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Heritable variation in the phaseolin protein of nondomesticated common bean, *Phaseolus vulgaris* L.

J. Romero-Andreas and F. A. Bliss

Department of Horticulture, University of Wisconsin, Madison, WI 53706, USA

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Summary. Crude protein extracts from single seeds of nondomesticated Mexican bean accessions were analysed by SDS polyacrylamide gel electrophoresis for variability in phaseolin protein. Six new phaseolin types; 'M1', 'M2', 'M3', 'M4', 'M5', 'M6', which contained polypeptides within the same range of molecular weights (51,000 to 45,000 daltons) as occur in the 'S', 'T' and 'C' phaseolin types of cultivated beans were identified. No 'T' and 'C' types were found among the nondomesticated Mexican accessions, and the 'S' type occurred in less than 7% of the seeds screened. Genetic analyses of F_2 progenies from crosses between 'Sanilac' ('S'), and five of the 'M' types showed that each 'M' phaseolin phenotype was allelic to the 'S' type and expressed codominantly.

Key words: *Phaseolus vulgaris* L. – Phaseolin – Seed protein – Genetic variation

Introduction

Phaseolin, the major globulin fraction of the seed protein of common bean, usually accounts for 30 to 50% of the total protein (Ma and Bliss 1978). Electrophoretic screening and genetic analyses of several hundred cultivars and Plant Introductions have revealed primarily three allelic phaseolin variants (Romero et al. 1975; Brown et al. 1981). The 'S' type occurred most frequently (69%), followed by the 'T' type (25%), and the relatively rare 'C' type (6%) (Brown et al. 1982). We now report that extensive genetic variability for phaseolin type occurs in nondomesticated common bean germplasm.

Materials and methods

Seeds of 23 accessions of nondomesticated bean, *P. vulgaris* were obtained from the collector, Howard Scott Gentry (Botanist, Agricultural Research Service, USDA), (1969) and were stored until this study was undertaken in 1980. One other accession from Argentina, PI 266910, was also included.

All seeds from each sample were inspected and if the seed lot was uniform in color and size, 3–9 seeds were analysed. If variability was evident, three or more seeds of each color and size were chosen. A small chip weighing from 2 to 4 mg was removed from the end of each seed distal to the shoot-root axis (SRA). Seed proteins were extracted from the crushed pieces. The remainder of each seed containing the SRA was then planted, germinated and a plant grown.

Seed proteins were extracted for 30 min in 50–75 µl of a solution containing 0.5 M NaCl, and 1.0% sodium azide. Extracts were diluted 1:1 (v/v) in a buffered denaturing solution containing 0.63 M Tris at pH 6.8, 1% SDS (w/v) and 2% 2-mercaptoethanol (v/v), and heated 5 min in a boiling water bath; 5–8 µl were then applied to the well of the stacking gel (Romero-Andreas 1984). Proteins were separated according to molecular weight using the SDS-PAGE system of Laemmli (1970).

One plant from each of six accessions representing the six 'M' phaseolin types was chosen as a male parent for crossing to 'Sanilac', a white-seeded commercial cultivar having 'S' type phaseolin. F_1 seeds were planted in January, 1981, and F_2 seeds harvested between April and June, 1982.

Results and discussion

The initial screening of 276 single seeds from 23 Mexican accessions and one South American accession using SDS-PAGE revealed six phaseolin patterns distinct from each other and from the 'T', 'S', and 'C' patterns of cultivated beans (Fig. 1). These new variants contained phaseolin with apparent molecular weights similar to the α, β, and γ phaseolin groupings of cultivated beans described by Brown et al. (1981). The

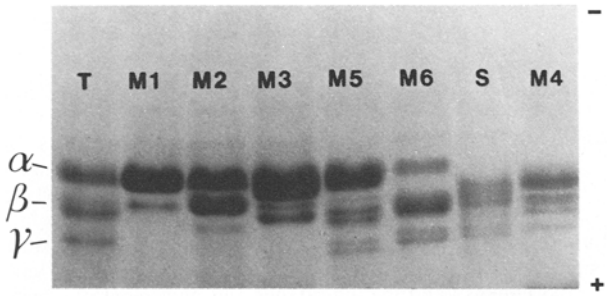

Fig. 1. Electrophoretic patterns of the phaseolin fraction from six nondomesticated bean accessions (M1, M2, M3, M4, M5, and M6). T 'Tendergreen' and S 'Sanilac' are the most common phaseolin patterns in bean cultivars. The α, β, and γ families of polypeptides are indicated

Table 1. Genetic analyses of phaseolin variants in F_2 progenies of Sanilac (S) crossed to five accessions (M) of nondomesticated *Phaseolus vulgaris* L

Parent or generation	No. of seeds per phaseolin type ×			(df=2)[b]	$P>$
	S	'F_1'[a]	M1		
325679-6	0	0	12		
'Sanilac' × 325679-6	0	2	0		
F_2 observed	11	35	14	1.97	0.25
	S	'F_1'	M2		
318696-B2	0	0	12		
'Sanilac' × 318696-B2	0	3	0		
F_2 observed	13	31	16	0.37	0.75
	S	'F_1'	M3		
325688-2	0	0	12		
'Sanilac' × 325688-2	0	1	0		
F_2 observed	12	36	12	2.40	0.25
	S	'F_1'	M5		
318700-A3	0	0	12		
'Sanilac' × 318700-A3	0	1	0		
F_2 observed	11	29	20	2.77	0.25
	S	'F_1'	M6		
325680-5	0	0	12		
'Sanilac' × 325680-5	0	3	0		
F_2 observed	17	27	16	0.60	0.50

[a] 'F_1' is the F_1 phenotype which appeared to be similar to a mixture of equal amounts of maternal and paternal phaseolins
[b] Expected ratio for each F_2 was 15 S: 30 'F_1': 15 M type

six variants were designated 'M1' through 'M6'. The South American accession, PI 266910, showed a 'T' phaseolin pattern. The absence of 'T' and 'C' types, the relative rarity of the 'S' type, and the fact that none of the six new phaseolin variants has been observed in either Mexican or other cultivated beans were unexpected (Brown et al. 1982; Romero-Andreas, unpublished data). The different phaseolin phenotypes found in this sample of nondomesticated beans suggested extensive genetic diversity of phaseolin structural genes not present in the cultivated plant materials.

The phaseolin phenotypes of all the progeny seeds were identical to the phenotype of their respective parent. It was assumed during the initial screening that any protein having an apparent molecular weight between 45 and 51 kd, the range observed for the three phaseolin types characterized previously, was phaseolin. SDS-PAGE of phaseolin extracted from the S_1 seeds of each 'M' phaseolin type showed that all of the 'M' phaseolins exhibited the same differential solubility properties as the 'S', 'C' and 'T' phaseolin and did not coelectrophorese with other seed proteins (gels not shown).

Plants of PIs 318696, 318700, 325679, 325680, 325688 and 325690, representing the six phaseolin types were crossed to the cultivar 'Sanilac'. However, because the 'M4' phenotype (PI 325690) could not be distinguished clearly from the 'S' phenotype on a 1-D SDS gel, analysis of that cross was not continued. All F_1 seeds tested showed a phaseolin phenotype similar in appearance to a mixture of maternal and paternal phaseolins. The resulting F_1 plants were indeterminante with secondary branching and displayed purple flowers resembling more closely the male than the female parent.

Sixty F_2 seeds from each of five F_1 plants were analysed by SDS-PAGE. Only three phaseolin phenotypes, the maternal and paternal electrophoretic patterns and a pattern resembling the F_1 phenotype were seen in each F_2 population. The segregation ratios of each of the five F_2 populations did not deviate significantly from an expected 1 : 2 : 1 ratio (Table 1).

The three allelic forms of phaseolin found most commonly in cultivars and the additional variants from nondomesticated beans provide considerable variability within a narrow range of molecular weights and isoelectric points. Phaseolin subunits tend to be grouped within three molecular weight classes, but in the 'M4' type the 51 kd subunit was absent, and the 'M1', 'M2', and 'M3' phaseolins lacked the lower molecular weight components.

Whether the wild bean of Mexico is the progenitor of the cultivated bean is unknown. Unless we assume that the 'M' phaseolin types were associated with undesirable horticultural characteristics, and therefore were eliminated from domesticated populations, it is difficult to explain the absence of the 'M' phaseolin types in cultivated beans.

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