Additional Evidence on Gene Flow Events in *Phaseolus vulgaris* in Costa Rica

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We present here evidence on gene flow between wild and cultivated forms of common bean in Costa Rica in addition to our previous work (González-Torres et al. 2003).

Seeds were collected from natural populations in the Central Valley of Costa Rica as previously reported (González-Torres et al. 2003). We focus on 226 weedy materials selected initially on morpho-agronomic characteristics, which phenotype is inherited from possible hybridization between wild and cultivated materials. A similar procedure has been used by Papa & Gepts (2003). The analyses were conducted on: 1) morpho-agronomic evaluation; 2) biochemical analysis of phaseolin by SDS-PAGE (Gepts et al. 1986), and isozymes: Diaphorase (DIA) and Peroxidase (PRX) according to Ramírez et al. (1987), and 3) molecular marker analysis: eight microsatellite primers reported by Gaitán-Solis et al. (2002), and cpDNA polymorphisms by PCR- RFLPs (Chacón-Sánchez, 2001).

The wild populations showed mainly two phaseolin patterns, Simple-4 and S (Table 1). In cultivated materials, the phaseolins T, Sb and Simple-4 were also observed although in low frequency.

Table 1. Morphological, biochemical and molecular markers used and No. individuals analyzed for each parameter.

Biological status	Seed average weight (g)	Phaseolin type	Isozymes		Microsatellites		cpDNA haplotypes
			Pattern[1]	Allele[2]	Primer	Allele	
Wild	6 N=443	"Simple-4" "S" N=402	DIA –1 N=227	**PRX 100** **N=204**	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 N=134	160 ***80*** 162 110 163 146 137 122	G, H N=97
Weedy	13 N=226	"C" "CH" "H" ***"S"*** "X-7"[3] **"Simple-4"** N=191	DIA-1 DIA-2 DIA-4 N=170	**PRX 100** PRX 98 **N=170**	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 N=142	160, 177 ***80*** 162, 183 110, 106 163, 189 146, 150 137, 174 122, 135	G, H J, K, L N=100
Cultivated	23 N=188	**"S"** "X-7" "CH" N=186	DIA –2 DIA –4 N=150	PRX 98 N=150	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 N=35	177 ***80*** 183 106 189 150 174 135	J, K, L N=33

[1] According to Sprecher (1988); [2]according to Koenig & Gepts (1989); [3] Phaseolin pattern for further checking.

In Figure 1, the shortest bar represents mainly wild characteristics and the longest bar is a description of cultivated materials. The bars show exchange among individuals for the following markers: shared SSR alleles, change in cpDNA haplotypes, 100-seed weight, isozymes and phaseolin patterns. In individuals 1 and 2, all the evaluated parameters are "wild" and they have a hybrid SSR allele, which suggests a cross

of wild material with pollen of cultivated material. The seed size of the individual 3 could be a phenotypic consequence of more than one past event of gene flow from cultivated material to wild form, because all evaluated parameters are "wild" including hypocotyl color (purple), purple flower, 85 days to flowering and growth habit IV. Besides, its F2 displays a weight of 10.3 g, which suggests that it has acquired "wild" characteristics but conserves the "cultivated" seed size. Individual 8 has hybrid isozymes, "wild" microsatellite alleles and phaseolin, but it has a "cultivated" chloroplast haplotype. Individual 9 has the same characteristics as individual 8 but it has "wild" isozymes. These materials represent cases of repeated gene flow of cultivated materials crossed with wild forms. Individual 14 is hybrid (PRX enzyme and one SSR locus), meaning that it comes from recent flow of "wild" pollen to a cultivated form. The evaluation of these 22 cases from Costa Rica indicates that all materials are indeed products of hybridizations showing that the methodology implemented in the selection of the intermediate materials was the appropriate one. Papa et al. (2003) found that the contribution of cultivated parental population was significantly higher than the wild parental one in Mesoamerica, while the direction of gene flow in the evaluated individuals in our study was evidenced mainly from wild material to the cultivated type. The presence of gene flow events in the other direction was observed at lower frequency.

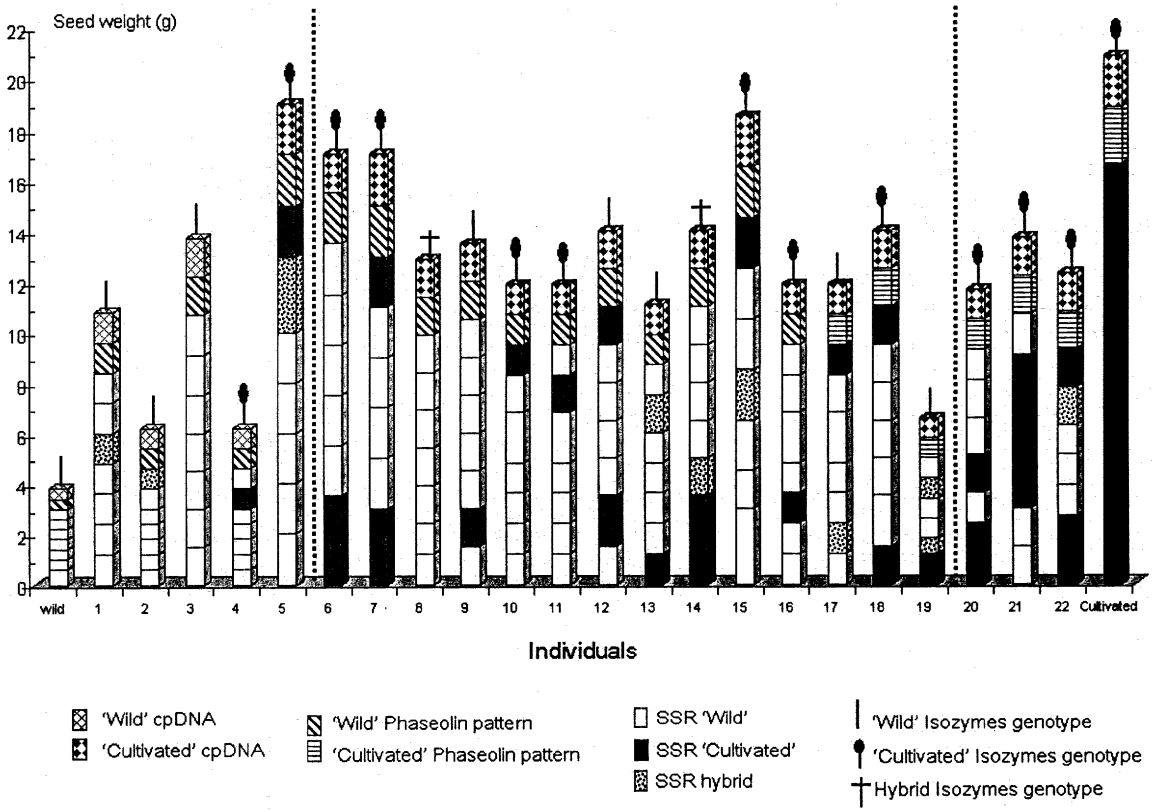

Figure 1. Graphical representation of individuals with their respective markers.

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