Genetic Diversity in Caribbean Accessions of Common Bean.

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Introduction

The introduction of common beans (*Phaseolus vulgaris* L.) into the Caribbean was postulated to have occurred from both the Andean and Mesoamerican centers of origin first from South America along the "Arawak Arc" of the Leeward Islands and the Greater Antilles before the arrival of Columbus and second from Central America along trade routes from the Yucatan to Cuba and Hispaniola (today the countries of Haiti and Dominican Republic) or around the Caribbean coast (Gepts et al., 1998, Castiñeiras et al., 1991). There is a range of small to medium to large seeded landraces in the Caribbean with predominant colors being small black in Cuba and lowland regions of Haiti and red mottled in the Dominican Republic and highland regions of Haiti (Beaver and Molina, 1997). Minor color classes include small-seeded white or brown beans, medium seeded tan, pink or yellow beans and large seeded light red kidney beans or pink striped kidney beans (Voysest 2000). The dispersal pattern and diversity of beans in the Caribbean makes this secondary center of diversity a likey region for inter-gene pool introgression. To test this hypothesis, we analyzed random amplified polymorphic DNA (RAPD) banding patterns and microsatellite (SSR) alleles in a total of 129 red, red mottled, pink or yellow seeded landraces from the Caribbean and performed diversity analysis to determine their genetic structure.

Materials and Methods

➢ The common bean genotypes inlcuded 112 traditional varieties (or selections thereof) from the Caribbean (65 from Dominican Republic; 18 from Haiti, 26 from Puerto Rico, and 1 each from Cuba, Panama and Jamaica) and 16 check varieties of which ten were advanced breeding lines from CIAT or UPR and six were varieties from Colombia, Peru and the USA (Figure 1).

➢ Phaseolin storage protein was analyzed from total seed proteins that were extracted from 0.10 g of peeled, finely-ground, oven-dried seed. Proteins were fraction-ated by boiling for 5 min and loading onto 6% stacking / 12% running SDS-PAGE (polyacrylamide) mini-gels which were stained with Coomasie Blue dye (Figure 2).

➢DNA was extracted from the young trifoliate leaf of a single seedling from each accession using an ammonium acetate extraction technique.

➢Standard RAPD and Microsatellite PCR reactions as well as agarase and polyacrylamide gel electrophoresis conditions were used for genotyping. Agarose gels were photographed. Silver-stained PAGE gels were scanned after drying overnight (Figure 2).

➢ A total of 26 RAPD primers and 27 microsatellite markers (19 genomic and 8 gene-based markers) were selected to evaluate the genotypes. Band sizing and allele calling was done based on comparisons to size standard ladders that were used two times per gel.

➢RAPD bands and microsatellite alleles were scored for presence or absence and molecular weight, respectively. Datasets were used to estimate genetic distance based on complete clustering in the case of RAPDs and Dice genetic similarity in the case of SSRs. Dendrograms were constructed using SAS or NTSYS.

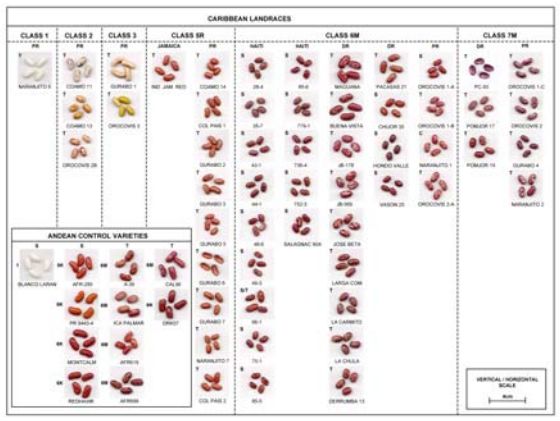

Fig 1. Caribbean grain types analyzed in this genetic diversity study.

Notes: Phaseolin type (C, S or T) indicated for each accession. Seed class designation according to CIAT (1987), where number indicates color (1= white; 2 = cream; 5= pink, 6 = red and 7 = purple) and letters indicate pattern or shape (M = mottled, R = striped and K = kidney). Country abbreviations are PR = Puerto Rico, DR = Dominican Republic. Vertical and horizontal scale as indicated in legend.

Results

➢ Phaseolin analysis: two predominant patterns were found, namely the Andean "T" allele and the Mesoamerican "S" allele. The "C" variant was also observed for a single traditional variety from Puerto Rico.

➢ RAPD analysis: detected three main groups: I) medium to large-seeded genotypes resulting from inter gene pool introgression. II) small seeded Mesoamerican genotypes. III) large seeded Andean genotypes.

➢ Microsatellite analysis: uncovered two major groups corresponding to Andean and Mesoamerican gene pools. Within each group, the Puerto Rican genotypes tended to cluster separately from the Dominican landraces while some genotypes with similar seed colors or those from the same collection site clustered together showing the fine-scale differentiation possible with this marker type.

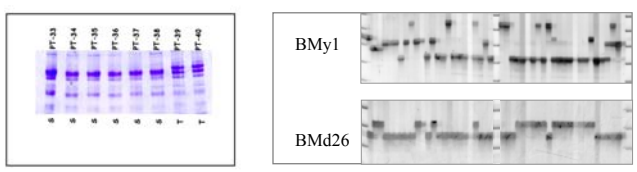

Fig 2. Polymorphisms for phaseolin and SSR markers among Caribbean genotypes.

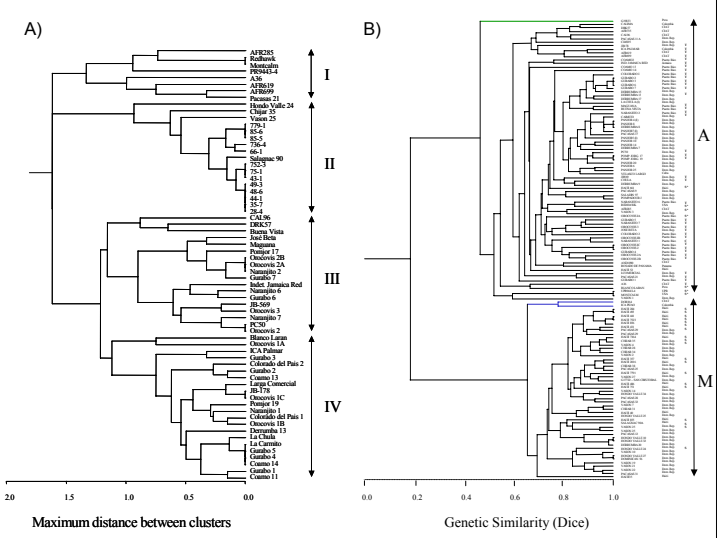

Fig. 3. Dendrogram of relationships among Caribbean accessions of common bean uncovered by A) RAPD and B) microsatellite marker analysis.

Discussion

➢ *Origin of Caribbean beans:* Caribbean germplasm with an Andean affinity were more similar to Calima than G19833 in the microsatellite analysis, indicating they were probably derived from the Northern Andes rather than from the Central Andes. Caribbean genotypes with a Mesoamerican affinity were more closely related to each other than they were to the black seeded and small red seeded control genotypes DOR364 and ICA Pijao, indicating that Caribbean Mesoamerican genotypes represent distinct germplasm from Central American Mesoamericans. The origin of the striped kidney class appear to be different from the origin of other kidneys as these are found in different groups in the RAPD dendrogram.

➢*Evidence of inter-gene pool introgression and gene-pool gradient:* The germplasm assignments of the Caribbean genotypes were generally supported by the phaseolin analysis, with "T" phaseolin being predominant in the Andean germplasm group and "S" phaseolin being predominant in the Mesoamerican germplasm group, however it was notable that some genotypes that were associated with the Andean group in the dendrogram had "S" type phaseolin. These results suggested that some Caribbean Andean germplasm has had an inter-genepool origin and that "S" type phaseolin has been introgressed into an Andean background. Such inter-genepool hybridization may explain how the red mottled seed coat color was transferred from the Andean genepool into Mesoamerican-like germplasm. The genetic analysis conducted in this study also agrees with the hypothesis of an east-to-west increase in the proportion of Andean beans within the Caribbean.

➢*Conclusions:* this study found that Caribbean landraces of common bean are diverse and are divided into two distinct groups one with a Mesoamerican phenotype and the other with an Andean phenotype. Genotyping with microsatellites, RAPDs and phaseolin markers however showed that some introgression has potentially occurred between these two groups or gene pools. Because pyramiding Mesoamerican and Andean disease resistance genes may provide broader and/or more durable resistance we postulate that the recombination between Andean and Mesoamerican traits found in Caribbean germplasm may provide a unique source of gene combinations useful to breeding programs and that Caribbean germplasm is worthy of further collection and analysis.

References

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