MITOCHONDRIAL RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN WILD *Phaseolus vulgaris* L.

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Restriction fragment length polymorphisms (RFLPs) of mitochondrial DNA (mtDNA) from the common bean, *Phaseolus vulgaris*, distinguish genotypes representing the two major gene pools, the Mesoamerican and the Andean (3,4). In this study, mtDNA was used to compare the amount of diversity in wild beans to that in the cultivated, in order to understand how and when the mitochondrial genomes of the two gene pools became distinct.

MATERIALS AND METHODS

Six wild bean accessions sampled across the range of distribution of wild *P. vulgaris* (Table 1) were obtained from Drs. D. Debouck and J. Tohme at CIAT, Colombia. Two cultivated bean lines from the MSU Malawi collection were used as the representatives of the cultivated Mesoamerican and Andean controls.

The procedures and conditions for mtDNA isolation, digestion and electrophoresis, Southern blotting, nick translation and hybridization were as described (4). Nine restriction endonucleases were utilized to digest the mtDNAs: AsnI, BamHI, DraI, EcoRI, EcoRV, HindIII, PstI, SalI, and XhoI. Southern blots were consecutively hybridized to five cosmid clones and strip-washed after each hybridization. The clones (C2, C3, C4, C6, C8) were provided by Dr. C. D. Chase (University of Florida, Gainesville). They contained random inserts from the bean mitochondrial genome ranging in size from 29 to 38 -kb.

RESULTS AND DISCUSSION

The restriction fragment profiles for all the enzymes were complex, and relatively uniform across the eight lines tested, as expected for an intraspecific comparison. Differences in ethidium bromide-stained gels were observed repeatedly in the case of DraI but were not as clear with the other enzymes and thus are not considered in this analysis. Hybridization experiments provided more conclusive results. Six out of nine enzymes revealed 20 polymorphisms. The scores (pattern #1 or #2) of the eight bean lines with respect to the polymorphic enzyme x probe combinations are organized in Table 1. The first six RFLPs differentiated the gene pools of the Mesoamerican and Andean cultivated beans. The other 14 RFLPs distinguish one or two of the wild bean lines from all of the others. This is a good indication of the higher variability of mtDNA in the wild material.

For RFLPs # 1 and 2, all six wild beans are similar to the cultivated Andean beans, while for RFLPs # 3 and 4 they are similar to the cultivated Mesoamerican beans. Maximum parsimony would dictate that the first two RFLPs were due to mutations occurring in the Mesoamerican gene pool, with the other two RFLPs due to mutations occurring in the Andean gene pool. These mutations would have occurred after domestication but they must have happened early enough such that all examined cultivated beans of an individual gene pool were the same (4).

As to the fifth polymorphism, it obviously occurred before domestication since the wild beans themselves exhibit both patterns. Surprisingly, however, accession w3 from northern Peru showed the same pattern (#1) as the Mesoamerican wild (w1, w2) and cultivated beans rather than pattern 2 seen in the other Andean wild (w4,5,6) and cultivated beans. Consistent with this observation, Koenig and Gepts (5) found that allozyme allelic frequencies in accessions from Colombia and northern Peru are more similar to those of Mexican accessions than to those of populations from southern Peru and Argentina. A sixth polymorphism (RFLP # 6), detected with AsnI and probe C2 differentiated the cultivated Mesoamerican and Andean beans used in this study, and was also obvious in the wild beans, indicating that the mutation happened before domestication. In this case, however, only accession w2 from Guatemala showed the same pattern as the Mesoamerican control.

Our results confirm previous studies of phaseolin (2,6) and allozyme (5) diversity showing that wild bean accessions from Mesoamerica and Colombia contain higher genetic variability than those from the southern Andes.

Table 1. The 20 mtDNA RFLPs detected in the cultivated and wild bean lines.

RFLP Number	Enzyme	Gel or Probe	Bean Lines*							
			cM	w1 G12878 Mexico	w2 G19906 Guatemala	w3 G21245 Peru	w4 G23455 Peru	w5 G23442 Bolivia	w6 G19892 Argentina	cA
1	Dra I	Gel	1	2	2	2	2	2	2	2
2	EcoR I	C4	1	2	2	2	2	2	2	2
3	Dra I	Gel	1	1	1	1	1	1	1	2
4	EcoR I	C2	1	1	1	1	1	1	1	2
5	Dra I	C3/C6	1	1	1	1	2	2	2	2
6	Asn I	C2	1	2	1	2	2	2	2	2
7	EcoR V	C3	1	2	1	1	1	1	2	1
8	Dra I	Gel	1	1	2	1	1	1	1	1
9	Dra I	Gel	1	1	1	2	1	1	1	1
10	Dra I	C8	1	1	2	1	1	1	1	1
11	EcoR I	C2	1	1	1	2	1	1	1	1
12	EcoR V	C3	1	2	1	1	1	1	1	1
13	EcoR V	C8	1	2	1	1	1	1	1	1
14	Asn I	C3	1	1	1	2	1	1	1	1
15	Asn I	C3/C6	1	2	1	1	1	1	1	1
16	Asn I	C6	1	1	1	2	1	1	1	1
17	Asn I	C8/C4	1	1	1	2	1	1	1	1
18	Asn I	C8	1	1	1	2	1	1	1	1
19	Hind III	C4	1	1	1	1	1	1	2	1
20	Xho I	C6	1	1	1	2	1	1	1	1

* cM, cA are the cultivated Mesoamerican and Andean controls, respectively, and w1 to w6 are the wild bean accessions with their respective CIAT number and country of origin.

The significantly higher mtDNA variability of accession w3 is quite peculiar. Koenig and Gepts (5) have shown with allozyme data that this same accession (DGD 1962) from northern Peru is genetically distinct from 82 other wild bean accessions that clustered into two major groups.

The similarity of mtDNA from the cultivated Andean beans to mtDNA of the south Andean wild accessions, especially w4 and w5, is remarkable. These data suggest that the south Peru - Bolivia area may be the site of initial domestication of the Andean beans. On the other hand, the mtDNA patterns of the Mesoamerican cultivated beans are most similar to those of the wild bean accession from Guatemala, which, in a similar fashion, may point to a specific area of bean domestication in Mesoamerica.

Finally, it was surprising to see that the mtDNA of the bean accessions from the extremes of the range of distribution of wild *P. vulgaris*, w1 and w6, shared one polymorphism that differentiated them from all other wild beans (Table 1, RFLP # 7). It is unclear whether this RFLP arose twice independently or if it is due to common descent.

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