Characterization of a resistance gene cluster in cassava

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INTRODUCTION

Cassava, *Manihot esculenta* Crantz (*Euphorbiacee*), is one of the most important tropical food crops. Viral and bacterial diseases are major contributors to yield reductions. More than 30 genes have been characterized from different plant species that provide resistance to a variety of different pathogen and pest species. The largest group of plant resistance genes contains a Nucleotide-Binding Site (NBS) and Leucine-Rich Repeat (LRR) domains. The NBS-LRR family of R-genes can be further subdivided into two subfamilies TIR and non-TIR. The well conserved regions of the NBS domains have been used for a PCR based strategy to isolate putative R-gene sequences with degenerate primers in several plants. Most of the R-genes are clustered in the genome of several species.

Here a PCR based strategy using NBS and TIR primers was used to isolate R-gene candidates (RGCs) in cassava.

MATERIALS AND METHODS

PCR amplification and cloning

Tissues from TMS30572 and CM2177-2 were used as source of DNA for PCR amplification. Different primers (S2, AS3, AS4,Motif1,TIR1, TIR5, PRS3) were used.

BAC library screening and fingerprinting

A cassava BAC library was obtained from Fregene et al. (unpublished results) from TMS 30001 and hybridized. The BAC clones were isolated and DNA was extracted using the REAL kit (Qiagen). DNA was digested with *Hind*III. After image analysis, DNA was transferred onto nylon membranes (Hybond-N$^+$, Amersham) and hybridized with each radiolabeled RCa probe.

Sequencing one BAC containing five NBS sequence

Sequence was determined using a shotgun cloning strategy.

RESULTS AND DISCUSSION

Amplification and cloning of TIR and non-TIR NBS

10 classes of NBS RGCs were obtained and were differentiated into two groups. The first one grouped the RCa6 to RCa10 together with the RPS2 gene from *Arabidopsis*. The second group is formed by the RCa1 to RCa5 together with N and L6 R-genes.Two classes of RGCs isolated using TIR primers were also characterized in cassava.

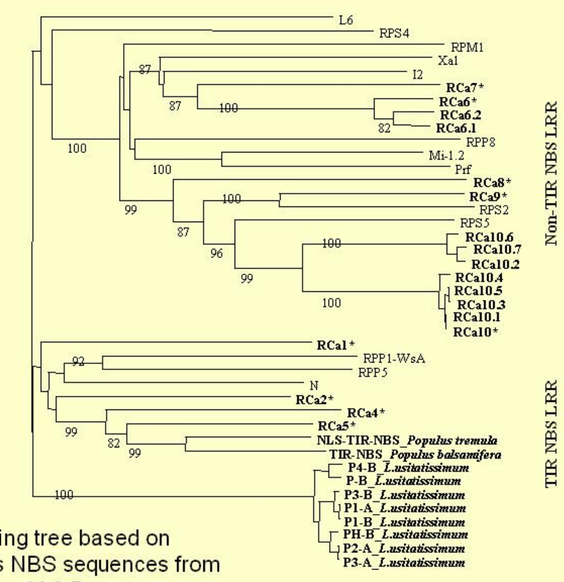


Figure 1. Neighbor-joining tree based on alignement of 10 RGCs NBS sequences from cassava and RPS2, N and L6 R-genes

BAC library screening and fingerprinting

• A total of 42 BACs hybridized. Some RGCs belonging to TIR and non-TIR NBS-LRR hybridized with the same BAC clone suggesting the interspersion of TIR and non-TIR NBS sequences in the cassava genome.

• The majority of RGCs hybridized to only a few BACs except RCa6 that hybridized to 27 BAC clones (Fig. 2). Almost all BAC contigs or singletons showed only one or two copies of NBS. The contig 2 and contig 6 contain five NBS sequences suggesting the presence of a cluster of R-genes in cassava (Fig 2).

•The BAC end sequences showed significant similarity with retrotransposable element, cinnamyl-alcohol dehydrogenase and other proteins. Homology with R-genes or with putative NBS-LRR type resistance protein was also observed for 6 BAC end sequences.

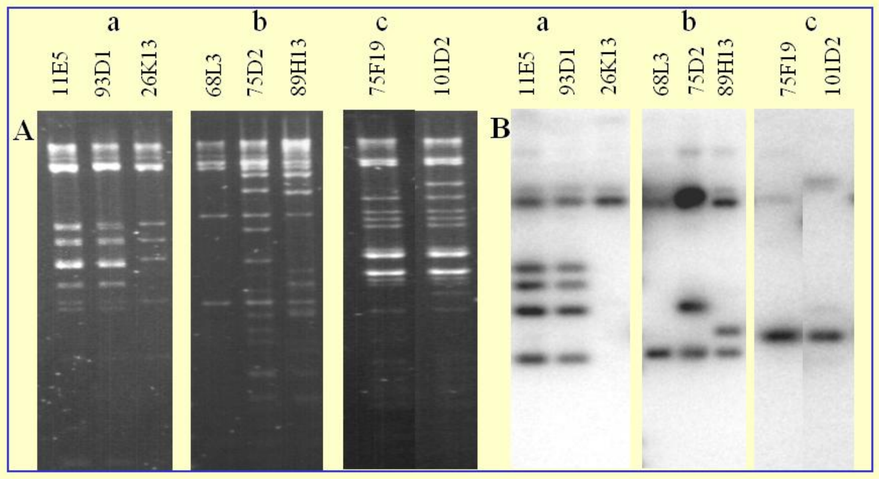


Figure 2. BAC DNA digested with *Hind*III. a , b and c represent different contigs, numbers indicate the corresponding BAC. **A.** BACs fingerprinting. **B.** Hybridization of BACs with the probe RCa6.

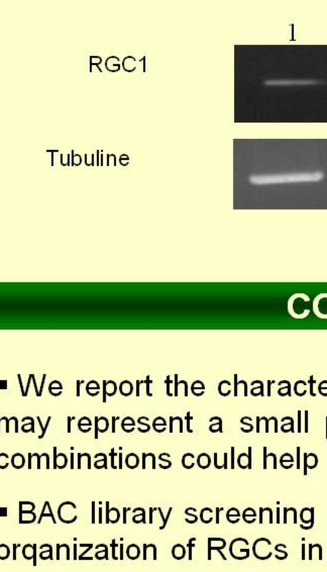
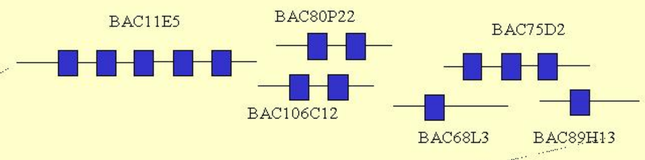


Figure 3. Genetic linkage group J showing the position of the RGCs candidate-containing BACs.

• Primers designed from BAC end sequences were tested as new markers for genetic mapping. Some BACs were localized in linkage groups (E, J) (Fig 3). No QTL was associated with resistance to cassava diseases in these linkage groups.



Sequencing of BAC11E5

BAC11E5 contained five NBS sequence. BAC sequencing analysis results in the identification of two complete RGCs, RGC1 and RGC2, encoding for two proteins of 1024 and 1025 amino acids respectively. They showed 92% of identity at the amino acid level. The LRR domain is formed by 16 imperfect LRR varying in length from 13 to 31 amino acids .

• To conduct expression assays, a set of primers specific to RGC1 were designed and tested by RT-PCR. A fragment of 800pb was amplified from mRNA from the resistant variety Mbra685 inoculated and non inoculated with *Xam* (CIO151) confirming the expression of RGC1 (Fig. 4).

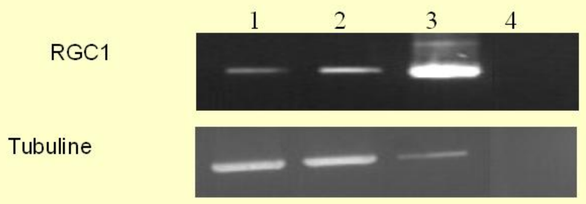


Figure 4. Expression of RGC1 by RT-PCR from (1) stem tissue of the resistant variety, (2) healthy tissue, (3) genomic DNA and (4) negative control.

CONCLUSIONS AND PERSPECTIVES

▪ We report the characterization of a set of resistance gene candidates in cassava that may represent a small portion of the entire set present in the genome. Other primer combinations could help in identifying other NBS sequences.

▪ BAC library screening and Southern analyses provided new insights into the genomic orqanization of RGCs in cassava. We showed the presence of 5 NBS sequence in one BAC clone, suggesting the existence of an RGC cluster. Several BACs containing RGCs were mapped on linkage group J suggesting the presence of a chromosome rich in R gene like sequence. At least 14 R-gene like sequences were identified. It will be interesting to determine the specificities located on linkage group J.

▪ Two completes RGC genes were identified. To test wether they are functional R genes we must determine their resistance specificity (ies) and study their expression pattern.

▪ The identification and characterization of RGCs will allow studies on the evolution of R-gene like sequences in cassava and their implications for plant pathogen interactions. We provide with new markers that can be used in genetic mapping as tools for map-based cloning of R-genes in cassava.

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