Functional validation of two resistance gene candidates RXam1 and RXam2 to cassava bacterial blight employing RNAi.

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INTRODUCTION

To overcome diseases plants have evolved resistance genes that recognize pathogens and activate defense responses. In Cassava, previous studies have identified two candidate genes that might confer resistance to Cassava bacterial blight caused by the gram-negative bacteria *Xanthomonas axonopodis pv. manihotis* (Xam) and have been named *RXam1* and *RXam2*. Mapping studies have demonstrated that *RXam1* and *RXam2* co-localized with QTLs that explain 13% of the resistance to Xam strain CIO136 and 62 % of the resistance to Xam strain CIO151, respectively. *RXam1* encodes a protein with Serine/threonine kinase (STK) and Leucine Rich Repeats (LRR) domains, and *RXam2* codes for a protein containing a nucleotide binding domain (NBS), which is typically present in proteins conferring resistance. In order to validate the function of these genes we will use intron hairpin RNA interference (ihpRNA) to silence the expression of these genes in cassava resistant plants and we will evaluate the loss of resistance in these transgenic silenced plants.

PCR AMPLIFICATION AND CLONING

PCR was performed for both *RXam1* and *RXam2* using forward primers containing CACC at 5' end, that allows directional cloning of blunt PCR product in pENTR/D-TOPO® vector, suitable for creating a gateway entry clone. Primers generate a 301bp product for *RXam1* and a 618bp product for *RXam2*.(Fig. 1)

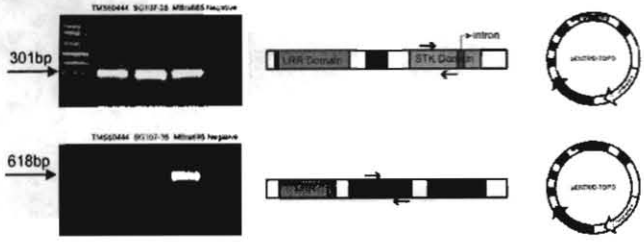

Fig 1 Amplification and cloning of *RXam1* and *RXam2* fragments in pENTR/D-TOPO. PCR Products of *RXam1* (top) and *RXam2* (bottom). Schematic representation of *RXam1* and *RXam2* fragments cloned in pENTR/D-TOPO.

FRAGMENT CLONING IN SILENCING VECTOR

Gene fragments cloned in pENTR/D-TOPO were sequenced using M13 forward primer to confirm the presence of the insert. Sequenced clones were used to perform an LR recombinase reaction in order to be cloned in pHellsgate12, a suitable gateway silencing vector for plants(Fig. 2).

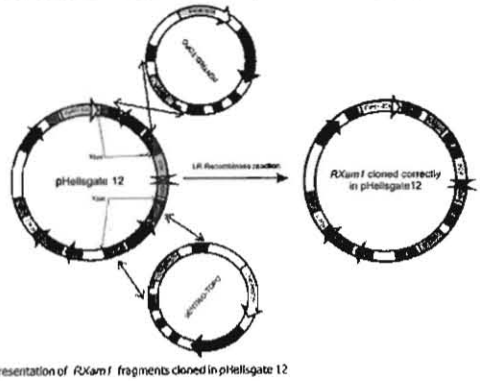

Fig 2 Schematic representation of *RXam1* fragments cloned in pHellsgate 12

To confirm the presence of sense and antisense fragments of *RXam1* and *RXam2*, the vector was digested independently with the restriction enzymes XhoI and XbaI, each one excises the sense or antisense fragment with their respective recombination sites generating a fragment of 1427pb for *RXam1* and 1744 for *RXam2*. These fragments were excised and eluted from an agarose gel and used as template for PCR (Fig. 3).

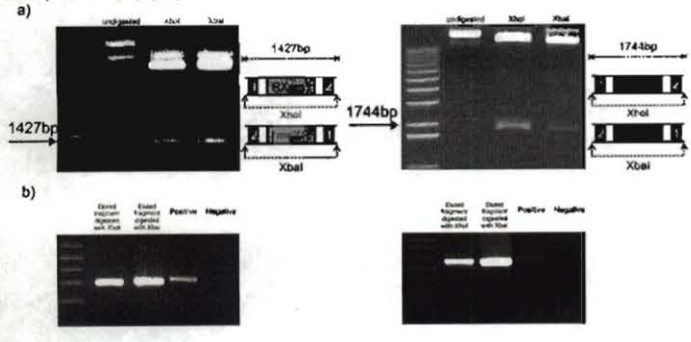

Fig 3. a) Digestion of cloned fragments of *RXam1*(left) and *Rxam2* (right) in pHellsgate with XhoI and XbaI. b) PCR using as template eluted bands of 1427 for *RXam1* and 1744 for *RXam2*

PERSPECTIVES

Resistant cultivars to Xam will be transformed with the cloned fragments of *RXam1* and *RXam2* using *Agrobacterium tumefaciens*. We expect to observe increased susceptibility due to silencing of the resistant gene candidate (RGC; Fig. 4). Further studies will allow the evaluation of Agrobacterium-mediated transformability in resistant cultivars SG107-35 and MBra685.

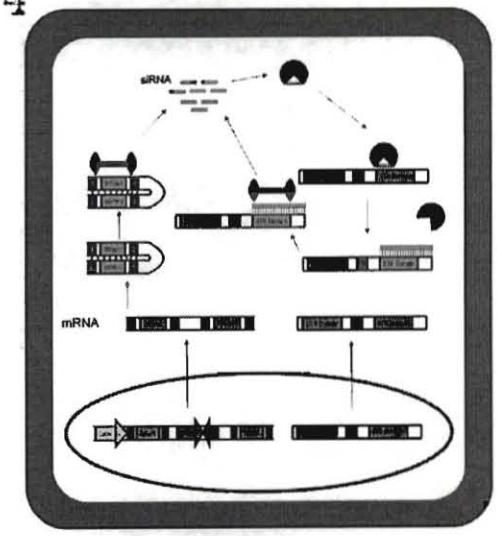

Fig 4 Schematic representation of resistant gene silencing and expected results

The cassava cultivar model for transformation is TMS60444, however we do not know if it is resistant to Xam CIO151 or Xam CIO136. However preliminary results show that RXam2 is expressed in TMS60444 suggesting that we can validate the function of this RGC in TMS60444 (Fig. 5)

Fig. 5 (Top) Nested RT-PCR of *RXam2* on cDNA of TMS60444 of 0-7days post inoculation with XamCIO151, (Bottom) RT-PCR of the constitutive gene elongation factor 1 α.

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