Application of a methodology for characterizing germplasm of common bean (*Phaseolus vulgaris* L.) using single nucleotide polymorphisms (SNPs)

UNIVERSIDAD NACIONAL DE COLOMBIA, PALMIRA CAMPUS
E.mail : c.quintero@cgiar.org

GENERAL OBJECTIVE

Apply a quick and reliable methodology for large-scale characterization of germplasm of common bean using Single Nucleotide Polymorphisms (SNPs).

SPECIFIC OBJETIVES

- **Characterize the genetic diversity of some genotypes of wild (core collection) and cultivated (donor parents of genes and commercial varieties) *P. vulgaris*.**
- **Compare with previous work, the results of characterizing the wild *P. vulgaris* core collection using SNPs to validate and prove its use in bean diversity studies.**
- **Study the relation between phenotypic characteristics of common bean and the presence/abscence of SNP alleles using an association mapping approach.**

EXPECTED RESULTS

- **Understanding of the informative potential of SNP markers for biodiversity studies and/or their utility for classification of bean germplasm on a large scale screening.**
- **Increased knowledge on existing genetic diversity within the primary gene pool of *P. vulgaris*.**
- **Rational use of the genetic diversity characterized through the SNPs in the development of new cultivars.**

METHODOLOGY

The study materials were sown in the screenhouse of the Mesoamerican Genetic Bean Program. The protocol of de Dellaporta *et. al.* (1983) modified by González *et. al.*, (1995) was followed for the extraction of DNA. SNPs will be detected using the single base extension (SBE) methodology. Starting from genomic DNA, the fragment containing the SNP will be amplified via PCR. Excess dNTPs and primers of the PCR product will be removed. Then, the amplified fragment will be annealed with an SBE primer with a 20-22 base oligonucleotide (ZIPcode or Tag) attached at its 5' end. The 3'end of the SBE primer anneals to the base that immediately precedes the SNP. The single base extension is carried out using ddNTPs labeled with biotin. Streptavidin is conjugated to the biotin label on the oligonucleotide, which is hybridized with polystyrene microspheres (5.6µM in diameter) bonded to ssDNA sequence complementary to the ZIPcode or Tag. The reaction is read in a flow cytometer (Luminex^{100}), which detects each microsphere by its unique fluorescent signal and the presence or absence of the SNP (streptavidin-SBE product). Data will be scored with the Masterplex GT (Miraibio, Inc) package with which the mean fluorescence intensity emitted by each of the samples will be determined, analyzed and used to define the SNPs alleles belonging to each individual under study.

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