

METHIONINE CONCENTRATION IN SOYBEAN PROTEIN FROM POPULATIONS SELECTED FOR INCREASED PERCENT PROTEIN¹

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ABSTRACT

When a component of grain, such as protein or oil, is changed quantitatively through plant breeding, it is important that the quality of that component be maintained. The current study was conducted to determine if protein quality, as measured by methionine concentration, had changed as a result of recurrent selection for increased protein in four soybean [*Glycine max* (L.) Merr.] populations.

Selected cycles from four populations were grown in replicated field experiments in 1975 and 1976. Two populations, designated IA and IB, were grown in one environment; the other two, designated IIA and IIB, were grown in another environment. In 1978, populations IA and IIA were tested together in a replicated field experiment in two environments. Percent protein of the seed and methionine concentration of the protein were determined in all experiments.

Differences in methionine concentration among selected cycles of the four populations tested in 1975 and 1976 were generally small. Therefore, selection for high percent protein in the soybean populations did not result in large changes in methionine levels in the protein. Population IA protein had a lower mean concentration of methionine than population IIA.

When populations IA and IIA were tested together in two environments in 1978, differences in methionine percentage of the protein among cycles of selection were nonsignificant, as before. However, the average methionine concentration of the two were similar and location by cycle interactions were highly significant. These results suggest that the differences in methionine concentration, previously observed between populations I and II, were a result of environmental influences. The lack of relationship between protein percent and methionine concentration of protein demonstrates that methionine is not likely to decrease as a result of selection for higher protein, at least not in early cycles of selection.

Additional index words: Protein quality, Selection, *Glycine max* (L.) Merr.

PLANTS are an important source of edible protein, but seed proteins lack sufficient quantities of one or more of the amino acids which are essential for proper animal nutrition. Thus, seed protein is usually considered to be of lower quality than animal and marine proteins and frequently requires synthetic amino acid supplementation if it is used in animal diets.

Concentrations of sulfur-containing amino acids, cystine and methionine, are notably low in soy protein. Of these, methionine limits protein quality most (3). Examination of the soybean [*Glycine max* (L.) Merr.] germplasm collection for Maturity Groups V through X revealed the methionine content among the genotypes evaluated to be between 1.1% and 1.6% of the total protein (4). Methionine level in the soy proteins currently grown is about 43% of that in the standard FAO (Food and Agricultural Organization) hen's egg (14). Thus, soy protein would need to contain about 3% methionine to provide the methionine equivalent of egg protein. Synthetic methionine is routinely used as a supplement to diets containing soybean meal to improve their nutritional quality.

There have been no reported efforts to increase percent methionine in soy protein through breeding. This is due primarily to the difficult and time-consuming nature of methionine assays and to a perception that genetic variability for the trait among soybean lines in the germplasm collection is limited (7, 12). Also, percent methionine in protein and protein concentration in the seed have been reported to be negatively correlated in some leguminous species: *Phaseolus vulgaris* (1, 7), and *Vicia faba* (10, 11). Although this negative relationship has not been found in soybeans (5, 6), the fact that it occurs in other legumes makes its occurrence in soybeans not unlikely.

Brim and Burton (2) successfully used recurrent selection to increase protein concentration in soybean seeds. Protein quality was not considered in the selection process. However, amino acid analyses of selected lines from early cycles of selection indicated that a decline in protein quality had not accompanied the increase in percent protein (C. A. Brim, personal communication). The purposes of the study reported here were to determine (a) the levels of methionine in seed protein from the soybean lines selected in six cycles of recurrent selection, and (b) the existence of a relationship between percent methionine in seed protein and protein concentration in soybean seeds.

MATERIALS AND METHODS

Two populations, each divided into A and B subpopulations, were used in recurrent selection experiments (2) to increase seed protein. Population I was derived from a cross of

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two highly adapted experimental lines, D55-4110 and N56-4071. The IA base population (Cycle 0 or C₀) originated from a set of 247 F₄ lines derived from the cross. The IB base population (C₀) was derived from the test population of the second cycle of selection in IA. Population II was derived from a cross between a highly-adapted experimental line to nine plant introductions, followed by a backcross of the F₁ plants to the adapted parent. The base populations for both IIA and IIB were derived from 477 F₁ progenies of the backcross. The A subpopulations of populations I and II were begun initially and continued throughout the selection process with effective population sizes four times larger than those of the B subpopulations. A detailed description of the population origins and selection procedures is published elsewhere (2).

The materials used in this study were composite populations of the lines selected from each cycle of four recurrent selection programs for increased percent protein in soybeans. In IA and IB, composite populations from six (C₀ to C₆) and four (C₀ to C₄) cycles of selection, respectively, were tested. In populations IIA and IIB, five (C₀ to C₅) and four (C₀ to C₄) cycles, respectively, were used. All selected lines were subjected to the same degree of inbreeding before making the composites. Ten replications of the composite populations of each cycle were evaluated for percent protein and oil and yield in several year-location environments. A description of the testing procedures has been published (2).

Five of the 10 replications of IA and IB populations in each environment were analyzed for percent protein. Methionine concentration was determined for the protein samples from one environment (Plymouth, N. C., 1975). Combinations of Replicates 1 and 2, Replicates 3 and 4, and Replicate 5 provided three samples from each selection cycle for methionine analysis. All 10 replications of the IIA and IIB populations in each environment were analyzed for percent protein. Methionine concentration was determined for the protein samples of nine replications from one environment (Kinston, N. C., 1976). Methionine analysis was performed on three samples for each cycle, which were obtained by combining sets of three replicates. In combining replicates, seed samples from each replication were ground separately. Equal quantities of each were then mixed together in a container and homogenized on twin shell dry blenders for 2 hours.

The information concerning methionine concentrations was obtained for Populations IA and IB grown in one environment and for Populations IIA and IIB grown in a different environment. We compared the two populations when grown under the same environmental conditions to obtain information on possible environmental effects on methionine concentration.

In 1978, composite populations from Cycles 0, 2, 4, and 6 cycles of selection in populations IA and IIA were tested in four replications at each of two locations, Clinton and Clayton, N.C. A split-plot design was used with populations as whole plots and cycles as subplots. Protein percentage and methionine concentration of the protein were determined for mature seed from each plot. All samples were prepared for analysis as in the previous experiments.

Samples containing 6 ± 0.5 mg protein were weighed to 0.01 mg into 10-ml ampules and covered with 4 ml 6N HCl and 4 ml propionic acid. Necks of the vials were heated and drawn to less than 1 mm I.D. The vials were placed into a desiccator which was evacuated until the acid began to bubble and the vacuum was released with N₂ gas. The procedure was repeated three times. Necks of the vials were sealed under 2 kg/cm² pressure and heated at 121 C in an autoclave for 2 hours. Samples were taken to dryness under vacuum at 40 C in a rotary film evaporator and taken up in 10 ml sample dilution buffer. Samples were clarified by centrifugation (10,000 × g for 5 min) and 0.50-ml aliquots were introduced

Table 1. Mean percent protein per cycle of selection in populations IA and IB and the mean concentration of methionine in the protein; Plymouth, 1975.

| Cycle | IA | | IB | | |
|----------------|--------------|-------------------------------|----------------|--------------|-------------------------------|
| | Protein % | Methionine g/100 g protein | Cycle | Protein % | Methionine g/100 g protein |
| C ₀ | 44.9 | 1.07 | | | |
| C ₁ | 46.0 | 1.03 | | | |
| C ₂ | 46.0 | 1.11 | C ₀ | 45.3 | 1.17 |
| C ₃ | 46.7 | 1.07 | C ₁ | 45.7 | 1.18 |
| C ₄ | 47.1 | 1.15 | C ₂ | 45.6 | 1.42 |
| C ₅ | 46.5 | 1.12 | C ₃ | 46.1 | 1.57 |
| C ₆ | 47.5 | 1.06 | C ₄ | 46.5 | 1.39 |
| L.S.D. | | | | | |
| 0.05 | 0.7 | N.S. | | 0.9 | N.S. |

Table 2. Mean percent protein per cycle of selection in populations IIA and IIB and the mean concentration of methionine in protein; Kinston, 1976.

| Cycle | IIA | | IIB | |
|----------------|--------------|-------------------------------|--------------|-------------------------------|
| | Protein % | Methionine g/100 g protein | Protein % | Methionine g/100 g protein |
| C ₀ | 43.8 | 1.26 | 45.0 | 1.45 |
| C ₁ | 44.6 | 1.33 | 46.2 | 1.38 |
| C ₂ | 44.9 | 1.27 | 46.0 | 1.25 |
| C ₃ | 45.8 | 1.24 | 46.7 | 1.29 |
| C ₄ | 46.6 | 1.33 | 47.2 | 1.27 |
| C ₅ | 47.4 | 1.21 | | |
| L.S.D. | | | | |
| 0.05 | 0.4 | N.S. | 0.4 | N.S. |

into the amino acid analyzer (Beckman³ model 119 with automatic sample injection). Resin and buffers were those recommended by Spackman et al. (13). In our laboratory the hydrolysis procedure using propionic acid gave higher methionine values than either the standard 16-hour hydrolysis in 6 N HCl at 110 C (9) or the 2-hour hydrolysis at 145 C in 6 N HCl (8). All three methods gave comparable results for the other amino acids.

RESULTS AND DISCUSSION

As reported previously (2), percent protein increased linearly with selection in all four populations. In the 1975 Plymouth environment, the composite population of selected lines from Cycle 6 (C₆) had an average percent protein 47.5 compared to 44.9 for the beginning C₀ population (Table 1). In the IB population, C₄ was 46.5% compared with 45.3% for C₀. In the 1976 Kinston environment, C₅ of population IIA averaged 47.4% protein compared to 43.8% for C₀, and in IIB, C₄ was 47.2% compared to 45.0% for C₀ (Table 2).

The magnitude of the observed methionine concentrations of the protein are within the range of those previously reported (Tables 1 and 2). The highest, 1.57 for C₃ of IB, is very close to the highest value,

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Table 3. Mean percent protein per cycle of selection in populations IA and IIA and the mean concentration of methionine in the protein, 1978.

| Cycle | IA | | IIA | |
|----------------|---------|-----------------|---------|-----------------|
| | Protein | Methionine | Protein | Methionine |
| | % | g/100 g protein | % | g/100 g protein |
| C ₀ | 49.4 | 0.91 | 46.0 | 1.12 |
| C ₁ | 49.8 | 0.96 | 48.5 | 0.97 |
| C ₂ | 49.8 | 1.03 | 49.6 | 0.95 |
| C ₄ | 51.0 | 0.99 | 50.8 | 1.04 |
| L.S.D. 0.05 | N.S. | N.S. | 1.9 | N.S. |

1.61, reported for lines in the germplasm collection of Maturity Groups V to X (4). The variation in methionine concentration of protein among cycles was not statistically significant at the 0.05 probability level in any of the four populations, and with the exception of population IB, the differences in methionine concentration among cycles of selection within a population were small. For example, in population IA concentrations ranged from a low of 1.03 in C₁ to a high of 1.15 in C₄ (Table 1). Thus selection for high percent protein in the soybean populations did not result in large changes in methionine levels.

When the A populations were tested simultaneously in the 1978 environments, the protein concentrations were greater. Population IA showed a smaller total increase in protein from 49.4% to 51.0% (Table 3), and population IIA showed a greater increase, from 46.0% to 50.8%. The latter increase may be partially due to the additional cycle of selection (C₆ in 1978 vs. C₅ in 1976).

As in the earlier tests, the variation among cycles in methionine concentration in the protein was not statistically significant. Methionine concentrations were lower in the 1978 tests. In Population IA, the average methionine concentration of the protein in 1978 was 0.97% compared to 1.10% in 1976 (averaged over Cycles 0, 2, 4, and 6). The 1978 methionine concentration for population IIA was 1.01% compared to 1.29% in 1976 (averaged over Cycles 0, 2, and 4).

These results show that protein quality is probably affected by environmental factors. When IA and IIA were grown together in 1978, they were quite similar with respect to methionine concentration. Also, variation due to the interaction between locations and cycles within populations was highly significant in the 1978 analysis. This suggests that differences between

IA and IIA in 1975 and 1976 tests were a result of environmental and not genetic differences. The lack of correlation between percent protein in seeds and methionine concentration in the protein is evidence that protein quality is not likely to decrease significantly as a result of selection for higher protein, at least not in six or fewer cycles of selection.

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