GENETIC ANALYSIS OF CROSSES BETWEEN CULTIVATED TEPARY BEAN AND WILD *PHASEOLUS ACUTIFOLIUS* AND *P. PARVIFOLIUS*. -

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Introduction: Cultivated tepary bean (*Phaseolus acutifolius*) has several wild relatives. First are the wild accessions within the species itself, these include two variants (var. *acutifolius* and var. *tenuifolius*) and second there are the wild accessions belonging to the closely related species, *P. parvifolius*. The genetic diversity within cultivated tepary beans is small, therefore the objective of this research was to study the variability generated by crossing cultivated tepary beans by several of their wild relatives. These crosses were analyzed for polymorphism and segregation in the F2 generation using common bean microsatellites. The crosses can be expected to incorporate added genetic diversity into the cultivated tepary beans which may be useful for breeding this neglected crop and will also be used to generate recombinant inbred lines that can be analyzed for biotic and abiotic stress tolerance genes segregating in the populations derived from these crosses.

Methodology: We used three contrasting tepary parents to develop two reciprocal F2 populations (Table 1). Parents included the genotypes G40022 (cultivated *P. acutifolius* from Arizona, USA), G40186 (wild *P. parvifolius* from Jalapa, Mexico) and G40240 (wild *P. acutifolius* var. *tenuifolius* from Durango, Mexico). Crosses were made with hand emasculation and both the F1 and F2 plants were grown in 9-inch pots in the greenhouse and single harvested. The F3 seed from each of 120 F2 plants was harvested from the greenhouse and was field-planted at CIAT headquarters in semester 2002A and 2002B. Seed scarification was used to increase the germination rate on both these sets fo reciprocal cross populations. In the F2 generation, data was collected on a series of phenotypic characteristics and furthermore, leaf tissue was collected for each individual plant grown in the greenhouse so that DNA could be extracted with standard methods. Ninety-four plants were analyzed for each of the AP populations and fourty-six plants were analyzed for each of the AT populations. A total of 68 common bean microsatellite markers were tested for polymorphism on the parents of each population. Polymorphic microsatellites were run on all individuals of the population along with the parents.

Results and Discussion: Phenotypic analysis showed that both the AP and AT populations were segregating for several phenotypic traits and several of these appear to be simply inherited, notably stem color and flower color. Meanwhile, growth habit, plant height, flowering date, maturation date, leaf size, leaf color, pod size, yield and yield components were more quantitative traits in all the populations. Leaf shape was probably an oligogenically inherited trait because there were gradations between the narrow leaf of the wild tepary bean and the wider leaf of the cultivated tepary bean. For the molecular survey, the rate of parental polymorphism was roughly equivalent for both the AP (28 microsatellites or 40.6%) and AT (29 microsatellites or 43.3%) populations. Of these a total of 25 and 20 microsatellites were selected to run on the AP and AT populations, respectively. Significant segregation distortion was observed for 44%, 52%, 15% and

25% of these markers in the populations AP-1, AP-2, AT-1 and AT-2, respectively (Table 1). The average segregation distortion was higher for the cross between *P. acutifolius* and *P. parvifolius* (48.0%) than between *P. a.* var. *acutifolius* and var. *tenuifolius* (20.0%). The segregation distortion results suggest that there is a greater distance between the parents of the AP population than the AT population. Supporting this hypothesis was the observation of genetic incompatibilities and hybrid lethals and dwarfs in the cross between *P. acutifolius* and *P. parvifolius* but none between *P. acutifolius* var. *acutifolius* and var. *tenuifolius*. In each pair of reciprocal crosses the use of the cultivated *P. acutifolius* as the female parent reduced the amount of segregation distortion, while the use of the wild parent, either *P. parvifolius* or *P. acutifolius var. tenuifolius* increased the amount of segregation distortion. This may reflect the possibility that a cytoplasmic factor for incompatibility is more significant when the wild tepary beans are used as females than when the cultivated tepary bean is used as the female parent. An additional observation was that the maternal allele was always favored over the parental allele in all of the crosses used in this study, however this was more notable in the *P. acutifolius* x *P. acutifolius* var *tenuifolius* crosses than in the *P. acutifolius* x *P. parvifolius* crosses. Garvin and Weeden (1994; Journal of Heredity 85: 273-278) made a series of inter-varietal crosses between *P. a.* var. *acutifolius* and var. *tenuifolius* and studied the resulting populations with isozymes and RFLPs, finding a similar levels of segregation distortion in this type of population, however our analysis is the first that we know of to reveal the high levels of segregation distortion in the inter-specific crosses between *P. acutifolius* and *P. parvifolius*. Segregation distortion and hybrid lethal incompatibilities are common with both intra-specific (eg. Andean x Mesoamerican *P. vulgaris*) and inter-specific crosses (*P. vulgaris* x *P. acutifolius*) within the genus *Phaseolus* and cytoplasmic effects have been observed in other crosses between species within the genus, including in crosses between *P. acutifolius* and *P. vulgaris*.

Table 1. Number of microsatellite markers showing segregation distortion (SD) and average frequency of cultivated (C), wild (W) and heterozygous (H) genotypes for four F2 populations developed for the genetic analysis of tepary bean.

Pop Code	Type of Cross	Female Parent	Male Parent	No. Indiv	No. Markers		% C	% W	% H
					total	SD			
AP-1	Inter-specific (cultivated x wild)	G40022	G40186	100	25	11	31.6	22.8	45.6
AP-2	Inter-specific (wild x cultivated)	G40186	G40022	100	25	13	24.1	29.9	38.8
AT-1	Inter-varietal (cultivated x wild)	G40022	G40240	46	20	3	25.4	17.8	56.8
AT-2	Inter-varietal (wild x cultivated)	G40240	G40022	46	20	5	21.8	34.6	43.6