UTILIZATION OF MICROSATELLITE MARKERS IN DIVERSITY ASSESSMENTS FOR COMMON BEAN

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INTRODUCTION

Microsatellite markers have been selected as the marker system of choice because of their low cost, high efficiency, whole genome coverage, robustness and minimum DNA requirements. In addition, they are highly polymorphic, co-dominant, PCR based and easily detectable. In common bean there are a range of microsatellite markers of which several sets of gene-based and genomic SSRs have been developed and mapped at CIAT. The aims of the CIAT program have been 1) to evaluate a large set of mapped SSRs for their diversity value (D), polymorphism information content (PIC) and heterozygosity level on a representative set of common bean genotypes and 2) to use the microsatellites in diversity assessment. This effort has been justified based on the need to select the most polymorphic markers for future analysis and apply them for germplasm characterization especially in the CIAT genebank and with National program partners.

MATERIALS AND METHODS

Plant material: A total of 44 genotypes were used in this study, representing wild accessions (3), landraces (17), breeding lines and cultivars (23) along with one tepary bean as an outgroup. All of the genotypes were of interest for biotic and abiotic resistance and/or grain quality traits. The genotypes were grouped in 3 parental surveys that were carried out separately with common controls, namely DOR364, a Mesoamerican genotype; and G19833, an Andean genotype, included in each survey.

Microsatellite analysis: The genotypes were evaluated for allelic diversity at 130 microsatellite (57 gene-associated and 73 genomic) loci. Amplification used genomic DNA template that had been extracted based on the miniprep procedure from Afanador et al. (1993). Microsatellite amplification and detection conditions were as reported in Blair et al. (2003). Markers that did not amplify were not considered further. To resolve allelic diversity as fully as possible, the PCR products for each survey were separated by electrophoresis for 1.5 hours at 120 constant volts on silver-stained 4% polyacrylamide gels. Microsatellite alleles for the control genotypes (DOR364 and G19833) were sized by comparison to the 10 and 25 bp molecular weight standards (Promega). Alleles of the remaining genotypes were compared to the control bands for each microsatellite so that molecular weights (in nucleotides) could be determined across parental surveys. Null alleles were not used in diversity assessment.

Data Analysis: The microsatellite alleles were coded into a binary data matrix that was analyzed by multiple correspondence analysis (MCA), using the CORRESP procedure of SAS (SAS Institute, 1989). Total diversity (H_t), intra population diversity (H_s) and inter population diversity (H_{si}) were also determined.

RESULTS AND DISCUSSION

Results of the multiple correspondence analysis and analysis of intra-population diversity and geneflow among the two principal clusters of genotypes corresponding to the gene pools and the subclusters corresponding to races is shown in Table 1. Intra population diversity (Hsi) was higher within the Andean genepool than within the Mesoamerican genepool and this pattern was observed for both gene-based and genomic microsatellites. Furthermore, intra-population diversity within the Andean races (0.356 on average) was higher than within the Mesoamerican races (0.302). Within the Andean gene pool, race Peru had higher diversity compared to race Nueva Granada, while within the Mesoamerican gene pool, the races Durango, Guatemala and Jalisco had comparable levels of diversity which were below that of race Mesoamerica. The divergence of the larger number of races in the Mesoamerican gene pool (Durango, Guatemala, Jalisco and Mesoamerica) was low compared to the races in the Andean gene pool (Nueva Granada and Peru). The distinction between races within each genepool has been further analyzed in two additional studies focusing on population structure in the Mesoamerican genepool (Diaz and Blair, 2006) and the Andean genepool (Blair et al., 2007). In conclusion, microsatellite markers have been useful for distinguishing races within the two major gene pools, and therefore supplement information collected from previous studies.

Table 1. Observed intra (H_s) and inter population (H_{si}) diversity for genotypes belonging to wild and cultivated common beans, to Andean and Mesoamerican gene pools and to races within each gene pool.

Category	N	Observed Heterogeneity			Value
		Gene-based (57)	Genomic (73)	Total	
Total	44	0.444	0.593	0.527	H_t
Species/Status[1]	44	0.429	0.575	0.511	H_s
Cultivated *P. vulgaris*	40	0.432	0.583	0.516	H_{si}
Wild *P. vulgaris*	3	0.388	0.477	0.437	H_{si}
Tepary Bean *P. acutifolius*	1	0.000	0.000	0.000	H_{si}
Gene pools	40	0.343	0.486	0.422	H_s
Mesoamerican	30	0.319	0.481	0.410	H_{si}
Andean	10	0.412	0.500	0.461	H_{si}
Races	40	0.253	0.363	0.314	H_s
Nueva Granada	4	0.215	0.352	0.292	H_{si}
Peru	5	0.397	0.436	0.419	H_{si}
Introgressed	1	0.000	0.000	0.000	H_{si}
Durango	4	0.154	0.325	0.249	H_{si}
Guatemala	2	0.246	0.292	0.271	H_{si}
Jalisco	3	0.257	0.367	0.319	H_{si}
Mesoamerica	21	0.289	0.430	0.368	H_{si}

[1]Status distinguishes wild versus cultivated *Phaseolus vulgaris*.

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