Gene Pool Specificity, Paucity of Enzyme Variation, and Phaseolin Polymorphism in the Common Bean

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Abstract. The future of molecular markers in common bean (*Phaseolus vulgaris* L.) breeding and genetics lies in the discovery of more useful markers than now available. One-dimensional SDS/PAGE analysis revealed four phaseolin types, “S,” “T,” “C,” and “H,” in proportions of 23.5%, 49.2%, 24.8%, and 2.5%, respectively. Molecular heterogeneity of phaseolin subunits was not apparent. On the basis of the phaseolin types and seed size, ≈75% of the landraces from Malawi probably were introduced from the Andean primary center of common bean domestication. The remaining 25% were small-seeded and probably originated from the Meso-American center of domestication of common bean. In Malawi, some amount of hybridization has occurred between genotypes from the two centers of domestication.

Isozyme diversity has been studied in cultivated and wild genotypes of common bean (Bassiri and Adams, 1978a, 1978b; Koenig and Gepts, 1989; Weeden, 1984, 1986). Weeden (1984) successfully distinguished among snap bean cultivars using 10 isozyme loci. However, Sprecher (1988) reported that common bean appears to exhibit gene pool specificity with respect to at least the six enzymes investigated. Two gene pools, Andean and Meso-American, have been identified in common bean (Evans, 1976; Koenig and Gepts, 1989; Singh and Gutierrez, 1984). The two common bean gene pools are marked by alternate alleles at the six isozyme loci reported by Sprecher (1988). In other words, the polymorphisms for these enzymes occur between and not within gene pools. The differences in the two reports may be due to the type of genetic materials used in the two investigations. While Sprecher (1988) used landraces, Weeden (1984) investigated cultivars whose pedigrees included genotypes from the Andean gene pool.

Common bean is an important legume in Malawi. Most of the bean crop is grown using landraces of a wide range of seed sizes and

color patterns. This variation has been characterized on the basis of phenological, morphological, agronomic, and other quantitative traits (Adams and Martin, 1988; Ferguson and Mkandawire, 1993; Martin, 1984), as well as isozyme data (Sprecher, 1988). The common bean differs from other studied legumes, such as soybean (*Glycine max* L. Merr.), pea (*Pisum sativum* L.), and faba bean (*Vicia faba* L.), in that it stores protein primarily as vicilin (Lioi, 1989). Vicilin in common bean is also called phaseolin or globulin-1 (Brown et al., 1980, 1981, 1982). Ma and Bliss (1978) reported that phaseolin accounts for between 35% to 50% of N in the seed of field bean.

Phaseolin exhibits polymorphism in common bean. Electrophoretograms of fully dissociated phaseolin from various genotypes have revealed the occurrence of three predominant patterns described as “S,” “T,” and “C,” representing the respective first letters of the names of the genotypes in which they were first discovered, i.e., ‘Sanillac’, ‘Tendergreen’, and ‘Contender’ (Brown et al., 1981). Further analysis of the polypeptide subunits of phaseolin has revealed that the protein is capable of exhibiting microheterogeneity (Brown et al., 1980). Subsequently, variants of the primary polymorphs have been reported for some of the predominant phaseolin forms, including “Sd” and “Sb” for the “S” form (Koenig et al., 1988). In addition, novel phaseolin types have been discovered in both cultivated and wild common bean, including “A,” “B,” “H,” “I,” “J,” and “M” (Gepts et al., 1986; Koenig et al., 1988).

The objectives of our study were to 1) present evidence to corroborate the report by Sprecher (1988), 2) discuss the implications of

the paucity and gene pool specificity of isozymes in common bean breeding and genetics, and 3) investigate the phaseolin diversity and its geographic distribution in Malawi.

Materials and Methods

The 650 accessions used were collected from farmers' fields in the Dedza (southern region; lat. 14°22'30"S, long. 33°19'45"E) and Lilongwe (central region; lat. 13°59'15"S, long. 33°46'15"E) areas by Anne Ferguson in 1987, and from the northern section by Martin (1984). The materials used by Sprecher (1988) were collected from the northern part of Malawi. Three seeds per accession and plants raised from them were sampled and prepared for electrophoresis following the procedures used by Weeden (1984). Patterns for the following 23 enzymes were investigated: acid phosphatase (EC 3.1.3.2), aconitate dehydrogenase (EC 4.2.1.2), alcohol dehydrogenase (EC 1.1.1.1), aspartate amino transferase (EC 2.6.1.1), catalase (EC 1.11.16), diaphorase (EC 1.6.4.3), endopeptidase (EC 3.4.23.6), fluorescent esterase (EC 3.1.1), glucose phosphate isomerase (EC 5.3.1.9), glucose-1-phosphate transferase, glucose-2-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase (EC 1.1.1.49), isocitrate dehydrogenase (EC 1.1.1.41), leucine aminopeptidase (EC 3.4.11.1), malate dehydrogenase (EC 1.1.1.37), malic enzyme (EC 1.1.1.40), mannose-6-phosphate isomerase (EC 5.3.1.8), phosphoglucosyl transferase (EC 2.7.5.1), peroxidase (EC 1.11.1.7), 6-phosphogluconate dehydrogenase (EC 1.1.1.43), ribulosebiphosphate carboxylase (EC 4.1.1.39), shikimate dehydrogenase (EC 1.1.1.25), and superoxide dismutase (EC 1.15.1.1).

Samples were prepared for electrophoresis following the procedures of Brown et al. (1981) and electrophoresed by one-dimensional SDS/PAGE according to the method of Laemmli (1970) as modified by Ma and Bliss (1987). The phaseolin phenotypes of the various accessions were scored by comparing the patterns to those of reference genotypes obtained from the International Center for Tropical Agriculture in Colombia, South America. Six additional seeds were selected randomly from each accession and individually weighed.

Results and Discussion

Isozymes. Estimation of the frequency of isozymes was limited to those that showed consistent and scorable polymorphism (Table 1). The frequencies observed were similar to those reported by Sprecher (1988). Various allele combinations were identified, representing a more anodally located unit on the zymogram by F (for fast allele) and S (for slower migrating unit or allele). The two allelic combinations occurring most frequently were a) F-S-S-F-S-S and b) S-S-F-S-F-F for *Dia-1*, *Dia-2*, *Me*, *Prx*, *Rbsc*, and *Skdh*, respectively. Twenty-five accessions exhibited isozyme phenotypes that differed from the two predominant patterns at two or more enzyme loci. Sprecher (1988) was first to report

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Table 1. Allele frequencies of seven polymorphic loci in 402 Malawian accessions and their gene pool affiliation.

Locus	Allele frequency ^a		Gene pool affiliation ^b	
	F	S	Andean	Meso-American
<i>Dia-1</i>	0.86	0.14	F	S
<i>Dia-2</i>	0	0	S	S
<i>Me</i>	0.29	0.71	S	F
<i>Prx</i>	0.22	0.78	F	S
<i>Rbsc</i>	0.28	0.72	S	F
<i>Sdpr</i>	0.84	0.16	F	S
<i>Skdh</i>	0.27	0.73	S	F
<i>Mdh</i>	0.28	0.72	?	?

^aF, S = fast and slow alleles, respectively.^bThe gene pool affiliation as reported by Sprecher (1988).

the identical predominant allelic patterns as reported above, and associated pattern "a" with the common bean Andean gene pool and the "b" with the Meso-American gene pool. The 25 intermediate phenotypes observed could have arisen by intergene pool crossing with subsequent natural selection. As Martin (1984) reported, up to 2.2% outcrossing occurs among Malawian common bean landraces. Single seed weight was averaged over all the accessions that displayed either of the two predominant allelic patterns. The two averages were significantly different ($P \leq 0.01$), being 0.43 g/seed for pattern a and 0.30 g/seed for pattern b.

The separation of the accessions into two subpopulations corroborates the findings of Sprecher (1988). A significant difference was found for malate dehydrogenase (MDH), which appeared not to be gene-pool specific as reported for other enzymes. A factor analysis also indicated this independence when MDH produced the smallest loading coefficient in factor 1 but had the largest coefficient in factor 2 (Table 2). Furthermore, *Dia-1* and *Sdpr* (seed protein) were loaded with large negative coefficients in factor 1, indicating some association between them. Only two alleles per isozyme locus occurred in the Malawian accessions.

The reports of researchers in common bean breeding and genetics, including those cited in this report and the present study, indicate that enzyme variations have been conclusively observed at 15 out of ≈ 40 enzymes investigated. This number of polymorphic loci may be too few for applications such as gene tagging and genome analysis of quantitative variation. The utility of isozymes as markers in common bean research is further limited by the existence of isozyme allele-gene pool speci-

ficity. Researchers who work with genotypes from one gene pool will be severely restricted in the use of isozymes as markers in the breeding of common bean because they will have access to only one allele per locus (except where multiple alleles exist), unless the study involves genotypes from the two gene pools or materials such as those used by Weeden (1984). In the latter case, the researchers will have to contend with the problem of partial reproductive isolation of the two gene pools and the consequence of dwarf lethality (Singh and Gutierrez, 1984) attendant to intergene pool hybridization in common bean.

Until new enzyme polymorphisms are found, especially within gene pools, researchers interested in using molecular markers in common bean research will have to turn to alternative techniques, such as restriction fragment length polymorphisms and polymerase chain reaction to find additional markers.

Phaseolin. In the study of phaseolin, four of the 11 reference phaseolin types were present in the Malawi collection, the most frequent form being T (49.2%), followed by C (24.8%), S (23.5%), and H (2.5%) (Table 3). Gepts (1984) reported similar trends, i.e., 62% (T), 10.8% (C), and 17% (S), in a collection of 111 cultivars from various bean-growing countries in Africa. No H form was reported. The S form was found predominantly in the small-seeded genotypes, while the other three were associated with the large-seeded genotypes (Table 3). Similar findings have been reported by Gepts (1984), Gepts et al. (1986), and Koenig et al. (1988), who noted that common

beans from the Andean region of South America are characterized by large-seeded genotypes and the T, C, H, A, J, and I phaseolin forms, while beans from the Meso-American region tend to be small-seeded and exhibit the S or M phaseolin types. A few large-seeded genotypes showed the S phaseolin pattern usually associated with small seeds (Table 3). Although the two common bean gene pools show partial reproductive isolation (Singh and Gutierrez, 1984), an outcrossing rate of up to 2.2% was reported among the landraces from Malawi (Adams and Martin, 1990; Martin, 1984). This natural crossing with selection may be responsible for the occurrence of the noncharacteristic phaseolin types in certain genotypes.

As was concluded previously by Gepts (1988), cultivars from both Meso-American and Andean origins were disseminated to various parts of the world to a varying extent. In the case of Malawi, $\approx 75\%$ of the common bean grown appears to have Andean origin, while $\approx 25\%$ were probably introduced from the Meso-American source. The probable routes by which the landraces reached Malawi from the two primary centers of common bean domestication were via Brazil and western Europe (Gepts, 1984).

Of the four phaseolin types observed, no variant forms were apparent, and, as such, two-dimensional SDS/PAGE was not conducted to ascertain the molecular heterogeneity of the phaseolin subunits.

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Table 3. Geographic distribution of phaseolin types and association with seed weight of landraces of common bean from Malawi.

Site	Location ^a	Phaseolin types (%)				Total no. accessions
		S	T	C	H	
Chitipa North	N	5.7	58.6	35.7	0	70
Misuku Hills	N	34.0	46.8	10.7	8.5	47
Livingstonia Hills	N	35.2	43.7	18.3	2.8	71
South Ruku Plain	N	23.5	52.9	11.8	11.8	17
Mabulabo	N	1.9	77.3	18.9	1.9	53
Lilongwe area	C	20.2	53.4	24.5	1.8	163
Dedza Hills	S	30.6	38.9	28.8	1.8	229
Total						650
Percentage		23.5	49.2	24.8	2.5	
Seed wt (g)						
Minimum		0.11	0.21	0.16	0.23	
Maximum		0.60	0.92	0.95	0.52	
Average		0.30	0.44	0.46	0.40	
SE		0.01	0.01	0.02	0.04	

^aN = north, C = central, S = south; Lilongwe is located on lat. 14°S.

Table 2. Factor pattern for principal factor analysis of 402 common bean accessions from Malawi on the basis of isozyme or protein polymorphism at seven loci.

Locus	Factor 1	Factor 2
<i>Dia-1</i>	-0.73	-0.11
<i>Skdh</i>	0.84	0.08
<i>Me</i>	0.85	0.15
<i>Prx</i>	0.82	0.09
<i>Rbsc</i>	0.81	0.10
<i>Sdpr</i>	-0.81	-0.12
<i>Mdh</i>	-0.30	-0.95
Proportion of variance (%)	46.39	20.20

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