

Selection for increased percentage phaseolin in common bean

1. Comparison of selection for seed protein alleles and S_1 family recurrent selection

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Summary. Two selection methods were compared to determine which was more efficient for increasing percentage phaseolin in the common bean (*Phaseolus vulgaris* L.). A base population consisting of families segregating for six seed protein alleles ($Phas^S$, $Phas^C$, $Phas^T$, $phas^-$, lec^- , and $Arcl^+$), all of which have measurable effects on percentage phaseolin, was subjected to either three cycles of S_1 family recurrent selection for increased percentage phaseolin (PPS), or one cycle of selection for combinations of the protein alleles (PAS) known to have positive effects on phaseolin accumulation. One cycle of PAS resulted in an increase in percentage phaseolin that was equivalent to three cycles of PPS. Selection under both methods produced increases in several correlated traits including percentage total protein, phaseolin as a percent of total protein, mg protein/seed, and mg phaseolin/seed. The amount of nonphaseolin protein per seed decreased, while seed yield was unaffected by either selection procedure. By selecting for favorable seed protein alleles identified by electrophoresis, it was possible to rapidly increase percentage phaseolin without the need for field evaluation.

Key words: *Phaseolus vulgaris* (L.) – Selection methods – Recurrent selection – Biochemical markers – Percentage protein

Introduction

Total seed protein typically comprises from 19% to 30% of the seed weight in common bean (*Phaseolus vulgaris*

L.), and of that, 36%–50% is the globulin protein phaseolin (Ma and Bliss 1978). Phaseolin is important because, as the predominant protein fraction, it is a major contributor to available methionine. Gepts and Bliss (1984) studied the association between available methionine and phaseolin content and found that in white-seeded lines, available methionine was correlated positively with phaseolin as percent of flour and as percent of total protein.

Recurrent selection using S_1 families as the selection unit is well-suited for use with self-pollinated crops. Evaluation of selfed progenies exposes less desirable recessive alleles that can then be selected against. S_1 family recurrent selection has been used successfully in soybean to increase percentage seed protein (Miller and Fehr 1979; Brim and Burton 1979; Carter et al. 1982). Increases in percentage grain protein have also been achieved in wheat using recurrent selection among F_3 families (McNeal et al. 1978; Loeffler et al. 1983).

Quantitative traits are often controlled by many genes, each with small individual effects; and there may be a large component of variation due to environmental effects. Using conventional selection methods, gain from selection of quantitative traits with low heritability often will be modest because it is difficult to identify individuals that are genetically superior. Biochemical markers of quantitative trait loci are advantageous because they are not affected by the environment and the genotype of an individual can be determined accurately. This reduces the chance of carrying along undesirable alleles in a population because of errors in selection that are due to environmental effects and/or dominance. Therefore, desired gains for the trait of interest can be achieved in a shorter period of time. Stuber et al. (1982) compared selection for allozymes linked to loci associated with grain yield in maize with direct selection for grain yield using full-sib

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family recurrent selection and found that in some environments selection for allozymes was more effective.

Although phaseolin concentration is inherited quantitatively, there are discrete differences in the amount of phaseolin produced, which are associated with different phaseolin alleles. Also, differences in percentage phaseolin are associated with variation for various other protein fractions. The allelic composition of a particular seed with respect to phaseolin type and other protein fractions can be classified by the banding patterns on SDS-PAGE. In backcross-derived lines with S-, T-, and C-type phaseolin, T- and C-type seeds contained significantly more phaseolin and milligram phaseolin/seed than S-types (Hartana 1986). In the absence of lectin (*lec*⁻) the amount of phaseolin in seeds was increased among all three phaseolin types (Osborn and Bliss 1985; Hartana 1986). When the novel protein arcelin is produced, the accumulation of phaseolin is reduced by over 50% (Romero-Andreas et al. 1986). Lines homozygous for a recessive phaseolin-deficient allele (*phas*⁻), isolated from the *P. coccineus* cv 'Mexican Red Runner', produce no detectable amounts of phaseolin in their seeds.

Unlike isozymes and RFLPs, which must be linked closely to a quantitative trait locus to be useful, seed proteins are the actual gene products constituting the trait of interest, i.e., percentage phaseolin. Thus, they are a more powerful tool in selection experiments. In this study we used two selection methods, S₁ family recurrent selection and selection for favorable combinations of six seed protein alleles, to increase the percentage of phaseolin in common bean seeds. The objectives of the experiment were to compare the efficiency of these two methods and other associated genetic changes.

Materials and methods

Development of base population

The base population on which both selection methods were initiated consisted of seven breeding lines (2-4-1, 6-30, PP11-37, 809598-3, L12-56, SARC1-7, and BMC 3522). These lines were homozygous for various combinations of six different seed protein markers (*Phas*^S, *Phas*^C, *Phas*^T; and phaseolin-deficient (*phas*⁻) phaseolin alleles, lectin-deficient (*lec*⁻) and arcelin-1 (*Arc1*⁺) (Table 1). The *Phas*^C and *Phas*^T alleles and the *lec*⁻ allele generally produce positive effects on the amount of phaseolin, while the *Arc1*⁺ and *phas*⁻ alleles have strong negative effects on percentage phaseolin. Individuals with S-type phaseolin usually produce less phaseolin than those with either the C- or T-type. However, two of the parental lines, 2-4-1 and 6-30, have S-type while being high in phaseolin, presumably because they carry a gene(s) that enhances the production of phaseolin (Sullivan and Bliss 1983a; 1983b).

The seven parental lines were intermated in a half-diallel design that resulted in 20 F₁ progenies (one cross was not successful). These 20 F₁'s were crossed in a partial diallel design in which crosses were made only between F₁'s that had no parents in common. The resulting 95 double crosses and the parental lines were planted in 1985 at the Hancock Agricultural Research

Table 1. Genotype, mean percentage phaseolin and effect of each parental genotype on percentage phaseolin compared to the standard cultivar 'Sanilac'. Plants were grown in 1987 at the Hancock Agricultural Research Station, Hancock, Wisc.

Parent	Genotype	% Phaseolin	Effect ^b
2-4-1	<i>Phas</i> ^S ^a	12.93	2.57
6-30	<i>Phas</i> ^S ^a	11.78	1.42
PP11-37	<i>Phas</i> ^C	11.75	1.39
809598-3	<i>Phas</i> ^T	11.44	1.08
L12-56	<i>Phas</i> ^S and <i>lec</i> ⁻	12.12	1.76
SARC1-7	<i>Phas</i> ^S and <i>Arc1</i> ⁺	4.40	-5.92
BMC 3522	<i>phas</i> ⁻ and <i>lec</i> ⁻	0.00	-10.36
'Sanilac'	<i>Phas</i> ^S	10.36	0.00

^a These two lines carry unknown gene(s) for higher percentage phaseolin

^b Relative to Sanilac

Station, Hancock, Wis., in a completely randomized design with two replications. Selfed seed was collected from each double-cross family, using seed from both replications. These S₁ seeds of the 95 families constituted the cycle 0 (C₀) population.

From the base population, two selection programs were initiated based on different selection criteria. In the first (designated PPS), selection was among S₁ families, which were evaluated for percentage phaseolin by rocket immunoelectrophoresis. In the second (designated PAS), individuals were selected that had favorable combinations of the seed protein alleles, without regard to percentage phaseolin per se.

S₁ family recurrent selection (PPS)

In 1985, S₁ seeds of the 95 C₀ families were evaluated, and the 20% (19 families) with the highest percentage phaseolin were selected as parents to form the C₁ population. Remnant seeds of the 19 families were planted in the greenhouse in the fall, and ten plants from each family were intermated to plants of other families, using a chain crossing design. During the winter, two to three plants from each successful cross were selfed, and the resulting seeds were bulked to provide enough seeds for replicated field testing. The resulting 120 S₁ families and the parental lines used as checks were planted in 1986 at the Hancock Agricultural Research Station in a randomized complete block design with three replications. Each plot consisted of ten plants spaced 10 cm apart with 0.76 m between plots. A guard plant was planted at the front and back of each plot to provide equal competition among test plants. Mature seeds from each plot were again evaluated for percentage phaseolin, and the best 20% (24 families) selected as parents for the C₂ population. The 24 families were intermated and selfed as before to produce 120 S₁ families for replicated field testing. These S₁ families were evaluated for percentage phaseolin, and the best 20% (24 families) were selected.

Selection for seed protein alleles (PAS)

In the second selection method, families from crosses among the five parental lines having positive alleles for percentage phaseolin (2-4-1, 6-30, PP11-37, 809598-3, and L12-56) were chosen from the C₀ population. Within these 12 families S₁ seeds were screened by SDS-PAGE (Laemmli 1970; Ma and Bliss 1978) to determine their genotype for the six alleles (*Phas*^S, *Phas*^C, *Phas*^T, *phas*⁻, *lec*⁻, and *Arc1*⁺). Using these data individuals were selected that were homozygous for the *Phas*^T allele and heterozygous for *Phas*^C/*Phas*^T, *Phas*^T/*Phas*^S, and *Phas*^C/*Phas*^S. All

of the individuals selected were homozygous for both the *lec*⁻ and *arc1*⁻ alleles. Each of these four categories was subdivided into two groups, one having 2-4-1 as one of the parents, and the other with 6-30 as one parent. This was done in an attempt to incorporate into the population any alleles for high percentage phaseolin that these two parents contain. Five plants of each of the eight lines were grown in the greenhouse and intercrossed in a chain-crossing design. Three plants of each of the resulting F₁'s were selfed to produce S₁ families for field testing.

Field evaluation

To compare progress from the two selection methods, the original parental lines, selected families from C₀, C₁ and C₂ of the PPS (percentage phaseolin selected) population, and S₁ families from the C₀ selections of the PAS (protein allele selected) population were evaluated in a field experiment. The experiment was planted in the summer of 1987 at the Hancock Agricultural Research Station in two adjacent randomized complete blocks each with three replications. Each plot consisted of ten test plants spaced 10 cm apart and bordered by guard plants at each end. The distance between plots was 0.76 m.

Plots were harvested as they matured, and data were recorded on the following traits: maturity (number of days from planting to 50% dry pods), seed yield per plot (g/plot), 50 seed weight (g), percentage protein and percentage phaseolin (mg phaseolin/100 mg flour). Algebraic manipulations of these data were applied to determine mg phaseolin/100 mg protein, mg protein/seed, mg nonprotein/seed, mg phaseolin/seed, and mg nonphaseolin/seed.

Data for seed yield per plot were adjusted by a covariance analysis, using number of plants per plot as a covariate. Percentage protein for each plot was determined from a sample of ground flour by near-infrared reflectance (Pacific Scientific, Model 101S, Cereal Grain Analyzer).

Rocket immunoelectrophoresis

The percentage phaseolin (mg phaseolin/100 mg flour) was determined from a crude protein extract using rocket immunoelectrophoresis (Laurell 1966). A 50-seed sample from each plot was ground using a Udy cyclone mill. Proteins were extracted by stirring approximately 0.1 g of flour in 5 ml of acid salt buffer (0.5 M NaCl, 0.5 M glycine, 0.025% NaN₃, pH 2.4) for 2 h at room temperature. The procedure for quantitation of phaseolin was that of Sun et al. (1978). Purified phaseolin for use as a standard and for antibody production was obtained using the methods of Sun and Hall (1975), except that 1.5 volumes, instead of 4 volumes of cold distilled water were added after the initial extraction. After centrifugation, an additional 2.5 volumes of distilled water were added to the supernatant to precipitate the phaseolin. Antibodies against purified phaseolin were produced from rabbits using the methods of Sun et al. (1978).

Results

S₁ family selection (PPS)

The mean percentage phaseolin of the selected families increased from C₀ to C₂ (Table 2) by an average, of 1 percentage point per cycle (Fig. 1), or a net gain of 8% per cycle. The mean of the C₂ selections was 54% higher than the mean of the parental lines. There was, however, a marked reduction in the phenotypic variance from C₀ to C₂ ($\sigma^2_p = 3.65$ and 0.87, respectively), which will lead to less gain per cycle in future generations. Changes in

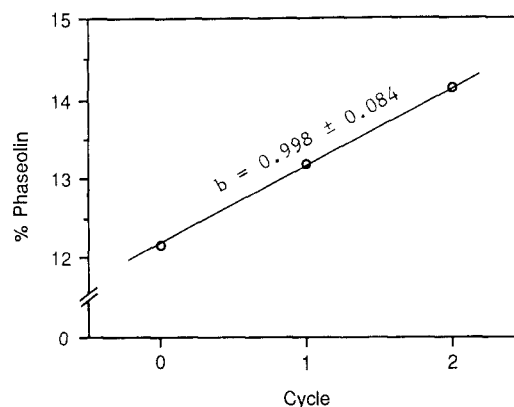


Fig. 1. Average rate of gain (regression coefficient and standard error) for three cycles of selection for increased percentage phaseolin

other seed and protein fractions were associated with increases in percentage phaseolin (Table 2). Means for percentage total protein, mg phaseolin/100 mg protein, and phaseolin/seed were all increased. Nonphaseolin protein/seed decreased, but the ratio of phaseolin:nonphaseolin protein increased with each cycle, and was double that of the parents in C₂. The amount of the protein and nonprotein seed fractions was not changed by selection for increased percentage phaseolin.

In other recurrent selection experiments, Sullivan and Bliss (1983 b) observed increases in the phaseolin concentration (mg/g flour) of bean seeds that were due to either higher amounts of phaseolin/seed or less nonprotein/seed. Miller and Fehr (1979) found that both the carbohydrate and oil fractions of soybean were reduced when selection for higher percentage seed protein was practiced. In this population, improvement in percentage phaseolin was due mainly to increases in phaseolin/seed, which is advantageous since reductions in the nonprotein fraction, which are not compensated for by increases in protein, can result in smaller seed size and lower yields.

Three important traits, maturity, seed weight, and seed yield, were unaffected by selection for increased phaseolin (Table 2). Similar results for maturity were obtained in a population of wheat selected for high grain protein percentage (Loeffler et al. 1983), however, later maturity was associated with increases in seed protein in a soybean population (Miller and Fehr 1979). Decreased seed weight and seed yield often are reported to be associated with increased total seed protein (Dudley et al. 1974; Brim and Burton 1979; Carter et al. 1982; Loeffler et al. 1983). In contrast, McNeal et al. (1978) reported that in five of nine spring wheat populations studied, there was either no change or an increase in grain yield.

Selection for seed protein alleles (PAS)

In one cycle of selection for favorable combinations of the seed protein alleles, percentage phaseolin was in-

Table 2. Means for several seed and agronomic characters for parental lines and selected families from three cycles of selection for percentage phaseolin and one cycle of selection for seed protein alleles

Parents and selections from cycles	% Phaseolin	% Protein	mg Phaseolin per 100 mg Protein	Seed fractions	
				Protein mg/seed	Nonprotein mg/seed
<i>Selection for % phaseolin (PPS)</i>					
	y	y	y	y	y
Parents	9.2 c	22.9 b	39.5 c	47.3 a	159.2 a
C ₀	12.2 b**	23.7 ab**	51.3 b**	49.6 a**	159.9 a**
C ₁	13.2 ab**	24.0 ab	55.0 ab**	49.1 a**	155.2 a**
C ₂	14.2 a	24.6 a	57.6 a	51.7 a*	158.7 a**
<i>Selection for protein alleles (PAS)</i>					
Parents	9.4 b	22.9 b	40.2 b	47.9 b	160.6 a
C ₀	13.9 a	24.3 a	57.1 a	55.2 a	171.7 a
Protein fractions			Maturity	Seed wt.	Seed yield
Phaseolin mg/seed			(days)	(mg/seed)	(g/plot)
<i>Selection for % phaseolin (PPS)</i>					
	y	y	y	y	y
Parents	19.1 c	28.1 a	104.5 b	206.5 a	220.6 a
C ₀	25.4 b**	24.2 b	115.9 a	209.5 a**	250.8 a
C ₁	26.9 ab**	22.1 b	113.2 a*	204.3 a**	253.3 a
C ₂	29.8 a	22.0 b*	111.4 ab**	210.4 a**	244.3 a
<i>Selection for protein alleles (PAS)</i>					
Parents	20.1 b	28.5 a	103.5 b	209.3 a	225.5 a
C ₀	31.5 a	23.7 b	116.4 a	226.8 a	265.6 a

*, ** Mean is significantly different from PAS C₀ selections based on a *t*-test at the 0.05 and 0.01 levels respectively

y Mean within columns followed by different letters are different ($P \leq 0.05$, Duncan's multiple range test). The PPS and PAS populations were analyzed separately

creased 4.5 percentage points or 48% over the mean of the parents. All other protein fractions increased as well, except for the nonprotein fraction of the seed, which was unchanged, and mg nonphaseolin protein per seed, which decreased. Maturity was delayed among the selected lines, while seed weight and seed yield were not significantly higher than that of the parents. The objective of this experiment was to select only those individuals with protein allelic combinations that would produce the highest amount of phaseolin. It was not surprising then that using this procedure resulted in a six-fold decrease in the phenotype variance for percentage phaseolin (from $\sigma_p^2 = 3.65$ in C₀ to $\sigma_p^2 = 0.58$ for the PAS C₁ population).

Comparison of the two selection methods

After only one cycle of selection for favorable combinations of the seed protein alleles, the increase in percentage phaseolin was one and one-half times higher than for the first cycle of S₁ family selection. Even after three cycles, the average percentage phaseolin among the PPS C₂ selected families was not significantly higher than the PAS

C₀ families (Table 2). For the other traits, the PAS C₀ selections were generally significantly higher than the PPS C₀ selections, and in some cases were also higher than PPS C₁ and/or C₂. The means for the traits mg nonphaseolin protein per seed, maturity, and seed yield were similar among the C₀ selections from the two populations.

Discussion

The percentage phaseolin in common bean seeds was increased when either of the two selection methods was used; however, selection for favorable protein alleles was more efficient than S₁ family recurrent selection. In the environment in which this study was conducted, one cycle of selection for protein alleles produced as much gain as three cycles of S₁ family selection. In a comparable selection study of grain yield in maize, Stuber et al. (1982) found that one cycle of selection for allozymes was equivalent to one and one-half to two cycles of full-sib family selection for yield, depending on environment.

Both selection procedures resulted in a greatly reduced phenotypic variance for percentage phaseolin; selection for protein alleles more so than S_1 family selection. Although the maintenance of long-term selection response is an important consideration when choosing a breeding method, plant breeders are also interested in short-term gain. S_1 family recurrent selection is a useful method for this purpose and is easily managed with self-pollinated crops. The advantage of selecting for specific seed protein alleles is that selection can be done in early generations, without the need for field testing. One cycle of S_1 family selection requires 12 months to complete, whereas one cycle of protein allele selection takes only 7 months. Also, since selection is practiced at the level of the primary gene product, the chance of losing valuable favorable alleles because they are masked by other factors is reduced.

If our goal is to increase the percentage phaseolin in common bean to the same level as the major soybean seed protein, glycinin, which comprises about 20% of seed weight (Nielsen et al. 1988), one might then ask, now that the six favorable seed protein alleles have been combined, what is the next step? Continued selection among the selected families would yield only small increases at best, since the phenotypic variance among the lines is very low. The PAS population could be expanded by intercrossing, then selection continued for percentage phaseolin. Some of the selections from the PPS C_2 population may have different favorable alleles for phaseolin accumulation and could be crossed with families from the PAS population to further increase percentage phaseolin. Another approach might be to find new markers, such as isozymes, RFLP's, or other seed protein alleles, which could be used to increase the frequency of additional, currently unmarked, favorable alleles.

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