

Determination of gene flow events in natural "wild-weedy-cultivated" complexes in gene pools of *Phaseolus lunatus* L.

Suárez-Barón, H^{1*}, C Martínez-Garay^{2*}, RI González-Torres³, MC Duque³, DG Debouck⁴ & J Tohme³

Universidad del Quindío¹, Universidad del Tolima², CIAT Agrobiodiversity Project³, Genetic Resources Unit⁴

AA 6713 Cali-Colombia r.gonzalez@cgiar.org

Introduction

Gene flow events in natural populations of Lima beans have been reported along its broad distribution in the Americas (Mexico to Argentina) (Baudoin et al. 2004), as a result of its facultative reproduction system that allows varying levels of allogamy up to 48% (Maquet et al. 1996, and Zoro Bi et al. 2005). We were interested to test evidence of gene flow events using weedy materials from six populations of Campeche (Mexico) (Debouck 1979) and ten populations from Cajamarca (Peru) (Debouck et al. 1987).

Materials and Methods

The natural populations were chosen and analyzed using the same methodology as described by González-Torres et al. (2003, 2004) (Table). We evaluated the participation of nuclear genome through 18 microsatellite *loci* (Gaitan-Solis et al. 2002), and lectin patterns (Gutiérrez Salgado et al. 1995). In order to establish the direction of the gene transfer we studied 25 non-coding regions of chloroplast DNA using RFLPs (Fofana et al. 1999, Chacón 2001, and González-Torres et al. 2003). On the other hand, the concentration of HCN has been evaluated (Essers et al. 1993) as an antinutritional compound in order to assess some consequences of gene flow in these populations.

In addition, using all markers we found additional evidence for the two major gene pools: different banding patterns such as M1 and A1/A4 in lectins, three defined SSR *loci* within each gene pool, and contrasted chloroplast haplotypes. The observed genetic similarity of the weedy materials with cultivated types suggests that the pollen flow could be inferred from cultivated materials towards wild types.

Accession	Country	Gene pool	Biological status	Average seed weight (g) Color of seed testa	Lectin patterns	Haplotype of chloroplast	HCN concentration (ppm)
G25737	Mexico	Mesoamerican	Wild N=30	14,4 (wild type)	M1 (16/30)	H3 (25/30)	1902,3
G25789					M2 (9/30)	H1 (5/30)	
G25705			Cultivated N=45	43,4 (colored)	M4 (1/30)	H1 (6/45)	71,5
G25786					M1 (35/45)	H3 (39/45)	
G25787					M2 (2/45)		
G25706	Weedy N=24	25,3 (wild type; colored)	M4 (4/45) M8 (1/45)	H3 (24/24)	1118,5		
G25913	Peru	Andean	Wild N=30	13,8 (wild type)	M1 (13/24)	H4 (24/24)	3213,7
G25914					M2 (4/24)		
G25826			Cultivated N=61	94,1 (colored)	M8 (5/24)	H4 (30/30)	239
G25831					A1 (15/30)		
G25943					A2 (14/30)		
G25944					Unknown pattern (1/30)		
G25947							
G25948	Weedy N=58	47,4 (wild type; colored)	A1 (44/59) A4 (9/59) A6 (5/59) Unknown pattern (1/59)	H4 (47/61) H2 (14/61)	1460,7		

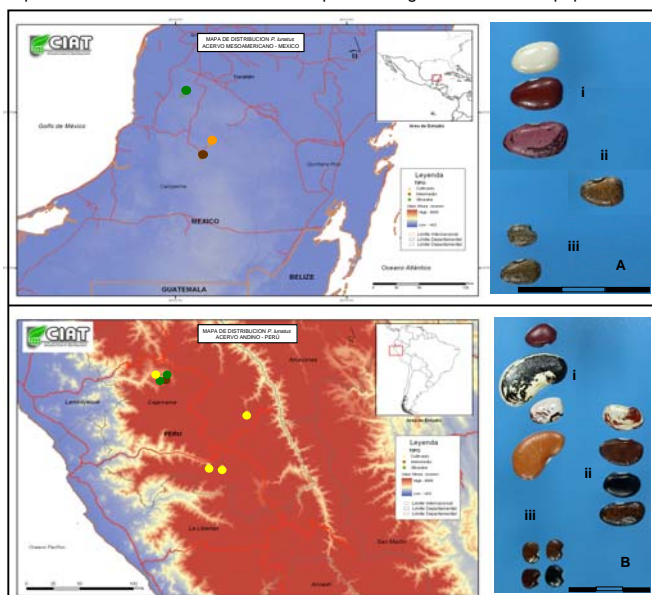


Figure 1. Distribution of complex wild-weedy-cultivated lima bean common bean in the gene pools Mesoamerican (A) and Andean (B) (ArcMap V1.0) (i) wild type, (ii) weedy type, and (iii) cultivated type.

Results and Discussion

Morphoagronomic markers such as seed weight and color of seed testa help verify individual cases of gene flow. The biochemical and molecular characteristics of the weedy materials indicated that they were indeed hybrids between cultivated and wild forms. Specific SSR alleles of the nuclear genome were found in each biological form among both gene pools and these were shared by weedy materials. In relation to the direction of gene transfer, using cpDNA data, the movement of pollen seems to occur in both ways. Using ADMIX software (data not shown) to determine the nuclear genome contribution of each biological form, the main direction in the Mesoamerican gene pool was that of wild pollen towards cultivated forms (1.3 times higher) in contrast with the Andean gene pool, where the main direction was from cultivated to the wild form (3 times higher). The analysis of multiple correspondence (Figure 2) shows in addition the clearly defined trend for each gene pool obtained utilizing ADMIX.

The inheritance of HCN trait in *P. lunatus* is still unclear, yet suggesting a polyfactorial inheritance with dominance in the wild (Baudoin et al. 1991), for that reason this information could be related to infer gene flow events with other markers such as seed weight and lectins. The higher HCN concentration of wild types than those found in the cultivated forms, but intermediate levels on weedy forms was exhibited in both gene pools as well as the absence of relation to the color of seed testa with the cyanide content according to Baudoin et al (1991). Therefore, the whole evaluated weedy forms are indeed hybrids resulted of gene flow events among wild and cultivated types. There by the results suggest that the pollen directions are similar than those found with ADMIX.

The separate events of domestication affecting both gene pools evidenced elsewhere (Gutiérrez Salgado et al. 1995) have been confirmed by our study, also in relation to the concentration of HCN and patterns of lectins displayed by wild and cultivated materials.

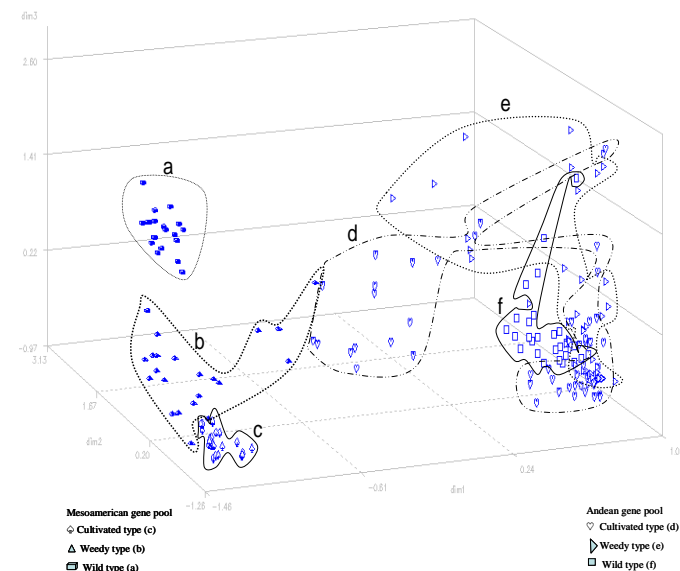


Figure 2. Analysis of multiple correspondence (AMC) in the Mesoamerican and Andean gene pools

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* First authorship is shared