**PHASEOLUS VULGARIS BAC LIBRARY: IDENTIFICATION AND CHARACTERIZATION OF BAC CLONES CONTAINING THE INSECTICIDAL LECTIN LOCUS**

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Most of the species belonging to the genus *Phaseolus* contain in their seeds a family of homologous lectin and lectin-related proteins (Arcelin/Phytohemagglutinin/alpha-Amylase Inhibitor), which are encoded by a single locus, the APA locus. In common bean (*P. vulgaris* L.) this locus shows a high variability. The three major components are present together only in some wild accessions, while most genotypes contain only phytohemagglutinin (PHA) and alpha-amylase inhibitor (alpha-AI) and some wild Mesoamerican accessions contain only PHA and Arcelin (ARC). This variability indicates a complex evolution of the APA locus, which might be better understood by the isolation and comparison of the entire locus from genotypes with different sets of APA components.

Today, the most commonly used system for constructing large insert libraries is the bacterial artificial chromosome (BAC) system.

The BAC library was construct using *P. vulgaris* accession G12949, CIAT, which contains the entire multigene lectin family Arc/PHA/alpha-AI and shows high resistance against seed eating insects. High-molecular-weight DNA was partially digested with *Hind*III and cloned in the *Hind*III site of pIndigoBAC5 vector. The library consists of 30,720 clones with an average insert of 135 kb, providing a coverage of 6 genome equivalents. The library is maintained and grown on eighty 384-well microtiter plates.

To identify BAC clones containing APA genes, the entire library, was gridded onto two 22.5 x 22.5 cm high-density filters double-spotted in a 4 x 4 array and probed with PHA, Arc4-II and alpha-AI clones isolated from the same genotype (1). After filter hybridization about 50 BAC clones were identified as positives. Among them, only 39 were confirmed as positives after a second screening using the two-lectin specific PCR primers P1 and P2 (2). In addition, to produce a contiguous chromosomal region covering the APA locus, the resulting 39 positive BAC clones were fingerprinted for contig assembly. Our preliminary results confirm that the APA locus is very long, probably more than 250Kbp, in fact to reconstruct the entire locus at least 4 overlapping clones appear necessary. However, to obtain a more detailed figure on the APA length and organization the sequencing of a first BAC clone containing a large part of the APA locus is in progress.