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1) Subunit composition of glycinin from various samples of cultivated and wild soybean.

The major storage protein of 11S class of soybean seeds, glycinin, has a complex subunit structure. Each of the six subunits is composed of two protein molecules (acidic and basic), linked via disulphide bonds (Badley et al., 1975). Depending on subunit, the acidic moiety molecular weight varies from 37,000 to 42,000, with one exception (m.w. 10,000). By this parameter, the basic moieties of the subunits are more homogeneous (m.w. close to 20,000) (Moreira et al., 1979).

Plant storage protein genes are studied intensively. These genes seem to be an appropriate model for studying the regulation mechanisms of protein biosynthesis, gene structure, and evolution. It has been shown that multiple genes coding individual glycinin subunits may be grouped in several families (Goldberg et al., 1981). The variability of subunit structure within the corresponding protein families and the evolutionary interrelatedness of the subunits has not been studied in detail. On the basis of such data, mechanisms of soybean storage protein evolution may be elucidated, as well as the species specificity of its structure. Such information also is necessary for improving soybean agronomic traits, i.e., the methionine content, by selection.

In this paper, we compare the subunit composition of purified glycinin isolated from the seeds of various soybean cultivars and wild forms of these plants.

Materials and methods

Protein extraction and isolation of glycinin. Seed samples were obtained from the All-Union Institute of Plant Breeding (USSR, Leningrad). Protein extraction was carried out according to Hill and Breidenbach (1974). The protein extract (3-5 mg of protein in 200 µl of sodium phosphate buffer, containing 0.4 M NaCl and 10 mM of 2-mercaptoethanol, pH 7.5, buffer A) was layered on a sucrose density gradient (10-35% w/v, buffer A) was centrifuged for 18 hr at 36,000 r.p.m., rotor SW-41, "Beckman", at 20°C. Protein content in each of 30-40 fractions was estimated as described by Sedmac and Grossberg (1977), and

the relative content of 11S component was estimated by the area (weight) of the peak, corresponding to glycinin in the sedimentagram. In order to isolate glycinin, the total extract was fractionated with ammonium sulphate and isoelectric precipitation in 60 mM Tris-HCl (Wolf et al., 1962). After this procedure, glycinin (20-40 mg), containing admixtures of β-conglycinin and other proteins, was purified by passing through a column (1.5 x 10 cm) with concanavalin-A-sepharose ("Pharmacia") at a rate of 10 ml/hr in order to eliminate glycosylated admixtures. The protein of the first peak was precipitated with ammonium sulphate, dissolved and purified by passing through a column with DEAE-Sephadex A-50 (2.2 x 20 cm), as recommended by Mori et al. (1979). In the final stage, the material of 11S peak was separated by sucrose density gradient centrifugation under the conditions described above.

The amino acid analysis. Automatic amino acid analyzer Durrum D-500 was used. Glycinin samples were previously oxidated by performic acid for 4 hr, liophylized and then hydrolyzed by 5.7 M HCl for 24 hr at 110°C.

Electrophoresis and isoelectric focusing. Electrophoresis in 10% polyacrylamide gel with sodium dodecyl sulphate (SDS) was carried out according to the method of Laemmly (1970). Isoelectric focusing was carried out in "Multifor" ("LKB") in pH gradient 3.5-10 with 6 M urea for 4 hr at 1,000 V. Gel was stained as described by Jacle (1979).

Chromatography of glycinin subunits. Purified glycinin was reduced and subjected to S-alkylation with 4-vinylpyridin as described by Hermodsen et al. (1977). The reduced protein preparation was dialyzed against 50 mM sodium phosphate buffer with 6 M urea and 20 mM of 2-mercaptoethanol, pH 6.6. Chromatography (0.5-1.0 mg of protein) was carried out on "Mono Q2" column of fast protein liquid chromatography (FPLC) system ("Pharmacia"); the column was previously equilibrated with the same buffer. Elution was carried out with salt concentration gradient.

Results and discussion

The content of glycinin in the soybean varieties. Three major peaks with sedimentation coefficients 2S, 7S, 11S, respectively, and one minor peak (15S), corresponding to aggregated material, were observed. The amount of the aggregated material does not exceed 5%. Data on glycinin content based on the estimated proportion of the material of the 11S peak are presented in Table 1. The results demonstrate that glycinin content in most cultivated and semiwild varieties is 36-41%. The content of 11S protein in wild perennial species is lower.

Table 1. The content of the 11S component in different samples of soybean seeds (in % to extractable protein)

Sample No.	Cultivar	Species, subspecies	Content of 11S component, percent
1	Rannaja-10	*G. max*, ssp. *chinensis* Enk.	40.0
2	Ada	ssp. *manshurica* Enc.	40.0
3	Merit	ssp. *manshurica* Enc.	39.0
4	Richland	ssp. *manshurica* Enc.	37.0
5	Hardom	ssp. *manshurica* Enc.	38.5
6	Mandarin	ssp. *manshurica* Enc.	39.0
7	K-5683	ssp. *gracilis*	36.5
8	K-4937	ssp. *slavonica* Enk.	37.0
9	K-6910	*G. ussuriensis*	33.5
10		*G. canescens*	20-25+
11		*G. clandestina*	13-16+
12		*G. tabacina*	20-26+

+11S component content varies in individual samples.

Amino acid composition of glycinin samples. The results of the amino acid analysis of glycinin are presented in Table 2. The data allow us to conclude that the content of the individual amino acids is the same in all samples, and the deviations from the mean value do not exceed the experimental error. In general, the results obtained correspond to those of Moreira et al. (1979).

The subunit composition of glycinin of soybean varieties. Fig. 1a demonstrates patterns of glycinin SDS - polyacrylamide gel electrophoresis. One can see that all glycinin patterns, including wild soybean species, are formed by the same acidic and basic subunits with similar electrophoretic characteristics. Fig. 1b shows the results of the isoelectric focusing of glycinin. The pattern reveals noticeable heterogeneity of glycinin polypeptides. Such heterogeneity for isolated glycinin subunits was first reported by Moreira et al. (1981). Similarity in the location of bands representing polypeptides of glycinin from different seed samples is obvious. To reveal the possible differences in subunit composition of glycinin, the acidic subunits were

Table 2. The amino acid composition of glycinin from various seed samples

Sample No.+	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg	Met
1	10.8	3.7	6.7	18.9	6.8	7.8	4.9	4.7	4.3	7.4	1.9	3.5	2.0	4.8	9.6	1.1
2	11.0	3.8	6.8	19.0	6.7	8.1	4.9	4.6	4.3	7.7	2.3	3.7	2.1	4.8	9.2	1.1
3	11.6	3.8	6.8	19.5	6.9	7.9	5.3	4.6	4.2	6.8	2.0	3.7	2.0	4.5	8.5	1.2
4	11.5	3.8	6.7	19.0	6.5	7.6	5.1	4.7	4.3	7.7	2.2	3.5	1.9	4.4	8.7	1.2
5	11.5	3.8	6.8	19.5	6.8	7.9	5.1	4.7	4.1	6.8	2.1	3.4	2.2	4.7	8.7	1.0
6	11.4	3.6	6.8	20.1	7.1	7.9	4.7	4.7	4.2	7.2	2.2	3.5	2.0	4.2	8.2	1.2
7	11.8	3.8	6.6	20.0	6.1	7.8	5.5	4.9	4.7	8.3	2.3	4.0	1.5	4.0	7.1	1.0
8	11.3	3.8	6.4	18.1	5.2	7.8	5.4	4.9	4.1	7.6	1.9	4.1	1.9	4.9	10.3	1.1
9	11.8	3.8	6.5	20.0	5.9	7.8	5.5	4.9	4.8	8.2	2.0	4.1	1.6	4.2	7.1	1.1

+Sample numbers are the same as in Table 1.

separated by FPLC. The typical chromatographic profile has six major peaks (Fig. 2). Fraction 1 corresponds to basic subunits with the admixture of 4-vinylpyridine. Fractions 1a, 2 and 3 correspond to polypeptides of the electrophoretic mobilities corresponding to those of A_1 subunit (Moreira et al., 1979). Fraction 4 corresponds to A_2 subunit, and fractions 5 and 6 contain subunits A_3 and A_4, respectively. The chromatographic patterns were roughly identical for all samples analyzed. It was of interest to study the subunit structure of glycinin of the wild species of soybean. It appeared that in both *Glycine max* and *Glycine ussuriensis*, the subunit structure of glycinin is very similar (Fig. 2b). Generally, it was found that glycinin from all the seed samples consists of the identical sets of polypeptides.

Conservatism of glycinin subunit composition. The data obtained suggest that the subunit composition of glycinin of cultivated soybean and its probable ancestor is characterized by pronounced conservatism. Staswic et al. (1983) point out that entries from the northern USDA collection also do not exhibit polymorphism. The available data concerning glycinin polymorphism are not numerous and matter rather for scientific than practical aspect of the problem. Thus, according to our data on glycinin structure conservatism, we can scarcely increase the glycinin methionin content by breeding due to the absence of the subunit polymorphism. As glycinin makes up more than 40% of the total protein, the improvement of amino acid composition becomes a quite complicated problem.

The conservatism of glycinin subunit composition seems to depend on its structure, because the storage proteins of other legumes reveal polymorphism. It is also known that other protein components of soybean seeds may be lacking, due to mutations (Orf and Hymowitz, 1979; Orf et al., 1978). The mechanism of glycinin synthesis via high molecular weight precursor (Barton et al., 1982; Epishin and Vinetsky, 1983) limits possible rearrangements, because any subunit substitution will affect both acidic and basic moieties. Besides that, substitution of any pair of subunits may alter the mechanism of assembling of the glycinin molecule. So, the glycinin conservatism probably reflects the strict order of subunit assembling into a glycinin complex.

As it was recently shown by Staswic et al. (1983), wild perennial species of the genus *Glycine* have a slightly different structure of 11S globulin. It is possible that the increase in the 11S protein content and a growth of the seed size are connected with the advantages of glycinin structure, which is

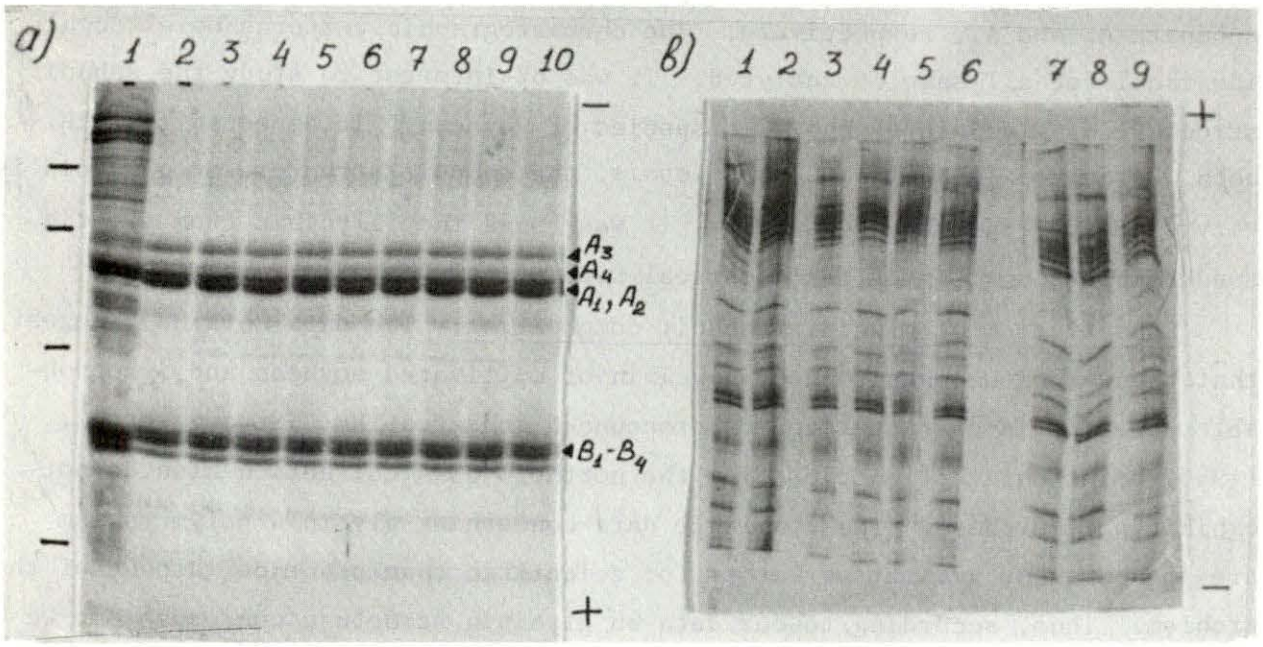

Fig. 1. a) SDS - electrophoresis of purified glycinin in 10% gel. The samples loaded: 1 - Rannaja-10, total protein; 2 - 8-preparations of glycinin; sample numbers correspond to those in Table 1; 9 - glycinin, sample 1; 10 - sample 9. To the left, the position of molecular weight markers are shown (from top to bottom: 67 kD, 45 kD, 25 kD, 12 kD). The bands of glycinin are marked according to Moreira et al. (1979).

b) Isoelectric focusing of purified glycinin in 10% gel. The sample numbers correspond to those in Table 1.

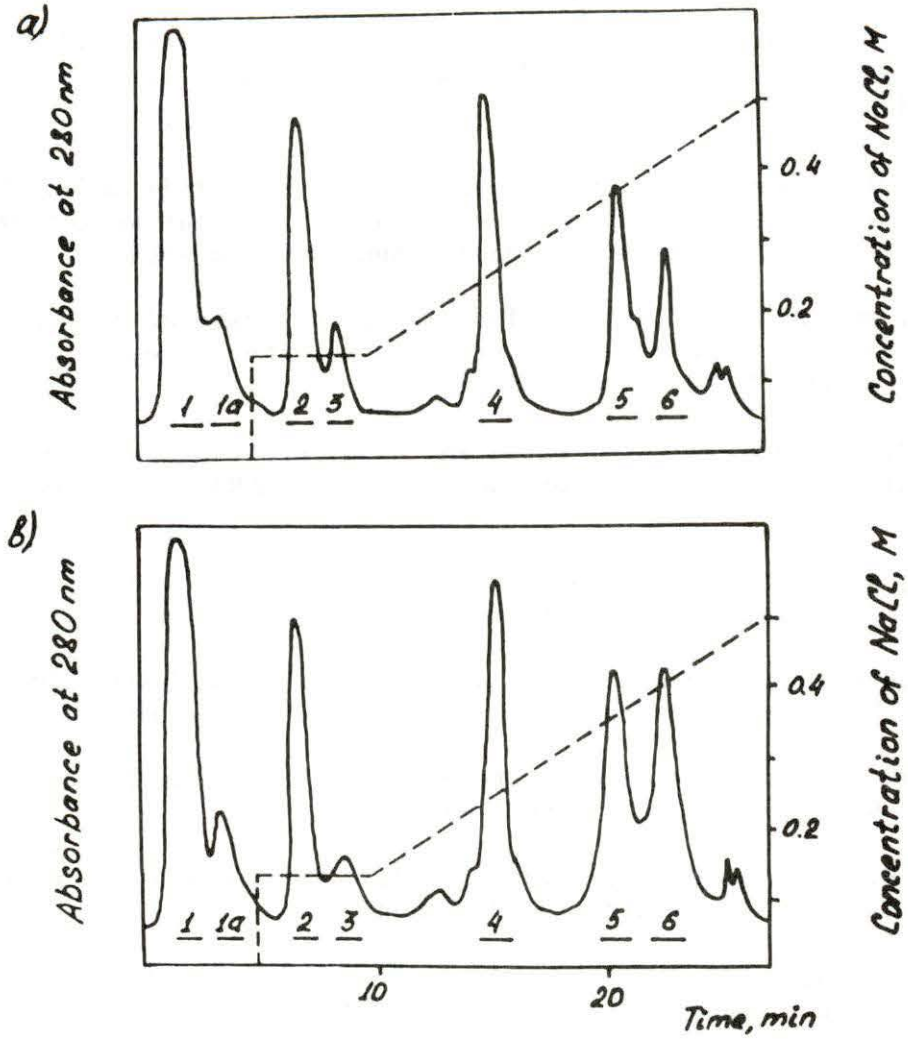

Fig. 2. The chromatographic profiles of reduced glycinin on "Mono Q" column. _____ - the absorbance at 280 nm; ----- - salt concentration. a) Sample 1 (*Glycine max*); b) Sample 9 (*Glycine ussuriensis*).

observed in the cultivated soybean. Evolutionary advantages of this structure seem to have developed in consequence of the genetical isolation during the domestication and as a result of artificial selection.

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