2	22.5	1.79
6. <i>Phaseolus vulgaris</i>	7	21.8	1.69
7. <i>Dolichos lablab</i>	1	26.3	1.28
8. <i>Vigna sinensis</i>	6	24.7	1.78
9. <i>Pachyrhizus erosus</i>	1	34.1	1.97
10. <i>Glycine max</i> (soybeans)	8	43.1	2.06
11. <i>G. soja</i> (wild soybeans)	5	44.9	2.43
12. <i>Amphicarpaea edgeworthii</i>	1	29.4	2.31
13. <i>Vicia faba</i>	2	30.8	1.49
14. <i>Cicer arietinum</i>	2	30.8	1.75
15. <i>Pisum sativum</i>	5	26.6	1.47
16. <i>Medicago sativa</i>	3	38.9	1.62
17. <i>Trifolium repens</i>	2	35.1	1.39
18. <i>Lotus corniculatus</i>	1	33.2	1.10
19. <i>Arachis hypogaea</i>	4	28.9	1.85

estimation for number of effective factors based on Wright's formula resulted in that three factors would be responsible for protein percentage inheritance in F_2 generation in the species cross. This result well agreed with Weber¹¹). Repeated backcrossing for certain times to soybean parent is needed because viny plant type dominates among single cross progenies. Studies on this problem are now under way, but one time backcrossing was still unsatisfactory based on observation of B_1F_2 population derived from a species cross, (Hakuho soybean cultivar \times a wild soybean strain) $F_1 \times$ Hakuho.

Amino acid profiles of the whole protein hydrolyzed from whole ground seeds of the genus *Glycine* species were measured with bioassay method.¹¹ Close similarity was indicated for 18 amino acid composition including sulfur-containing amino acids. It was considered that realization of marked increasing of sulfur-containing amino acid content could not be expected by any species cross within the genus *Glycine* even if the crossing is possible. Soybean seed protein as well as other pulse crop proteins is composed of relatively simple protein fractions (mainly of globulins, ca. 80% in quantity).

Soybean globulins contain mainly 11S and 7S fractions, relatively rich and poor for sulfur-containing amino acids, respectively. Variation of 11S/7S content ratio in soybean globulins is likely to cause varietal differences in the amino acid content in protein.

In fact, it is ascertained that there exist detectable genetic variations for sulfur-containing amino acid content among the population of soybean cultivars.⁹⁾ Therefore, it is natural that steady quality improvements could be attained with usual breeding procedures.

Recently, Kitamura and Shibasaki⁷⁾ revealed subunit structure of 11S protein which is known as a component of soybean globulins with relatively higher sulfur-containing amino acid content. Subsequently, Than and Shibasaki¹⁰⁾ succeeded in clarifying subunit structure of 7S protein which is a component of soybean globulins with relatively lower sulfur-containing amino acid content. Inheritance studies on 11S and 7S proteins as well as their subunits are now just at the beginning. The author believes however, that genetic operation procedures to increase 11S and decrease 7S in soybean seed protein will soon be established. In his laboratory a few soybean cultivars with distinctory

lower content of 7S subunit were discovered by Kitamura with densitometric survey of SDS electrophoreogram (unpublished).

Removal of anti-nutritional factors

During the stay at University of Illinois in 1975 through 1976, the author⁶⁾ discovered two soybean cultivars which were characterized by lacking of Kunitz trypsin inhibitor activity through an electrophoretic investigation of a number of soybean cultivars collected from all over the world. They were both Korean



Plate 1. Morphology of soybeans with no Kunitz trypsin inhibitor.

Left: Kum du (before maturity: late-maturing)

Right: Baik tae (at maturity: early-maturing)

cultivars named "Kum du" and "Baik tae". They had been numbered PI 157440 and PI 196168, respectively, in the United States. Among five different sources of "Kum du" and "Baik tae" derived from recent accessions from the Korean Agricultural Experiment Station and so on, only one source of the two varieties showed the inactivity of Kunitz trypsin inhibitor.

By further studies carried out later using DEAE-cellulose column chromatography, polyacrylamide slab-gel electrophoresis, immunochemical diffusion test and hybridization experiment, it was recognized that the two cultivars are unable to synthesize Kunitz trypsin inhibitor itself, and such a peculiar character-

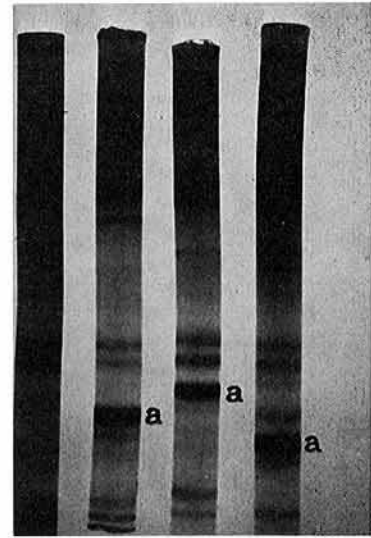


Plate 2. Electrophoretic polymorphism found in banding pattern of Kunitz trypsin inhibitor (the darkest band, a). By courtesy of Hymowitz, T.

Leftmost: Pattern of the inhibitor-free cultivars

Other: Pattern of the three polymorphic inhibitors

istic is conferred by a recessive gene. The details including descriptions on other properties will be published soon. It is very interesting that the entire synthesizing process of Kunitz trypsin inhibitor is controlled only by a single gene. This finding offers an expectation that other anti-nutritional factors, e.g., Bowman-Birk trypsin inhibitor, might also be under the control of a single gene.

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