Identifying QTLs and RGAs in cassava and their relationships with three *Phytophthora* species

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INTRODUCTION

Root and stem rots, a complex of diseases caused by several *Phytophthora* spp. (water molds), comprise a major constraint to cassava (*Manihot esculenta* Crantz) production. Losses of more than 80% occur particularly in Brazil, Colombia, Cuba, Kenya, Mexico, and Nigeria (Alvarez and Barragán 1998; CIAT 2000). Molecular markers form an important tool for breeding resistant cassava. A number of disease resistance genes have been cloned from several plant species, which encode components of signal transduction pathways, and their protein products share some common structural domains.

MATERIALS AND METHODS

Identifying QTLs. We identified QTLs associated with resistance to *P. tropicalis* (44), *P. melonis* (P12), and *P. palmivora* (P4). We had used phenotypic data from the cassava K family and genotypic data from the map generated from a female K parent, which has 192 markers (RAPD, RFLP, SSR, isoenzymes), distributed in 22 linkage groups (Fregene et al. 1997). Analysis and mapping of QTLs was done with Q-gene 3.06V software (Nelson 1997), using simple regression or Simple Point Analysis, where the dependent variable was reaction to pathogen, and the independent variable was the number of alleles on the locus marker, depending on the individual's segregation. Significance level was 5%. A QTL map was generated, and the phenotypic variance explained by each QTL was estimated by the regression coefficient (r^2).

Identifying RGAs. We amplified conserved regions of DNA, using PCR with degenerated NBS and Pto kinase primers, from three cassava genotypes resistant to *P. tropicalis* and *P. palmivora*. The clones obtained were sequenced and compared with known resistance genes. Specific primers were designed, based on the sequences, to permit amplification of DNA regions of parental material and resistant and susceptible individuals. Bands were separated by denaturing polyacrylamide gel electrophoresis and nondenaturing polyacrylamide gel.

RESULTS

Identifying QTLs. Five QTLs were identified. These explained from 0.2% to 8.3% of phenotypic variance. Two were associated with resistance to *P. palmivora*, and were located on linkage group O, close to markers rP1a (RFLP) and rSSRY 19 (microsatellite). Another two, associated with resistance to *P. melonis,* were located on linkage group G, the first being close to RFLP marker CPY 79, with additional effect from the male parent; and the other being close to RFLP marker rGY 170, located on linkage group H. The fifth QTL, associated with resistance to *P. tropicalis*, was located on linkage group E, close to microsatellite marker rNS217 (Figure 1).

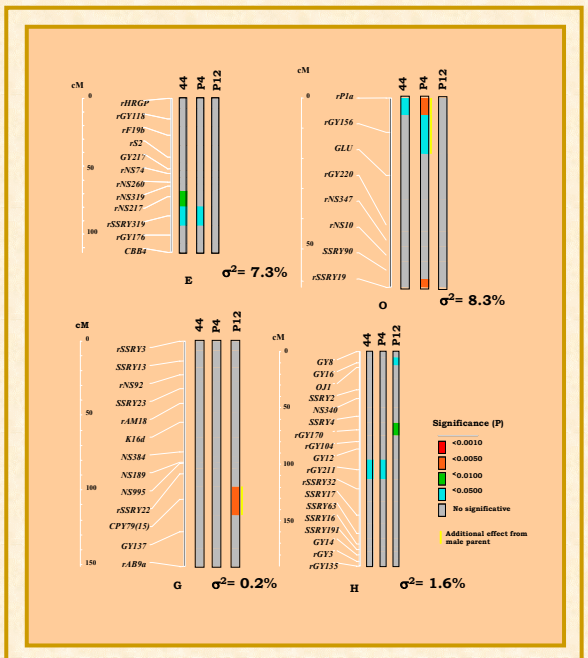

Figure 1. Map of QTLs identified in different linkage groups and associated with resistance of the cassava K family to *Phytophthora tropicalis* (44), *P. palmivora* (P4), and *P. melonis* (P12). Distances among molecular markers are in centimorgans (cM).

The K family presented individuals that were more resistant than their parents. This finding, together with the detection of QTLs associated with resistance to *Phytophthora* spp., shows that resistance alleles were inherited from both parents, which thus contributed to resistance in the progeny, and are therefore useful for combining resistance factors in the same genotype.

Identifying RGAs. We obtained 28 NBS and 2 Pto kinase clones by PCR. Of these, 5 showed homologous sequence with NBS-LRR resistance gene analogs (RGAs). Four (N33, N37, N38, and K1) showed open reading frames (ORF) with conserved motifs of the NBS region, which means they were considered as RGAs. Three different RGAs classes were identified, but these did not show association with resistance to *Phytophthora* spp. (Figure 2).

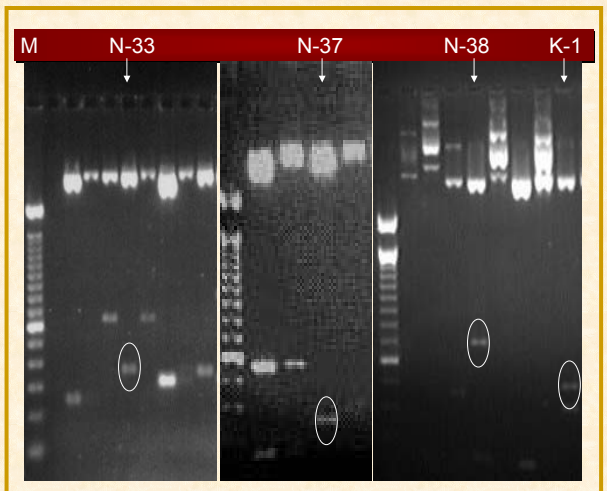

Figure 2. Clones obtained from resistant cassava genotypes MBra 1045 (N-33 and N-37), and MBra 532 (N-38 and K1), by amplification with degenerated primers NBS. M= 100 bp marker

A phylogenetic tree showed that clone N-37 showed homology with no-TIR genes, RPS2, and Mi, whereas clones K-1 and N-38 showed homology with TIR genes, L6, and RPP5. Clone N-33 was different to the other clones isolated (Figure 3). Specific primers, designed to amplify the sequences of the clones isolated from resistant genotypes, did not separate resistant individuals from susceptible ones.

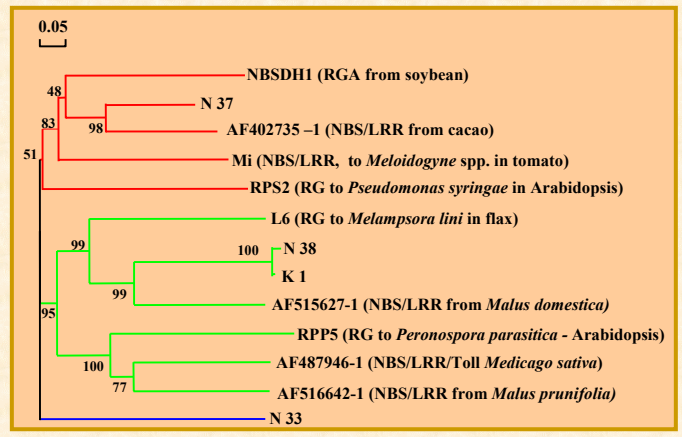

Figure 3. Phylogenetic tree of amino acid sequences made by parsimony and bootstrap analysis (5000 replicas) of RGAs N-33, N-37, N-38, and K-1 identified in cassava and compared with homologue sequences reported in GenBank and resistance genes from other species.

CONCLUSIONS

- Five QTLs, associated with resistance to *Phytophthora* root and stem rots, were mapped.
- RGAs of TIR and no-TIR subclasses, belonging to the NBS-LRR class, were identified in the cassava genome.
- Association was not found between the RGAs isolated and resistance to *Phytophthora* spp. in cassava.

ACKNOWLEDGEMENTS

The authors thank to Jaime Marín for helping with QTLs analysis and Elizabeth de Páez, for the edition of the English text.

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