Food Legumes—Compositional and Nutritional Changes Induced By Breeding

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The desirable combination of ability to biologically fix nitrogen and the capacity to accumulate abundant seed protein makes the food legumes attractive at a time when nitrogen fertilizer costs are high and there is increasing demand for protein. The pulses (food legumes) include several genera and are distributed worldwide. However, their importance varies greatly between localities, often depending on the per capita income of consumers. Roberts (1) has pointed out that protein from food legumes comprises nearly all the dietary protein for up to 50% of the people in many less developed countries.

The efficiency of protein production (ratio of energy input to protein output) in food legumes compares favorably with other major food crops such as potato, corn, rice and wheat. However, if the food legumes are to become even more important protein sources, improvement in the yielding ability and nutritional value of the seed protein must be realized. A comprehensive understanding of the controls which limit the types and amounts of seed protein synthesized and of constraints which must exist in the chemistry of the storage proteins is necessary if these limitations are to be alleviated or removed by biological manipulation (2).

Serious consideration will probably be given to oilseed meals as a direct source of dietary protein, and the production and use of fish protein concentrate, single cell protein and extractable leaf protein as food sources will be explored. Fortification of these sources with synthetic amino acids is another important aspect. However, genetic enhancement of productivity and protein quality in traditionally accepted crop plants deserves particular attention, since by following this approach people requiring better sources of protein need not be induced to adopt new foods and no new economic infrastructure for processing, fortification and distribution is essential (3).

Legume Seed Proteins

Seed proteins are of two types—metabolic and storage. Legume seeds contain large amounts of storage proteins, the majority of which are globulin in nature, being soluble in dilute salt solutions and insoluble in water (2). Despite the preponderance of globulins, the albumin (metabolic) fraction has also been shown to be important for increasing the nutritional value of cultivars through breeding (4,5).

The storage proteins of Vicia spp. and Pisum sativum were originally named legumin and vicilin (6). However, these terms are sometimes used indiscriminately and we have questioned the wisdom of their broad usage to indicate similarities that do not exist. We have found that the major globulin fractions from Phaseolus vulgaris L., which have the solubility characteristics of the original definitions of legumin and vicilin, are not identical to legumin and vicilin from Vicia (7). Millerd (2) suggests that experimental evidence clearly shows these terms should be reserved for genera in the Fabaceae and Trifoliate.

Since the storage proteins are globulins, it is customary to use the properties of their solubility in dilute salt solution and insolubility in water to separate them from the metabolic proteins (8). After extraction with buffered sodium chloride and precipitation with ammonium sulphate, salt is removed by dialysis; the resulting precipitate is assumed to be only legumin and vicilin. The two fractions are then separated by the low solubility of legumin at its isoelectric point, pH 4.7.

However, this procedure has been shown to yield globulin fractions that either contain metabolic proteins or are a mixture of several globulins (9, 10), thus seriously limiting its use for quantitative studies. Millerd (2) concludes that if purification procedures similar to the Danielsson method are used to prepare proteins, data on subunit composition should be treated with caution.

McLeester et al. (7) have described a procedure for the extraction and preparation of two globulin fractions from Phaseolus vulgaris L. seeds with an ascorbate-NaCl medium. The two preparative fractions which we refer to as G1 and G2 globulins were shown to be completely separated from each other, with a high proportion of each fraction being extracted (11). This procedure has been modified to provide a method for quantitatively estimating the protein fractions in seeds of P. vulgaris genotypes differing in percentage seed protein (Yu Ma and F. A. Bliss, unpublished data). If genetic control of seed protein synthesis and accumulation is to be utilized for modifying protein quantity and quality through breeding, analytical procedures for obtaining pure protein species are essential.

Glycoproteins are widespread among the pulses and include substances that either directly or indirectly affect the nutritional value of the seeds. They have been found in the globulin protein fraction of beans, where they constitute the largest single portion of storage protein as in the case of glycoprotein II, isolated and described by Pusztai and Watt (12).

According to Millerd (2) the 8S protein isolated from Phaseolus aureus by Ericson and Christeels (13) corresponds to glycoprotein II. Racusen (14) found a similar fraction to constitute 35% of the total seed protein of P. vulgaris. Recent evidence (10) suggests that G1...
Changes Induced By Breeding

Globulin protein, which we have found to be the major constituent of *P. vulgaris* seed protein, is similar if not identical to glycoprotein II.

A general feature of these globulin protein fractions that are major constituents of the seed protein appears to be a deficiency of the sulphur-containing amino acids. Methionine and cystine have been found to be present in small or trace amounts or to be absent (10, 12, 15-17). The well-known fact that legume seed protein is generally poor in methionine and cystine is attributable to the small amounts of these amino acids in the major fraction(s) of the seed storage protein. The glycoproteins also include substances such as hemagglutinins, protease inhibitors, toxicants, phytoecyanins and enzymes (12).

Toxic Constituents of Food Legumes

The terms “toxic factor” and “toxicant” are commonly used when referring to natural substances that are contained in the seeds of some food legumes and that are deleterious when consumed by humans. While some plant substances may produce violent reactions when ingested and become lethal beyond a given level of intake, more subtle effects such as inhibition of growth and a decrease in food efficiency are more commonly observed (18).

Although the toxicants found in the food legumes vary as to their chemical composition, those that are of a protein nature are of particular interest. Food legumes are widely consumed as a source of protein, and there is considerable interest in improving the quantity and quality of seed protein through biological manipulations. Attempts to alter protein composition should consider the consequences in the broadest sense of nutritional modification.

Protease inhibitors. In every instance where protease inhibitors have been isolated in a high state of purity, the active substance has proved to be a protein (19). Their wide distribution in the food legumes has stimulated great interest in their nutritional effects. While the action of several enzymes may be inhibited by legume proteins, inhibition of trypsin often causes the more severe problems.

It has been suggested that trypsin inhibitors may interfere with nitrogen utilization and availability of sulfur amino acids, specifically methionine (20). However, Almquist and Merritt (21) found that rather than a specific interference with release of methionine, action of the inhibitor may involve a general interference with digestion so that a substantial amount of the most limiting amino acid (which is often methionine) is excreted unabsorbed, or is absorbed too late to be of value.

It has also been suggested that growth depression caused by trypsin inhibitor may be due to endogenous loss of essential amino acids (22). Since legumes are low in methionine and cystine, loss of these amino acids would be particularly acute (19). Others have postulated that the effect of trypsin inhibitor is to increase the metabolic conversion of methionine to cystine, which would intensify the body’s need for methionine. There seems to be evidence that trypsin inhibitors may also affect amino acid incorporation during protein synthesis within the body (19).

The significance of trypsin inhibitors varies greatly. As Bressani (23) pointed out, not all legume foods are improved by heating, and if this effect is to destroy trypsin inhibitors, either all legumes do not contain them or concentration and activity varies between species and cultivars. Little is known about the effect of variable heat treatment on trypsin inhibitor content and activity. However, some legume protein fractions devoid of anti-tryptic activity still inhibit growth because of other factors.
Phytohemagglutinins. Although phytohemagglutinins (lectins) have been known for nearly 100 years, only recently have they received much attention. While they occur in a wide variety of plants, they are detected frequently among the Leguminosae (Table I). Lectins derived from beans belonging to the genus Phaseolus have been studied because of their effects on a wide variety of biological systems, which include: 1) ability to initiate mitosis in cultures of normal human leucocytes; 2) stimulation of the synthesis of RNA in human peripheral blood lymphocytes; 3) binding and, in some cases, an inactivation of certain types of tumor cells; 4) beneficial effect in the treatment of a plastic anemia; 5) protection of human lymphocytes against the toxic effects of nitrogen mustards; and 6) involvement as a factor that contributes to the poor nutritive value of unheated legumes (15).

It is generally recognized that phytohemagglutinins are proteins. Often they have been classified as globulins because their electrophoretic mobility is similar to that of human serum globulin, although most do not require the presence of neutral salts for solubilization (24). Most purified lectins are known to be glycoproteins, but a notable exception is conconaval A from jack beans in which no carbohydrate could be detected (25). Although most lectins are rich in aspartic acid, threonine and serine, they are very low in or devoid of sulfur-containing amino acid (25), and there appear to be wide variations in amino acid patterns between the different legume lectins (24).

The impact of selection upon altering the levels of lectins as well as total seed protein and methionine/cystine content of the seed protein must be considered in studying the effects of breeding for nutritional improvement. Levels of agglutinins in plants are thought to be genetically controlled (24), and Jaffe et al. (26) have reported that agglutinating activity in P. vulgaris against blood cells of different animals is inherited as a single dominant trait. Significant differences in toxicity between red and white bean varieties have been observed in animal feeding trials, with the effects being due to the presence of different types of hemagglutinins (27).

The mechanism of intoxication caused by hemagglutinins is not well known (24), although interference with several metabolic processes is likely. Poor digestibility associated with low food absorption and poor nitrogen retention have been shown. Evidence for interaction between bean lectin and intestinal cells is based on the observation that the agglutinin is strongly bound when shaken with a suspension of homogenized intestinal rat tissue (24). The specificity of action of the lectins on blood cells from various animals is comparable to that of antibodies (24); however, nonspecific lectins are also known. In some cases it has been possible to identify fractions that show toxicity without agglutinating activity (25).

Proper autoclaving of legumes destroys the agglutinating activity as well as other inhibiting factors (24). However, even with cooked beans, methionine supplementation is often necessary for normal animal growth (27). Dry heat is considerably less effective than is autoclaving for improving nutritional value, and it is of practical importance to know the exact conditions and duration of heating which allow complete destruction of toxic action with a minimum of heat damage to the proteins (28).

### Prospects for Nutritional Improvement

The Protein Advisory Group has recommended urgent research attention to six major food legumes: dry bean, pigeon pea, cowpea, chick pea, broad bean and garden pea. While increased yield and improved consumer qualities should be primary objectives, genetic improvement of important nutritional factors must also be considered. Such factors include increased protein percentage, higher methionine and cystine levels, augmented lysine levels where feasible and improved protein digestibility (29).

The nutritional deficiencies characteristic of most food legumes cannot be regarded solely as a result of amino acid imbalance due to low levels of methionine and/or cystine. Since legume-grain protein is often the natural supplement to cereal-grain proteins, it has been suggested that nutritional improvement must take into account the role they play in diets rather than as a single food (23). As such, additional legume intake can provide higher levels of total protein, lysine, tryptophan and methionine.

Attempts to improve the nutritional value of the seed protein by biological manipulation must also consider the impact of breeding strategies on alteration of levels of various toxic substances which may also contribute to total seed protein. The complexity of the problem points to the necessity for a comprehensive treatment of improvement of nutritional value.

Elucidation of the physical and chemical properties of major protein constituents, mechanisms controlling synthesis and the basis of poor digestibility of some protein fractions can provide the information necessary to implement optimum methods for genetic alteration. Until such information is available, genetic improvement will proceed using...
traditional procedures suitable for quantitatively inherited traits.

**Heritable Differences in Legume Seed Protein**

The phenotypic expression of percentage seed protein and amino acid content of legume seeds varies depending on different genotypes and environmental factors (29-35). The protein content of the seeds is usually between 18 and 32%, methionine between 0.03 and 0.11 g/gN and cystine between 0.01 and 0.07 g/gN (23). Despite rather large environmental effects, heritable differences for percentage protein and levels of sulphur-containing amino acids have been reported in several species (29, 34, 36-40).

To date there have been no reports of major genes that either control total protein synthesis or alter the amino acid composition of legume seeds in a manner similar to the *opaque* genes of maize and sorghum and the genes altering lysine content of barley.

Depending on the populations studied and the methods of estimation, heritability values in *Phaseolus vulgaris* have ranged from 0.05 to 0.82 for percentage protein and from 0.10 to 0.85 for sulphur-amino acid content (29, 34, 38, 39). Pandey and Gritton (33) reported broad sense heritability estimates for percentage protein of *Pisum sativum* to be 0.54 to 0.67 and narrow sense heritability estimates to be from 0.45 to 0.67.

In cowpea (*Vigna unguiculata*), methionine content was found to be of medium heritability (0.46-0.54), and cystine content (0.27-0.34) and percentage protein (0.29) showed low heritability (40). Based on the genetic variation for protein, methionine and cystine content present in the legume species that have been studied, it appears that these nutritionally important traits can be altered if appropriate breeding procedures are employed.

In addition to the associations between various toxic substances and the seed storage protein and amino acids, the relationships between these nutritionally important factors and seed yield must also be considered. This is particularly critical since yields of food legumes are uniformly low and little progress has been made toward substantial increases. Since negative correlations between seed yield and percentage protein have been found (29, 31, 34, 38), the possibilities of developing high yielding cultivars that are also high in percentage protein have been questioned (41). However, the negative correlations are usually small (i.e., greater than -0.50), suggesting that recombinants (high yield, high protein) should occur.

Conclusive evidence must come from genetically variable populations that are subjected to appropriate selection procedures, rather than from homoygous genotypes (which may or may not be adapted) grown in selected environments. While it has been suggested that total protein yield (seed yield x percentage protein) might be a useful selection criterion, this is not entirely appropriate since attention must also be given to various consumer-preference traits such as seed size, shape and color, in addition to total yield. Percentage protein is also important since consumers are more likely to eat a given quantity of beans based on their availability rather than consume an amount adequate to provide the necessary protein intake.

Negative relationships between percentage protein and methionine per unit of protein have usually been found in legumes. However, the magnitudes of estimates have been small enough to suggest that strains high in protein and also inherently rich in methionine can be developed.

Adams (41) has suggested that evidence relating to whether variation in levels of methionine and cystine is due to genes controlling the synthesis of these amino acids per se, or whether it depends upon regulator genes that control the ratios of the various protein fractions in the seed, is essential to the plant breeder.

It appears likely that variation in the levels of these amino acids will result from the combined expression of both types of genes. Their effects on the production of amino acids, specific protein fractions and total seed protein will likely be resolved when the biochemical and physiological bases of individual traits are understood.

**Alteration of Seed Protein of *Phaseolus vulgaris***

Genetic variability of percentage protein and methionine content. Four strains of bean have been studied extensively because of known variation in percentage protein and methionine content of the dry seed.

### TABLE II. PROTEIN AND AVAILABLE METHIONINE LEVELS IN FIVE BEAN STRAINS

<table>
<thead>
<tr>
<th>Identification</th>
<th>% Protein</th>
<th>% Available Methionine of Total Protein</th>
<th>mg Protein Seed</th>
<th>mg AM&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 207227</td>
<td>21.5</td>
<td>0.197</td>
<td>0.16</td>
<td>39.6</td>
</tr>
<tr>
<td>P1 302542</td>
<td>25.1</td>
<td>0.260</td>
<td>1.036</td>
<td>99.7</td>
</tr>
<tr>
<td>BBL 240</td>
<td>28.1</td>
<td>0.339</td>
<td>1.206</td>
<td>105.9</td>
</tr>
<tr>
<td>P1 229815</td>
<td>31.9</td>
<td>0.272</td>
<td>0.853</td>
<td>101.4</td>
</tr>
<tr>
<td>Sanilac</td>
<td>24.5</td>
<td>0.235</td>
<td>0.959</td>
<td>49.7</td>
</tr>
</tbody>
</table>

<sup>1</sup>AM = Available methionine.

### TABLE III. RESULTS OF BIOLOGICAL EVALUATION OF FOUR BEAN STRAINS USING RATS<sup>2</sup>

<table>
<thead>
<tr>
<th>Diet</th>
<th>Experimental Variable</th>
<th>Total Weight Gain (g)</th>
<th>Feed Conversion</th>
<th>PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Casein</td>
<td>9.6</td>
<td>3.2</td>
<td>3.24</td>
</tr>
<tr>
<td>2</td>
<td>P1 207227</td>
<td>35.0</td>
<td>6.8</td>
<td>1.48</td>
</tr>
<tr>
<td>3</td>
<td>P1 302542</td>
<td>35.9</td>
<td>6.5</td>
<td>1.48</td>
</tr>
<tr>
<td>4</td>
<td>BBL 240</td>
<td>43.4</td>
<td>6.0</td>
<td>1.62</td>
</tr>
<tr>
<td>5</td>
<td>P1 229815</td>
<td>32.5</td>
<td>7.3</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>Sanilac&lt;sup&gt;3&lt;/sup&gt;</td>
<td>39.5</td>
<td>7.6</td>
<td>1.44</td>
</tr>
</tbody>
</table>

<sup>2</sup>Mean values for groups of six rats given the experimental diet for 28 days.

<sup>3</sup>Values taken from Kakade and Evans (1965).
TABLE IV. BROAD SENSE HERITABILITY ESTIMATES FOR SEVERAL TRAITS IN THREE BEAN POPULATIONS GROWN IN WISCONSIN (W) AND COLOMBIA, S.A. (C)

<table>
<thead>
<tr>
<th>Population</th>
<th>Location</th>
<th>Percentage Protein</th>
<th>Percentage Available Methionine</th>
<th>Percentage Available Methionine, Percentage of Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBL 240 × PI 207227</td>
<td>W</td>
<td>0.69</td>
<td>0.52</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.73</td>
<td>0.78</td>
<td>0.64</td>
</tr>
<tr>
<td>BBL 240 × PI 302542</td>
<td>W</td>
<td>0.32</td>
<td>0.43</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.16</td>
<td>0.40</td>
<td>0.54</td>
</tr>
<tr>
<td>BBL 240 × PI 229815</td>
<td>W</td>
<td>0.71</td>
<td>0.56</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.46</td>
<td>0.80</td>
<td>0.73</td>
</tr>
</tbody>
</table>

TABLE V. RESPONSE TO SELECTION FOR HIGH AND LOW PERCENTAGE SEED PROTEIN, BASED ON INDIVIDUAL PLANT PHENOTYPE, WITHIN POPULATIONS RESULTING FROM THE CROSS BBL 240 × PI 207227

<table>
<thead>
<tr>
<th>Generation</th>
<th>Direction of Selection</th>
<th>Percentage Protein</th>
<th>Seed Yield (gm/Plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Actual</td>
<td>Predicted</td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>H</td>
<td>32.7</td>
<td>31.1</td>
</tr>
<tr>
<td>F5</td>
<td>H</td>
<td>28.7</td>
<td>24.8</td>
</tr>
<tr>
<td>F2</td>
<td>unselected</td>
<td>25.8</td>
<td>24.8</td>
</tr>
<tr>
<td>F1</td>
<td>L</td>
<td>25.4</td>
<td>24.0</td>
</tr>
<tr>
<td>F1</td>
<td>L</td>
<td>22.3</td>
<td>24.1</td>
</tr>
<tr>
<td>F1</td>
<td>L</td>
<td>21.0</td>
<td></td>
</tr>
</tbody>
</table>

TABLE VI. F2 AND F3 FAMILIES FROM THE CROSS BBL 240 × PI 207227 SHOWING THE POTENTIAL FOR SELECTING HIGH YIELDING, HIGH PROTEIN GENOTYPES

<table>
<thead>
<tr>
<th>Family</th>
<th>Seed Yield (G/Plant)</th>
<th>Percentage Protein</th>
<th>G Protein Per Plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1973</td>
<td></td>
<td></td>
</tr>
<tr>
<td>73-27</td>
<td>37.2</td>
<td>26.6</td>
<td>9.85</td>
</tr>
<tr>
<td>73-25</td>
<td>38.8</td>
<td>22.8</td>
<td>8.84</td>
</tr>
<tr>
<td>73-24</td>
<td>32.7</td>
<td>24.5</td>
<td>8.01</td>
</tr>
<tr>
<td>73-26</td>
<td>36.1</td>
<td>27.9</td>
<td>10.07</td>
</tr>
<tr>
<td>73-21</td>
<td>32.4</td>
<td>23.6</td>
<td>7.64</td>
</tr>
<tr>
<td>73-16</td>
<td>38.6</td>
<td>24.7</td>
<td>9.53</td>
</tr>
<tr>
<td>Sanilac</td>
<td>30.7</td>
<td>25.5</td>
<td>8.43</td>
</tr>
<tr>
<td>BBL 240</td>
<td>14.7</td>
<td>31.1</td>
<td>4.57</td>
</tr>
<tr>
<td>PI 207227</td>
<td>21.0</td>
<td>22.5</td>
<td>4.73</td>
</tr>
<tr>
<td></td>
<td>1974</td>
<td></td>
<td></td>
</tr>
<tr>
<td>74-16</td>
<td>36.5</td>
<td>22.2</td>
<td>8.10</td>
</tr>
<tr>
<td>74-14</td>
<td>39.9</td>
<td>27.5</td>
<td>10.86</td>
</tr>
<tr>
<td>74-28</td>
<td>33.5</td>
<td>20.3</td>
<td>6.81</td>
</tr>
<tr>
<td>74-21</td>
<td>43.5</td>
<td>21.5</td>
<td>9.40</td>
</tr>
<tr>
<td>74-24</td>
<td>40.9</td>
<td>20.9</td>
<td>8.55</td>
</tr>
<tr>
<td>74-19</td>
<td>34.5</td>
<td>23.8</td>
<td>8.21</td>
</tr>
<tr>
<td>Sanilac</td>
<td>29.1</td>
<td>23.0</td>
<td>6.69</td>
</tr>
<tr>
<td>BBL 240</td>
<td>17.3</td>
<td>30.6</td>
<td>5.29</td>
</tr>
<tr>
<td>PI 207227</td>
<td>26.2</td>
<td>22.3</td>
<td>5.84</td>
</tr>
</tbody>
</table>

*Indicates families having high mean yields and percentage protein.

These differ not only for protein traits but also for other economically important traits such as seed yield, size, shape and color; plant growth habit; maturity and adaptation; and origin. The navy bean cultivar 'Sanilac' has been used throughout as a standard since it is grown extensively, it is somewhat widely adapted and the percentage seed protein and methionine content is representative of Phaseolus vulgaris.

When the experimental strains were grown in uniform field trials in Wisconsin, differences in percentage protein, percentage available methionine and available methionine as percentage of protein were observed (Table II). Judged solely on percentage protein, PI 229815 was superior. However, when available methionine, percentage protein and seed size were considered, BBL 240 was the most promising parent. When seed was fed in 10% protein diets to rats, BBL 240 was superior with respect to weight gain, feed conversion and protein efficiency ratio (PER) (Table III). The relative differences among strains for percentage protein and methionine were similar when they were grown in Colombia, South America.

These results suggested that percentage protein and methionine content were genetically controlled. Experiments were conducted and others are underway to determine the heritability of these traits, to assess the possibilities for improving bean populations using genes from these and other sources and to implement breeding procedures that will best utilize the available genetic variability. Heritability based on individual plant observations was estimated using F2 and parental populations. The magnitudes of these estimates indicate that the traits are of medium heritability and that the levels of protein and methionine can be improved through selection (Table IV).

Selection for high and low percentage protein. The population resulting from the cross BBL 240 × PI 207227 was chosen to further investigate the results of selection for modification of protein quantity and quality. BBL 240 is a white-seed cultivar developed in the U.S. and grown for the edible pods. Although the seeds are large and high in percentage protein and methionine, seed yield is quite low. PI 207227...
originated in Colombia, South America. It has small black seeds and is high yielding but low in percentage protein and methionine.

In addition to the broad sense heritability estimates computed from the F2 and parental populations, regressions of F3 progeny on F2 parents and F2 progeny on F1 parents also indicated that protein and methionine content were moderately heritable. Selection was begun in the F2 generation by choosing 10% of the plants having the highest percentage seed protein and 10% having the lowest percentage protein as parents of the next generation. Three generations of directional selection have been completed and the results show that selection for percentage protein is effective for modifying this trait (Table V).

Realized heritabilities were 0.53±.17 and 0.45±.54 calculated from the high and low protein populations, respectively. Because of the necessity of increasing bean yield and percentage protein, yields were also recorded and summarized. Selection for high percentage protein without regard to seed yield resulted in low yielding populations, while yields of populations selected for low percentage protein were higher. Thus, the plant breeder must use procedures that consider yield as well as protein modification in practical improvement programs.

Six families that exceeded the yield of Sanilac were identified within the populations selected for high and low percentage protein in 1973 and 1974. Each year there was one family in which the percentage protein values of all individual plants exceeded the mean value for Sanilac, demonstrating the occurrence of families that combine high yield with improved seed protein content (Table VI). It appears that breeding procedures that first emphasize selection for high yield on a family basis and then selection for high percentage protein among individuals should be successful, provided there is substantial variability for these traits in the original population (42).

Biochemical basis for differences in methionine content. Bajaj et al. (5) found that the globulin to albumin ratio provided a good prediction of PER values in Pisum sativum. Because metabolic proteins (albumins) are richer in methionine per unit of nitrogen than storage proteins (globulins), seeds containing different proportions of globulin to albumin may vary in available methionine as percentage of protein. Since the altered amino acid pattern in opaque-2 maize endosperm appears to be due to a change in the proportion of the protein fractions that normally occur, particularly a lower zein content (43, 44), it has been suggested that breeding for reduced globulin to albumin ratio might be an appropriate method for increasing methionine content of bean proteins.

To further study the effects of selection for high and low percentage protein on the seed protein fractions that comprise the total protein, plants producing similar seed yield but differing in percentage seed protein were chosen from the F2 generation of BBL 240 x PI 207227 for comparison. The results suggest that changes in percentage seed protein resulting from selection are due largely to alteration of the globulin fraction and to a lesser extent the free amino acid content (Yu Ma and F. A. Bliss, unpublished results). The albumin fraction appears to be less affected by selection, but in one plant it may have been substantially altered. High percentage protein was associated with low available methionine, as determined by a microbial assay that employs Streptococcus zymogenes. This relationship appears to be due to increased globulin while the albumin remains largely unchanged, with the result being a larger ratio of globulin to albumin in high protein seeds.

Since globulins are poorer in methionine than is albumin, the net effect is a lower percentage of methionine per unit of protein. Reducing the globulin to albumin ratio may be a promising approach to raising methionine levels, since the synthesis of the various protein fractions appears to be under separate genetic control. However, we do not yet know whether high methionine can be combined with high percentage protein using this approach.

Variation in polypeptide subunits of the G1 globulin protein. After characterization of the G1 globulin protein in cv. 'Tendergreen' (45), which displays three distinct polypeptide subunits, the G1 fractions of the four protein-
modified strains and 'Sanilac' were studied using SDS acrylamide gel electrophoresis. The two strains that are richer in methionine (BBL 240 × PI 302542) were found to contain three distinct bands while strains having lower levels of methionine (PI 207227, PI 229815 and Sanilac) contained only two discernible bands (46).

Subsequent studies have shown that the slow moving band in the two-banded strains is modified and is hard to resolve from the middle band, rather than being absent. Genetic analyses have shown that the difference between the two-banded (PI 229815) and three-banded (polypeptide subunits) (BBL 240) strains is controlled by a single Mendelian gene that produces an intermediate phenotype in the F1 hybrid and 1:2:1 segregation for banding pattern in F2 seeds produced by a single individual F1 plant (46). There is apparently little or no maternal influence on expression of this gene (Table VII). The association between level of available methionine and polypeptide subunit structure of the GI globulin in the five protein-modified strains has stimulated our interest in determining the effects of this gene. This may offer a means for altering the levels of methionine in addition to genetically modifying the relative proportions of the various seed protein fractions.

**SUMMARY**

The desirable combination of ability to fix nitrogen biologically and the capacity to accumulate large amounts of seed protein makes the food legumes very attractive at a time when nitrogen fertilizer costs are high and there is an increasing demand for protein at a reasonable price. Genetic improvement of yield and nutritional value of the seed protein must be accomplished if the food legumes are to maintain their roles in traditional diets and become even more important protein sources. Glycoproteins which are found in the globulin fraction constitute a major portion of the storage protein of bean seeds. Since they are generally deficient in sulfur-containing amino acids, efforts to improve nutritional quality are likely to involve alteration of these proteins. Protease inhibitors and phytohemagglutinins, two major toxicants of legumes, often constitute a portion of the storage protein. Thus attempts to alter protein quality must consider these constituents as well as total protein and amino acid composition.

Seed protein percentage and sulfur amino acid content of legume seeds are generally found to be of low to moderate heritability. Improvement of these traits through selection has been shown to be possible with appropriate breeding procedures. However, because of low negative correlations between seed yield versus percentage protein and percentage protein versus methionine content as percentage of protein, selection for a particular trait should not be conducted without attention to the other affected traits. Possible approaches to the improvement of methionine content include lowering the globulin to albumin ratio and qualitatively altering the amino acid composition of specific protein fractions.

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