Estimation of gene flow on *Phaseolus vulgaris* L. using molecular markers: microsatellites and polymorphisms of chloroplast DNA

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Introduction

The natural dispersion of genetic material among populations through gene flow is a spontaneous process and may constitute an important evolutionary force to create genetic diversity. Gene flow can vary in magnitude and direction. Our plant model is preferentially autogamous but with a significant amount of outcrossing. Wild and cultivated forms of common bean are getting in contact in many places of the Americas. The present study aims to quantify gene flow in the wild-weedy-cultivated complex of common bean, resulting from cross pollinations in natural conditions in Costa Rica.

Materials and Methods

Seeds of such complex were collected in six natural populations during 1987, 1998 and 2003 in the Central Valley (González-Torres et al. 2003). A morphoagronomic evaluation (weight of 100 seeds in grams) was performed to select 732 individuals of each different biological form: wild, weedy and cultivated (Figure 1), out of which 217 individuals were weedy types possibly resulting from gene flow. A similar procedure has been used by Papa & Gepts (2003). We used as markers phaseolin and two *loci* of isozymes, and nine *loci of microsatellites* to evaluate the contribution of nuclear genome to gene flow. In addition, the direction of gene transfer was determinated by RFLPs-PCR on cpDNA (Chacón 2001). A multiple correspondence analysis (MCA) was performed to observe in a multidimensional plane the genetic structure of the population of evaluated individuals.

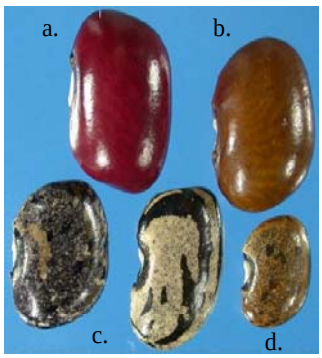

Figure 1. Biological forms evaluated:

a) Cultivated: seed size >22g, 'colored' testa.

b-c) Weedy: 8g < seed size < 22g. It could have 'colored', hybrid or *agouti* testa.

d) Wild: seed size < 8g, simple phaseolin, *agouti* testa, pod deshiscence.

Results & Discussion

The results obtained in the characterization of the populations are summarized in Table 1. The data analysis allowed to realize a graphical representation of the gene flow cases.

Biological form	Seed average weight (g)	Phaseolin type	Isozymes		Microsatellites		Chloroplast haplotype
			Pattern[1]	Allele[2]	Primer	Allele	
Wild	6 (2.5-7) N=443	"S-4" "S" "M1" "S-3" N=392	DIA –1 N=229	PRX 100 N=197	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 GATS91 N=134	160 80 164 110 165 147 138 122 224	G, H N=210
Weedy	13 (8-21.3) N=226	"C" "CH" "H" "S" "X-7" "S-4" N=196	DIA-1 DIA-2 DIA-4 N=157	PRX 100 PRX 98 N=182	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 GATS91 N=226	160, 177 80 164, 185 110 165, 189 147, 150 138, 148 122, 136 224, 243	G, H J, K, L N=170
Cultivated	23 (22-46) N=198	"S" "I" "X-7" "CH" N=198	DIA –2 DIA –4 N=64	PRX 98 N=29	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 GATS91 N=58	180 80 183 110 189 150 148 136 243	J, K, L N=53

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

In individuals 1 and 2 all the parameters are 'wild' and they have a hybrid SSR allele, which suggests a recent crossing of wild material with 'cultivated' pollen 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. Further, its F2 displays a weight of 10.3 g, which suggests that it has acquired "wild" characteristics but conserves the 'cultivated' seed size.

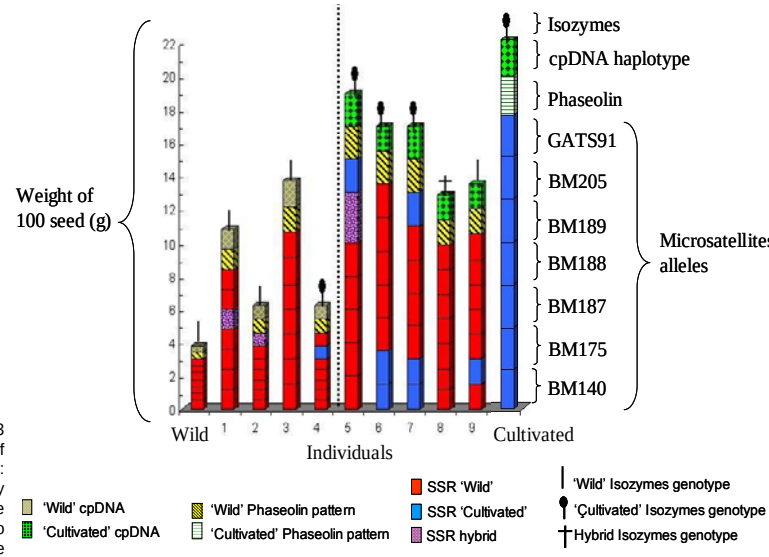

Figure 2. Graphic representation of markers used on a selection of individuals

Individual 8 has hybrid isozymes, 'wild' microsatellites 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.

The analysis of data showed, in the selected individuals, that 98% of the weedy types were indeed products of gene flow. The remaining 2% were due to phenotypic effects by environmental favorable conditions.

The principal direction of gene flow was that of wild pollen towards the cultivated materials (82%). However, the other direction of gene flow from the cultivated into the wild forms was observed in lower but significant frequency (González-Torres et al. 2004).

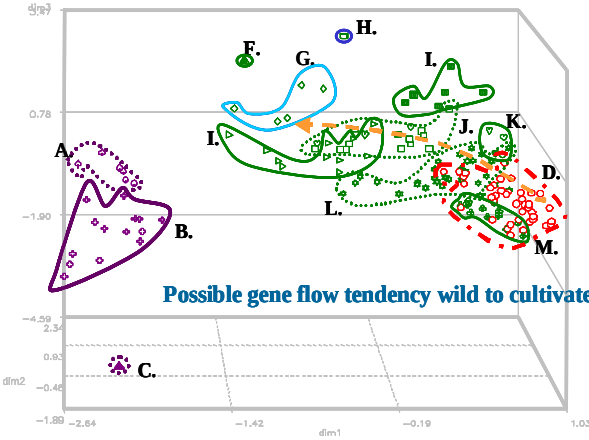

Figure 3. The population distribution with all markers using MCA. purple: cultivated type, green: weedy type and red: wild type.

The events of gene flow were mainly among materials belonging to the same gene pool (Mesoamerican). However, outcrossing between Mesoamerican and Andean gene pools was evidenced in 8% of the weedy materials.

Using all markers our distribution resulted in 13 groups (Figure 3) that explain 88.3 % of the total variation. As expected, the weedy materials were closer to the wild type. Group G showed only a 'wild' SSR allele, while the others had 'cultivated' SSR alleles. Group H had Andean phaseolin, chloroplast haplotype 'J' and size of seed up to 15 g.

Our methodology was useful to establish 214 real hybrids resulting from gene flow events out of 217 cases. The principal direction of gene flow was that of pollen of wild materials towards cultivated materials, the reverse direction being however significant. Outcrossing within a gene pool was dominant, while gene flow between pools was not negligible.

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