Mitochondrial genome size variation and restriction fragment length polymorphisms in three *Phaseolus* species

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Received January 25, 1991; Accepted March 7, 1991
Communicated by R. Hagemann

**Summary.** Restriction patterns of mitochondrial DNA (mtDNA) from three *Phaseolus* species were examined to estimate their relative genome sizes and to determine the level of interspecific variability and relatedness. Three restriction endonucleases that produced relatively simple profiles were identified and used to determine the genome size of the three species. Taking into account fragment stoichiometries, the average estimates across enzymes were 456, 324, and 400 kb, respectively, for *P. vulgaris*, *P. coccineus*, and *P. acutifolius*. Restriction fragment length polymorphisms (RFLPs) differentiated the species when the mtDNAs were digested with seven endonucleases and hybridized with five cosmid clones covering ca. 200 kb of mtDNA sequences. Proportions of shared restriction fragments between every two species were computed as F-values and demonstrated that *P. vulgaris* and *P. coccineus* are more related to each other than either is to *P. acutifolius*, and that the latter has a similar degree of relationship to the other two species.

**Key words.** Mitochondrial RFLPs – *Phaseolus* – Species relationships – Genome size

**Introduction**

The genus *Phaseolus* L. originated in the American continent and comprises about 30 species (Maréchal et al. 1978), of which approximately 90% occur in Mexico or adjacent areas of the USA and Central America (Smartt 1985). Four species have been most prominent as cultivated food crops: *P. vulgaris* L. (common bean, dry bean, snap bean), *P. coccineus* L. (runner or scarlet runner bean), *P. acutifolius* Gray (tepary bean), and *P. lunatus* L. (lima bean, Sieva bean). All four species are diploid (2n = 2x = 22) and have both domesticated and wild representatives. In the case of both *P. vulgaris* and *P. lunatus*, small-seeded and large-seeded forms are thought to have been domesticated independently in Mesoamerica and Andean South America (Kaplan 1965, 1981). The other two species appear to have been domesticated in Mesoamerica (Kaplan 1965), although Pratt and Nabhann (1988) suggest Aridoamerica (northwestern Mexico and the southwestern United States) as the area of domestication of tepary bean.

Several studies on morphological and pollen characteristics (Maréchal et al. 1978), interspecific hybridization (reviewed in Hucl and Scoles 1985; Mok et al. 1986), seed protein composition (Derbyshire et al. 1976; Sullivan and Freytag 1986) and their immunochemical reactions (Kloz et al. 1966; Kloz and Klozová 1974), and isozyme patterns (Bassiri and Adams 1978) have provided information on the relationships between the four cultivated species. The studies suggested that *P. vulgaris* and *P. coccineus* are most closely related, with *P. acutifolius* more distantly related and *P. lunatus* the most distant from the other three species. According to Harlan and de Wet’s (1971) gene pool nomenclature system, the primary gene pool of *P. vulgaris* comprises the genetic resources of the wild and domesticated populations, its secondary gene pool contains *P. coccineus* (and vice versa), and the tertiary gene pool includes the other species (Smartt 1985).

Comparative analysis of restriction fragment length polymorphisms (RFLPs) of organelle DNAs has enabled the resolution of species relationships in such crops as *Brassica* (Palmer et al. 1983), *Coffea* (Berthou et al. 1983), *Daucus* (DeBonte et al. 1984), *Aegilops* (Terachi and Tsunewaki 1986), *Lycopersicon* and *Solanum* (Mc-
Clean and Hanson 1986), and Pennisetum (Chowdhury and Smith 1988). The relatively small size of the chloroplast genome (120-180 kb) allows differences in restriction patterns to be seen clearly in agarose gels, permits restriction site differences to be mapped, and facilitates deduction of the events that resulted in the RFLPs. Due to the larger sizes and higher rates of rearrangements of plant mitochondrial genomes with respect to chloroplast genomes, measures of mitochondrial DNA (mtDNA) relatedness between plant species have been limited to the estimation of the proportion of shared restriction fragments between any two species. Plant phylogenies have been constructed using either the cladistic approach where chloroplast DNA (cpDNA) restriction sites are considered separate characters (e.g., Palmer et al. 1983), or the phenetic approach where relationships are expressed by a distance measure obtained from the number of shared restriction sites (more feasible with cpDNA) or shared restriction fragments (more common with mtDNA, e.g., Terachi and Tsunewaki 1986).

A study of mtDNA diversity within P. vulgaris indicated a high complexity (large size) of the genome and a very low level of variation (Khairallah et al. 1990). The infrequent RFLPs allowed the differentiation of cultivated common bean into its two major gene pools. In this study, we have utilized mtDNA RFLPs to determine the extent of mtDNA diversity among Phaseolus species in comparison to the diversity within the species. We have used these data to examine species relationships. For this reason, we have limited our study to the primary, secondary, and tertiary gene pool representatives: P. vulgaris, P. coccineus, and P. acutifolius. An additional objective of this research was to estimate the mitochondrial genome size of the three species because only rough estimates for P. vulgaris and P. coccineus are available.

Materials and methods

Plant materials

Small-seeded accessions of P. vulgaris were used to compare its mtDNA to that of the other two species because of the common Mesaoamerican origin in contrast to the South American origin of large-seeded common beans. Because no RFLPs have been detected in mtDNA from small-seeded lines of P. vulgaris (Khairallah et al. 1990), two were used as the source of mtDNA. These were lines 5-2 and 5-8 collected in Malawi (see Khairallah et al. 1990). A single accession each of P. coccineus and P. acutifolius was examined. These consisted of local cultivars collected at Francisco I. Madero, Durango, and San Pedro, Coahuila in Mexico, respectively, and were grown for seed increase at Durango, Durango.

Seeds were planted in vermiculite trays and allowed to germinate in darkness. The tepary bean seeds were first scarified to ensure a faster and more uniform germination.

Genome size estimation

The mtDNAs used for estimating the sizes of the three genomes were isolated as described in Khairallah et al. (1990), but were further purified through a cesium chloride (CsCl) gradient as follows. After overnight precipitation at -20°C, the mtDNA pellet was resuspended in 2-3 ml T10Et1 (10 mM TRIS, 0.1 M EDTA, pH 8.0) and 1.2 g solid CsCl/ml was added and allowed to dissolve. Bisbenzimide was added to a final concentration of 10-11 μg/ml and more CsCl was included to a final density of 1.67 g/ml, which corresponded to a refractive index of 1.3970±0.0008. The gradients were centrifuged for 18-20 h at 154,300 x g in a Sorvall TV 865 vertical rotor. The mtDNA band was removed and the dye was extracted with NaCl-saturated isopropanol. The mtDNA was then precipitated in 1/20 vol. of 5 M ammonium acetate and 1/2 vol. ice-cold isopropanol at -20°C overnight.

Samples of the mtDNAs were digested at 37°C for 5-7 h with NarI, SalI, or SstI [Bethesda Research Lab, Inc. (BRL)]. The resulting fragments were separated by electrophoresis in 0.5-0.7% agarose gels at room temperature for 20-35 h at constant voltage (30-40 V), using the TAE buffer system (0.04 M TRIS, 0.02 M sodium acetate, 0.001 M EDTA, pH 8.0, and 0.5 μg/ml etidium bromide). Three sets of molecular size markers were used: lambda DNA digested with HindIII, lambda DNA digested with HinfIII and EcoRI, and high-molecular-weight markers from BRL. The gels were photographed with Polaroid type 55 film using UV light or two different exposures. The negatives were then enlarged on 8 x 10 sheet film (Kodak TMAX 100) and were scanned on a Gilford Response II spectrophotometer using 500 nm visible light to read the percentage transmittance. The areas under the peaks were determined manually by counting the number of squares on graph paper in each peak.

Sizes of individual restriction fragments were estimated following the method of Schaffer and Sederoff (1981) using a computer program written in QuickBasic.

RFLP analysis

Procedures and conditions for mtDNA isolation, digestion, and electrophoresis, Southern blotting, nick translation, and hybridization were as described in Khairallah et al. (1990). Seven restriction endonucleases were used in this study: BamHI, Draf, EcoRI, HindIII, PstI, SalI, and XhoI. Southern blots were consecutively hybridized to five cosmids clones provided by Dr. C. D. Chase (University of Florida, Gainesville). The clones contain random inserts from the bean mitochondrial genome ranging in size from 29 to 38 kb.

For each enzyme × probe combination, the total number of fragments hybridizing per species (Nx, Ny) and the number of fragments shared by each pair of species (Nxy) were recorded. These were then added across clones for each enzyme and adjusted by counting fragments that hybridized to more than one clone only once. Using those figures, indices of relatedness (F-values) for the three pairs of species were computed according to Nei and Li (1979), where Fxy = [2 Nxy]/[Nx+NY]. Higher F-values indicate more relation between the species.

Results

Genome size estimation

Estimates of the mitochondrial genome size of the three bean species were obtained from restriction profiles of three enzymes by adding up lengths of individual fragments and accounting for their multiplicities. For these analyses, the enzymes NarI, SalI, and SstI were selected from a group of 11 enzymes because they produced the