Bacterial artificial chromosome (BAC) library resource for positional cloning of pest and disease resistance genes in cassava (Manihot esculenta Crantz)

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Tomkins J, Fregene M, Main D, Kim H, Wing R, Tohme J.

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Clemson University Genomics Institute (CUGI), 304 BRC, Clemson, South Carolina, USA.

Abstract

Pest and disease problems are important constraints of cassava production and host plant resistance is the most efficient method of combating them. Breeding for host plant resistance is considerably slowed down by the crop's biological constraints of a long growth cycle, high levels of heterozygosity and a large genetic load. More efficient methods such as gene cloning and transgenesis are required to deploy resistance genes. To facilitate the cloning of resistance genes, bacterial artificial chromosome (BAC) library resources have been developed for cassava. Two libraries were constructed from the cassava clones, TMS 30001, resistant to the cassava mosaic disease (CMD) and the cassava bacterial blight (CBB), and MECU72, resistant to cassava white fly. The TMS30001 library has 55, 296 clones with an insert size range of 40-150 kb with an average of 80 kb, while the MECU72 library consists of 92 160 clones and an insert size range of 25-250 kb average of 93 kb. Based on a genome size of 772 Mb, the TMS30001 and MECU72 libraries have a 5 and 11.3 haploid genome equivalents and a 95 and 99 chance of finding any sequence, respectively. To demonstrate the potential of the libraries, the TMS30001 library was screened by southern hybridization using a cassava analog (CBB1) of the Xa21 gene from rice that maps to a region containing a QTL for resistance to CBB as probe. Five BAC clones that hybridized to CBB1 were isolated and a Hind III fingerprint revealed 2-3 copies of the gene in individual BAC clones. A larger scale analysis of resistance gene analogs (RGAs) in cassava has also been conducted in order to understand the number and organization of RGAs. To scan for gene and repeat DNA content in the libraries, end-sequencing was performed on 2,301 clones from the MECU72 library. A total of 1705 unique sequences were obtained with an average size of 715 bp. Database homology searches using BLAST revealed that 458 sequences had significant homology with known proteins and 321 with transposable elements. The use of the library in positional cloning of pest and disease resistance genes is discussed.