GENETIC DIVERSITY OF THE CIAT TEPARY BEAN (Phaseolus acutifolius A. Gray) COLLECTION MEASURED WITH AMPLIFIED FRAGMENT LENGTH POLYMORPHISM MARKERS

L. C. Muñoz, M.W. Blair, D. Debouck

International Center for Tropical Agriculture, CIAT

Rationale

Tepary bean (Phaseolus acutifolius A.Gray) is in the tertiary gene pool of common beans (P. vulgaris L.) and as such represents a potential but difficult to use genetic resource for the improvement of common beans. The two species have been crossed, despite high embryo abortion, using congruity or recurrent backcrossing and these interspecific hybridizations have been used to incorporate common bacterial blight resistance into common bean. Notwithstanding their utility for the improvement of other species, tepary beans are a useful and interesting crop in their own right, especially for dryland agricultural systems. They are known to have high drought and salinity tolerance, good nutritional quality and a tradition of cultivation in Mexico, South western United States and Central America, that goes back 5000 years. Many landraces exist but there are no improved varieties. For this it is critical to have a baseline data on the diversity that exists within tepary beans. Previous authors (Scinkel and Gepts, 1989, Plant Breeding 102: 182-195; Garvin and Weeden, 1994; Crop Science 34: 1390-1395) have suggested that tepary beans seem to be less diverse than common bean or lima beans. They are thought to have had a single center of origin and to have been distributed from a few original sites across the present distribution. The objective of this research was to study the patterns of diversity within the species and its placement relative to other Phaseolus species that were used as outgroups, using amplified fragment length polymorphism (AFLP) markers. Relative to other molecular markers, AFLPs tend to be evolutionarily conserved markers and serve to reference different species relative to each other. Additional objectives of the study were to determine if P. acutifolius and P. parvifolius merit being separate species and if molecular markers can distinguish between the botanical varieties var. acutifolius and var. tenuifolius within the species P. acutifolius. AFLP markers have been applied before to study wild species of Phaseolus (Tohme et al., 1996; Crop Science 36: 1375-84) and lima bean, P. lunatus, accessions and their close relatives (Caicedo et al., 1999; Crop Science 39: 1497-1507) but not to tepary beans.

Materials and Methods

A total of 127 genotypes from the Genetic Resources Unit of CIAT were analyzed in the experiments. The outgroup consisted in 10 genotype from the Phaseolus genus including 4 P. vulgaris (common bean); 4 P. lunatus (lima bean); 1 P. coccineus (scarlet runner bean); and 1 P. glabellus genotype. For both common and lima beans a wild and a cultivated representative from both the Andean and Mesoamerican gene pools was included in the analysis. For the other species only a single representative was analyzed. A total of 117 tepary beans and their close relatives were analyzed, consisting in 49 cultivated P. acutifolius var. acutifolius; 44 wild P. acutifolius var. acutifolius; 12 P. acutifolius var. tenuifolius; and 12 P. parvifolius accessions. The genotypes were grown in the greenhouse and total genomic DNA was extracted from 2 g of fresh leaf tissue. AFLP fragments were generated with the Gibco BRL AFLP analysis system I kit. In a previous study, we chose the combination of E-AAG and M-CTT primers based on a survey of EcoRI (E) -Msel (M) adapters and primers with 3 selective nucleotides each. PCR products were run on 4% silver-stained polyacrylamide gels for 1, 1.5 and 2 hours to resolve as many fragments as possible. Bands were sized by comparison to a 50bp ladder molecular weight size standard. All the polymorphic AFLP bands between 100 and 400 bp were scored for presence or absence among the lines and used to calculate the similarity matrix. Genetic similarities between genotypes was determined with the Dice coefficient using the software packages SAS (SAS Institute, 1989) and NTSYS 2.02.

Results and Discussion

The AFLP combination used in this study had a good polymorphism rate, clear amplification profile and well-distributed range in PCR product sizes. The AFLP combination produced a total of 167 bands. Of these, 99.5% of the bands were polymorphic across all species although there was substantial monomorphism within the cultivated P. acutifolius. Both monomorphic and polymorphic band were used to determine the genetic similarity between genotypes. Figure 1 shows the dendrogram created for the AFLP bands. The structure of the dendrogram agrees with known taxonomic relationships for the six species represented in the study. P. lunatus was the most distant group, followed by P. glabellus and P. coccineus. P. vulgaris was the closest to the P. acutifolius -parvifolius clade. The level of similarity was around 35% between the five species/groups. Within both P. vulgaris and P. lunatus the distinction between Andean and Mesomerican gene pools was clear. The level of similarity between gene pools was higher in P. vulgaris (68%) than in P. lunatus (62%). Within the P. acutifolius – parvifolius
clade, all the accessions shared up to 54% similarity. Five groups could be distinguished within this clade: 1) cultivated *P. acutifolius* from Central and North America 2) cultivated *P. acutifolius* from North America (mainly Sonora and Sinaloa), 3) wild *P. acutifolius* var. *acutifolius* 4) wild *P. acutifolius* var *acutifolius* and *tenuifolius*; and 45) *P. parvifolius*. These five groups could be organized hierarchically into two supergroups, consisting of groups 1, 2 and 3 together and groups 4 and 5 together. The first grouping contained all the cultivated *P. acutifolius*, while the second grouping contained all the *P. acutifolius* var. *tenuifolius* and *P. parvifolius* accessions. The wild *P. acutifolius* accessions were distributed among the two groups, with some more allied to the cultivated accessions of the same species and others allied to the *P. parvifolius* group. Within the first group, the two cultivated groups (1 and 2) were related at 80% similarity and these were related to the wild accessions (group 3) within that group at 68% similarity. Within the second group, the *P. parvifolius* and *P. acutifolius* (both var. *acutifolius* and *tenuifolius*) were related at 64% similarity. The groups were distinguishable at 54% similarity.

The high genetic similarity detected with AFLP markers for all the cultivated tepary beans, seems to indicate that the crop may have arisen from a single domestication event that led to a genetic bottleneck which limits diversity within the cultivars. From this study, there is very little evidence for introgression from wild relatives into the cultivated gene pool after the initial domestication event. Tepary beans are known to have a very low crossing rate that limits the creation of new diversity within the crop. The lack of diversity within the cultivated tepary bean is a serious limitation for improvement of the crop although it belies some of the variability found for disease and insect resistance within the cultivars. Biotic resistances are often fast evolving characteristics so could be expected to have been generated by mutation even without a lot of initial diversity or inter-crossing. However, that lack of diversity in other characteristics such as plant morphology and adaptation range has serious implications for improving the species agronomically and using the species in inter-specific hybridization. The AFLP data presented here also clarifies the relationships within the *P. acutifolius – parvifolius* clade which has been controversial and suggest that *P. acutifolius* and *P. parvifolius* probably do not deserve to be different species, but could qualify as possible subspecies or varieties within the species. The high amounts of diversity found in the wild *P. acutifolius* and *P. parvifolius* accessions are an interesting resource for breeding tepary bean cultivars in the future.

**Figure 1.** Dendrogram showing the associations among 114 accessions of cultivated and wild tepary beans (*Phaseolus acutifolius* and *P. parvifolius*) and 10 other related species, based on UPGMA clustering for the AFLP banding pattern from primer combination E-AAG, M-CTT.