Variation in the Accumulation of Seed Storage Protein Among Genotypes of Phaseolus vulgaris (L.)

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ABSTRACT

Differences in the accumulation of total seed protein and globulin-1 (G1) protein were detected among three inbred lines of common bean. Total protein accumulation ranged from 2.3 to 3.7 milligrams per cotyledon pair per day among lines. In all lines the dry weight and protein accumulation ceased and a loss of chlorophyll in the cotyledons occurred when the moisture content had fallen to 50% of fresh weight. G1 was first detected and rapid accumulation began 14 days after flowering in two lines, whereas in the cultivar Endogava zurundi namame, rapid accumulation was delayed until 20 days after flowering. Rates of G1 protein accumulation ranged from 1.0 to 1.8 milligrams G1 per cotyledon pair per day among lines. G1 accumulation ceased 6 days before the end of total protein accumulation in Sanilac. A steady rate of protein accumulation was observed in Sanilac, but pauses in the accumulation of G1 and of total protein were documented in Endogava zurundi namame. The rate of G1 accumulation preceding and following the pause in Endogava zurundi namame was 2.7 milligrams G1 per cotyledon pair per day, nearly double that of the other lines.

The common bean, Phaseolus vulgaris L., is particularly well suited as a model system for studying specific gene expression because of the rapid accumulation of a single, easily isolated, tissue-specific protein, the G1 (7, 16). Since beans are an important food crop, improvement of protein quantity and quality is also of considerable interest.

Studies of the kinetics of protein accumulation in cotyledon and endosperm tissue of various plants suggest that several biological processes contribute to differences in protein content and quality among genotypes within species. The prevention or reduction of the synthesis of a single protein can modify seed protein content and quality. High lysine opaque-2 corn (Zea mays L.) and high lysine sorghum (Sorghum bicolor L.) result from reduced levels of the low lysine prolamine fraction and small increases in the accumulation of the other protein fractions (10, 24). An absence of agglutinating activity was reported in some lines of P. vulgaris (2). This corresponds to the absence or very low levels of the G2 fraction in protein extracts tested against anti-G2 serum by immunodiffusion (Mutschler and Bliss, unpublished data). Another P. vulgaris line which lacks one of the seed acid albumins (protein I) has been reported by Kloz (11).

Total protein content and quality may be affected by the times of onset and termination and the rate of synthesis of the component proteins. The onset of legumin synthesis was detected earlier in seed development in two lines of pea (Pisum sativum L.) having high legumin content (17). Endosperms of high and low protein lines of wheat (Triticum aestivum L.) showed similar rates of protein accumulation, but protein synthesis continued during seed drying in the high protein line (3). Differences in the accumulation of sugars, starch, and oil can also affect percentage protein in the mature seed. High and low protein lines of oats (Avena sativa L.) produced groats with similar nitrogen accumulation but different levels of starch and sugar deposition, resulting in final differences of percentage protein (6).

G1, as the largest protein fraction, has a great impact on the level of the limiting amino acid, methionine. The average G1 content of 10 related F1 bean lines studied by Ma and Bliss (15) was 42% of the total seed protein. The methionine contents of total cotyledon protein and of G1 protein were 1.10 and 0.88 mg methionine/100 mg of protein, respectively, whereas the average methionine content of all protein fractions except G1 was 1.47 mg methionine/100 mg protein. Mutschler and Bliss (20) reported that G1 of the mature seed of bean lines ranged from 33 to 50% of the total protein, suggesting genotypic differences in the control of synthesis and accumulation of G1 or total protein. Differences in G1 accumulation during early cotyledon maturation were found, indicating that its initial rate of synthesis varied among genotypes (19).

The use of rocket immunoelectrophoresis (13) provides a rapid, precise method for measuring G1 content in maturing (19, 25) and dry (18, 20) bean seed. Here, we relate the differences observed among genotypes in the accumulation of G1 protein in bean cotyledons to the differences in protein quantity and composition of mature seed.

MATERIALS AND METHODS

Plant Material. Plants of three genotypes, Endogava Z.N., WI 74-2047, and Sanilac, known to differ in percentage protein and G1 content, were grown in the field at Madison, Wisconsin. At least 35 plants per genotype were grown spaced 20 cm apart in three rows which were 1 m wide and 7.6 m long. Freshly opened flowers were tagged each day during the 1-week period of peak bloom. At least 15 pods were harvested every 2 days beginning 10 DAF. The seeds were removed and average fresh weight determined. At 10 and 12 DAF, whole seeds were frozen. At later sampling dates the cotyledons were separated, weighed, and frozen. The frozen samples were lyophilized and dry weight determined.

In previous work (19, 25), materials collected under field and Biotron conditions showed similar patterns for G1 initiation and accumulation. Additionally four genotypes including Endogava
Z.N. and WI 47-2047 showed similar relative differences in protein and G1 content when grown in replicated field tests during two years (18). Since experimental error increased as sample weights decreased, the cotyledons collected for the present study were pooled within days for each of the genotypes to provide sufficient sample weight. The dry seeds or cotyledons were ground to a fine flour for protein and G1 determination. Seeds were collected until maturation was complete, when the pods were completely dry and seed moisture was constant.

To assay for the presence of G1, the leaves, stems, roots, and pods of mature plants of the above three lines were dried and ground. Fresh roots of Contender, Sanilac, and Canadian Wonder were collected at 20, 15, and 10 days after germination, respectively, for the same purpose.

Protein Analysis. Total nitrogen content was determined in quadruplicate using the Microkjeldahl procedure (1) and protein calculated by multiplying per cent N by 6.25. G1 content was determined using rocket immunoelectrophoresis (19, 25), with the following modifications in the method of protein extraction. The extraction solution used was 0.5 M NaCl, 0.1% NaN3 (pH 2.4). The samples taken at 12 and 14 DAF (0.1 g) were ground in 1.5 ml of the extraction solution using a ground-glass rod. The rod was rinsed and the washings added to the sample to give a total extraction volume of 2.5 ml. The remaining flour samples (0.1 g) were extracted in 4 ml (16 and 18 DAF) or 6 ml (20 DAF and later) of the extraction solution. The samples were centrifuged and carbamylated as in Sun et al. (25). Triplicate samples were extracted; each extract was applied to gels in triplicate at least. Ouchterlony diffusion gels for all three genotypes were prepared for the extractions taken at 10 to 20 DAF, against a full- to one-sixty fourth-strength dilution series of anti-G1 and anti-G2 antibodies, to determine the onset of G1 and G2 synthesis.

Extracts of 100–200 mg of seedling and mature roots, stems, leaves, root nodules, flower buds, and pod tissue were made in a total of 2 ml extraction solution and tested by immunodiffusion for the presence of G1. Extracts of fresh seedling roots were prepared by grinding 4 g tissue in 16 ml extraction solution and soaking for 2.5 h at 4 C. These extracts were centrifuged at 12,350g (Beckman J-21) for 30 min, then dialyzed overnight against two changes of distilled H2O. The solutions were centrifuged at 12,350g for 30 min, and the pellets dissolved in 200 μl extraction solution to be tested for the presence of G1 and G2 by diffusion gels.

RESULTS AND DISCUSSION

Tissue Specificity. G1 and G2 proteins were found only in cotyledons, being detected neither in other plant parts nor in callus cultured from cotyledons sampled before or during G1 synthesis. These observations agree with several reports (9, 16), but conflict with that of Frame et al. (8) who found a protein in roots of the cultivar Canadian Wonder that cross-reacted with serum prepared against seed globulin. Protein isolated from the roots of Canadian Wonder, Sanilac, and Contender failed to cross-react with serum prepared against seed globulins.

Onset of Globulin Accumulation. G1 protein was first detected by immunodiffusion at 14 DAF in cotyledons of WI 74-2047 and Endogava Z.N. Sanilac cotyledons collected at 14 DAF were lost, but the heavy precipitate seen in those sampled at 16 DAF suggested that G1 had probably been present at 14 DAF. In previous analyses of Sanilac cotyledons collected from 10 to 20 DAF, G1 was first detected at 14 DAF (19). Analyses of Tendergreen cotyledons have shown G1 to be present in younger material (at 12 DAF) (19, 25). G2 protein was also detected differentially among the lines. G2 was first detected at 14 DAF in WI 74-2047 and at 16 DAF in Endogava Z.N. The large amount of G2 in Sanilac at 16 DAF suggests its presence in the cotyledons at 14 DAF, which agrees with the previous analysis (19).

G1 Accumulation. Rapid G1 accumulation proceeded immediately after the earliest detection of G1 in Sanilac and WI 74-2047, but began 6 days later in Endogava Z.N. (Fig. 1). Significantly different rates of G1 accumulation were found, the highest being 1.8 mg/cotyledon pair·day in Endogava Z.N. The rate for Sanilac was similar to that reported earlier, at 1.5 and 1.3 mg/cotyledon pair·day, respectively (19). A steady rate of G1 accumulation was found in Sanilac and WI 74-2047 (Fig. 1, A and B), but a pause was observed in Engogava Z.N. from 26 to 30 DAF (Fig. 1C). A corresponding pause in total protein accumulation was observed, as was an earlier pause in protein accumulation that coincided with the delay in rapid accumulation of G1 from 16 to 22 DAF (Fig. 1C). The smooth curves for G1 and protein accumulation were computed by least squares analysis. It is possible that the early termination of G1 accumulation in Sanilac, also at 26 DAF, may be analogous to the pause in accumulation in Endogava Z.N. The genotypic differences in G1 content were probably significantly different, considering the observed level of error variance, and since similar and significant differences were found among four inbreds in replicated trials (18). Since the differences among lines in G1 accumulation did not account for the differences in total protein, some of the other protein fractions also vary among these lines. Evidence for the variation of G2 content among lines has since been obtained (18).

Termination of Protein Accumulation. The total accumulated G1 protein differed among the three lines, with Endogava Z.N. having nearly twice as much as Sanilac (Fig. 1). In Endogava Z.N.
and WI 74-2047, the termination of G1 accumulation coincided with the end of dry matter and total protein accumulation and the loss of Chi in the cotyledons; these events occurred when moisture reached 50%. All three lines showed a rapid moisture loss from the 50% level so that the final moisture level of 13% was reached within 2 to 4 days. Per cent moisture in Endogava Z.N. and Sanilac decreased at similar rates from 14 DAF until the 50% moisture level, but since the initial moisture was higher in Endogava Z.N. than in Sanilac (87% versus 75%), the 50% level was reached 10 days later in Endogava Z.N. In Sanilac, G1 accumulation terminated 6 days before the moisture level reached 50%. Dry matter and total protein accumulation continued until the moisture dropped to 50%, however.

Endogava Z.N. had substantially more protein (28%) and G1 as a per cent of protein (47%) than the other lines (Table I). The high proportion of protein per seed resulted from a high rate of protein accumulation and slower accumulation of non-protein dry matter (Table II). The high level of G1 as a per cent of protein resulted from the high rate of G1 accumulation that occurred until the termination of total protein and dry matter accumulation. Sanilac had a relatively high rate of G1 accumulation until the early termination. At that point G1 comprised 45% of the total protein, but because of continued accumulation of other proteins, it accounted for only 38% at maturity (Table I). The final levels of percentage protein and G1 as percent of protein in Sanilac and WI 74-2047 were similar, although their accumulation patterns were different. In contrast to the high rate and early termination of G1 accumulation in Sanilac, WI 74-2047 showed a lower rate of G1 accumulation, which continued until the termination of total protein accumulation (Table II).

These results indicate that genotypic variability exists for differing in the onset of rapid protein synthesis, and in the rates and termination times of protein synthesis, particularly regarding G1 the major seed storage protein. Differential accumulation of G1 in relation to other protein fractions and non-protein dry matter accounted for the observed differences in G1 and total seed protein content at maturity.

The presence of pauses in accumulation of the constituents of bean seeds has been reported elsewhere. Carr and Skene (5) noted a pause in the accumulation of dry weight in the bean cultivar, Hawkesbury Wonder, as well as several other species. Pauses in dry matter accumulation may have also occurred in the bean cultivars, Tenderette and Charlevoix studied by Oliker et al. (21) and Popa et al. (23), respectively. In Belfast New Stringless (22) and Black Valentine (14), pauses in dry weight and nitrogen accumulation were present. Carasco et al. (4) observed these pauses in *Vigna unguiculata*, accompanied by a later change in the protein profile. Kloz et al. (12) cited evidence for nonuniformity of protein synthesis, with different proteins being synthesized during different maturation periods. It is possible that the pauses indicate the onset or termination of differential gene expression controlling synthesis of the various seed constituents.

Selection for high protein beans has shown initial success. Protein synthesis could also be altered by changing the amounts of the different protein fractions present. Changing the amount of G1 relative to the total seed protein should affect the sulfur amino acid content of the protein, since G1 normally contains low levels of methionine and cystine (15). Seeds low in G1 as a percent of protein should be richer in the sulfur amino acids. Alternatively, if genotypes were found that code for G1 richer in methionine than those currently available, it would be desirable to select for increased amounts of G1 protein.

No single genes similar to the high lysine mutants affecting the composition of cereal endosperms and concomitantly the seed protein have been found in beans, but substantial variability in G1 as a percent of protein has been observed (20). Genotypes that vary for initiation, rate of synthesis and/or termination of synthesis of G1 can be hybridized. Progeny may then be selected to obtain lines having more desirable protein quantity and quality.

**Table I. Time of Flowering, End of Protein Synthesis, Maturation Time, and Levels of G1 and Total Seed Protein in Three Lines of Phaseolus vulgaris L.**

<table>
<thead>
<tr>
<th>Line</th>
<th>Sanilac</th>
<th>WI 74-2047</th>
<th>Endogava Z.N.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering period (day of 1978)</td>
<td>127–133</td>
<td>134–142</td>
<td>124–131</td>
</tr>
<tr>
<td>Termination of protein accumulation (DAF)</td>
<td>32</td>
<td>36</td>
<td>38</td>
</tr>
<tr>
<td>Seed maturation (DAF)</td>
<td>36</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Protein content at maturity (% by weight)</td>
<td>22</td>
<td>22</td>
<td>28</td>
</tr>
<tr>
<td>G1 content at maturity (% of protein)</td>
<td>38</td>
<td>38</td>
<td>47</td>
</tr>
<tr>
<td>Seed weight at maturity (g)</td>
<td>0.28</td>
<td>0.25</td>
<td>0.24</td>
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</table>

**Table II. Accumulation of Seed Dry Matter, Total Protein and G1, and Loss of Moisture in Three Lines of Phaseolus vulgaris L.**

<table>
<thead>
<tr>
<th>Line</th>
<th>Sanilac</th>
<th>WI 74-2047</th>
<th>Endogava Z.N.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (mg/cotyledon pair-day)</td>
<td>10.4</td>
<td>10.8</td>
<td>9.8</td>
</tr>
<tr>
<td>Protein (mg/cotyledon pair-day)</td>
<td>2.3</td>
<td>2.5</td>
<td>3.7 (4.5)*</td>
</tr>
<tr>
<td>G1 protein (mg/cotyledon pair-day)</td>
<td>1.5</td>
<td>1.0</td>
<td>1.7 (2.7)*</td>
</tr>
<tr>
<td>Moisture loss* (percentage/day)</td>
<td>1.7</td>
<td>1.3</td>
<td>1.7</td>
</tr>
</tbody>
</table>

* Over-all accumulation rate 14–36 DAF. Numbers in parentheses indicate rate after pause.

**LITERATURE CITED**

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