A role of RXam1, a homolog of Xa21, in cassava resistance to *Xanthomonas axonopodis* pv. *Manihotis*

Lopez C[1]., Beltrán J[2]., Contreras E[1]., Chavarriaga P[2]., Tohme J[2] and Verdier V[3]

[1]*Universidad Nacional de Colombia, Departamento de Biología, Bogotá; Conservation and Use of Tropical Genetic Resources, CIAT, AA 6713 , Cali, Colombia; [3]UMR UP-IRD-CNRS 5096 Centre IRD, 911, Av Agropolis BP 64501, 34394 Montpellier Cedex 5, France*

INTRODUCTION

Cassava bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), is a major disease, endemic in Latin America and Africa, causing serious damage to cassava. In rice the *Xa21* gene confers a broad spectrum of resistance to *Xanthomonas oryzae* pv. *oryzae* and encodes a receptor-like kinase with LRRs in the putative extracellular domain.

We have identified and cloned a *Xa21* homologue in cassava: *RXam1*. This gene is associated with a QTL (XM5) explaining 13% of the resistance to *Xam* strain CIO-136.

***RXam1* is induced in two resistant varieties (MBra685 and SG 107-35) after challenging whit *Xam* strain CIO136, as well as whit strain CIO151.**

MATERIALS AND METHODS

BAC library (TMS 30001)

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Screening using a probe generated from specific *Xa21* primers (PCR250)

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Partial BAC sequence

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Sequence analysis: Identification of *RXam1*

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Expression of *RXam1* | **5' and 3' RACE PCR from cDNA of genotype resistant MBRA685**

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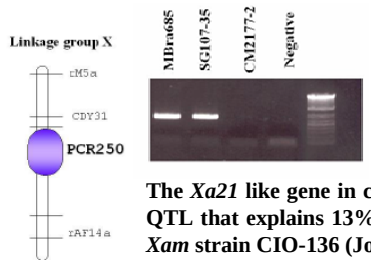
Cloning of *RXam1* gene

RESULTS AND DISCUSSION

Previously, using primers from the *Xa21* gene of rice a fragment (named PCR250) was obtained from cassava

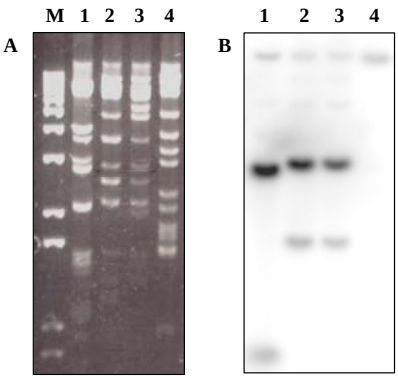


***Xa21* primer specific amplification (900 pb) in cassava resistant cultivars**

The *Xa21* like gene in cassava is associated with a QTL that explains 13% (P=0.004) of resistance to *Xam* strain CIO-136 (Jorge *et al*., 2000)

• Cassava BAC Library Screening

Four (1-4) positives clones were obtained from the BAC library (var. TMS3001 susceptible to *Xam* CIO-136) using PCR250 as a probe. All BACs showed one copy of PCR250. BACs 2 and 3 form a contig. The BAC 4 was a false positive.

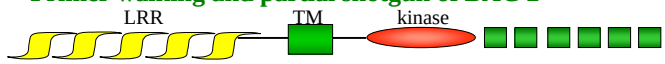


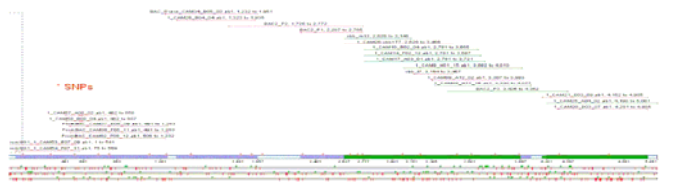
BAC fingerprinting

A. BACs digestion (*Hind*III)

B. BACs hybridization with PCR250 probe

• Primer walking and partial shotgun of BAC 2





Identification of putative full-length *RXam1* gene in the BAC 2

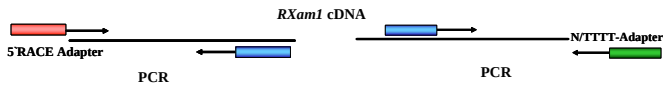
• Sequence analysis of *RXam1* gene

Analysis of *RXam1* sequences from TMS3001 (S) and MBRA685 (R)

	Complete ORF	Identity with *Xa21*	LRR domain	Ser/thr kinase domain	Sequenced region
TMS3001(BAC2)	No	34%	Yes	Yes	4.6 kb
MBRA685	Yes	35%	Yes	Yes	3.5 kb

In the intronic region of *RXam1* from different cultivars a high number of SNPs was detected

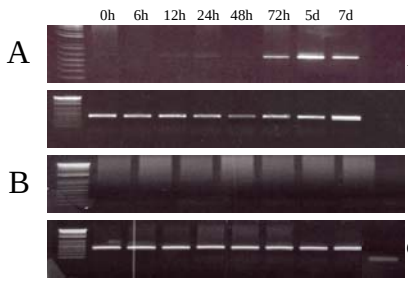
• 5' and 3' RACE PCR from cDNA of MBRA685



5' and 3' ends of full *RXam1* gene were completely sequenced

•Expression Analysis of *Rxam1*

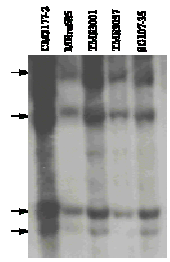
***RXam1* is induced by pathogen inoculation whit *Xam* strain CIO-136 in the resistant but no in the susceptible cultivar.**



A. Resistant cultivar (MBRA685)

B. Susceptible cultivar (TMS3001)

Copy number estimation of *RXam1* indicated that is a member of small gene family



• Cloning of *RXam1* gene from cassava resistant cultivar

Using specific *Rxam1* primers, a ~3.4 kb fragment was amplified from MBRA685 resistant cultivar from which it was cloned. Different clones are being sequenced for ORFs detection and confirmation.

PERSPECTIVES

• To isolate and characterize the *RXam1* promoter for further plant genetic transformation whit *RXam1*.

• Functional validation of *RXam1* gene by cassava genetic transformation.

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