**Identification of polymorphisms in resistance gene candidates in cassava (*Manihot esculenta* Crantz)**

**Identificación de polimorfismos en genes candidatos de resistencia en yuca (*Manihot esculenta*** [**Crantz**](http://www.ipni.org/ipni/idPlantNameSearch.do;jsessionid=5FAA60B5136A839CB9F197F682483C5F?id=351790-1&back_page=%2Fipni%2FeditSimplePlantNameSearch.do%3Bjsessionid%3D5FAA60B5136A839CB9F197F682483C5F%3Ffind_wholeName%3DManihot%2Besculenta%26output_format%3Dnormal)**)**

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Rec.: 02.09.11 Acept.: 05.06.12

**Abstract**

Cassava production can be detrimentally affected by diseases caused by different pathogens. To defend against viral, bacterial and fungal diseases, plants have developed a group of resistance proteins (R), which are able to recognize pathogen’s molecules. A wide repertoire of R proteins has been identified in a large group of plants. Even though conferring resistance to different pathogens, these R proteins have a few conserved domains. Taking advantage of the recent release of the complete cassava genome sequence, we identified cassava R-like proteins in this genome. With this information, primers were designed to amplify 13 genes showing similarity to known *R* genes. For 10 of them we obtained amplification in TMS30572 and CM2177-2 varieties, which represent the parents used in the construction of the cassava genetic map. After sequencing the obtained amplicons, we identified 37 SNPs (Single Nucleotide Polymorphisms) between these two cassava varieties, which represent 18 (48.6%) transitions and 19 (45.9%) transversions. The remaining are insertions/deletions (indels). This knowledge will help to develop appropriate strategies for the generation of CAPs (Cleaved Amplified Polymorphisms) markers to assess their segregation in the F1 population, allowing the localization of these markers on the cassava genetic map.

**Key words:** Cassava, genetic maps, genomes, *Manihot esculenta*, molecular markers, resistance.

**Resumen**

**La yuca** (*Manihot esculenta*) es **la base de la alimentación para más de 1000 millones de personas en el mundo.** La producción es severamente afectada por enfermedades ocasionadas por diferentes patógenos. Las plantas de yuca han desarrollado una serie de proteinas de resistencia R para defenderse de infecciones virales, bacterianas y fúngicas, las cuales son capaces de reconocer moléculas específicas de los patógenos. Un repertorio amplio de estas proteínas ha sido identificado en varias especies vegetales, no obstante, a pesar de conferir resistencia a patógenos diversos, presentan unos pocos dominios conservados. A partir de la reciente liberación de la secuencia completa del genoma de yuca se identificaron secuencias similares a proteínas R en este genoma. Con esta información se diseñaron cebadores para amplificar 13 genes *R*, logrando la amplificación de 10 de ellos en las variedades TMS30572 y CM2177-2, las cuales representan los parentales empleados en la construcción del mapa genético de yuca. A partir de la secuenciación de los amplicones obtenidos se identificaron 37 SNPs (Single Nucleotide Polymorphisms) de los cuales 18 (48.6%) corresponden a transiciones y 19 (45.9%) a transversiones. El restante corresponde a inserciones/deleciones. Este conocimiento permitirá desarrollar estrategias adecuadas para el desarrollo de marcadores moleculares tipo CAPs (del inglés Cleaved Amplified Polymorphism) para posteriormente evaluar su segregación en la población F1, permitiendo, de esta manera, posicionar estos marcadores en el mapa genético de yuca.

**Palabras clave:** Genomas, *Manihot esculenta*, mapas genéticos, marcadores moleculares, resistencia a patógenos, yuca.

**Introduction**

Cassava (*Manihot esculenta*) is the carbohy­drate source for more than 1000 millions of people that consume it daily, mainly in tropi­cal regions of Latin America, Africa and Asia (FAO, 2009). Although it is a wild crop, highly tolerant to abiotic (salinity, drought, acid soils) and to biotic stress conditions, there are some diseases affecting its production consi­derably (Ceballos, 2002). Among the main fungal diseases there are leaf spots caused by *Cercosporidium henningsii* or by *Phaeoramu­laria manihotis* species. Viral disease are ba­sically the cassava common mosaic (CsCMD) and the cassava vein mosaic (CVMD), which are present in South America but, generate important economic losses in the crop (Ceballos, 2002). One of the main limitants in the crop in Africa is the African mosaic virus (AMV) caused by a geminivirus (Ceballos, 2002). The main bacterial disease is the vas­cular bacteriosis produced by *Xanthomonas axonopodis* pv. manihotis, which is present in all the manihot cultivated regions (Lopez *et al.*, 2006). Although its importance, it has not been possible to identify the first gene for di­sease resistance in cassava. Having resis­tance genes allows strategy development for conventional or transformation genetic breeding in order to obtain varieties with longer resistance to a wide disease spectrum. The recent release of the cassava genome allows the identification of possible candidate genes for disease resistance in cassava.

Resistance proteins have the function of recognizing pathogens molecules and of acti­vating a transduction signaling pathway to restrict growth and colonization by the patho­gen (Zipfel, 2009). In the last 20 years, in different plant species more than 50 *R* genes have been cloned and characterized; they confer resistance to diverse pathogens like virus, bacteria, fungi, nematodes and insects (Hammond-Kosack and Kanyuka, 2007). Most of the *R* proteins have a Nucleotide Binding Site (NBS) and Leucine Rich Repeats (LRR) with additional domains in the amino terminus (Meyers *et al.*, 2003). It is believed that the role of these domains is monitoring the plant protein state of the target proteins of the pathogen virulence factors. Most of these proteins lack of signal peptides or hydropho­bic regions for tranmembral anchorage. Pro­teins with LRR domains are present in diver­gent beings as virus and eucariotes. It seems that the function of the amino-terminal part is associated with protein activity modulation, while the carboxy-terminal part is implicated in proteic interactions with another protein or ligand (Jones and Dangl, 2006). NBS-LLR proteins are subclassified in CC or TIR accor­ding to the presence of coiled-coil domains (CC) or domains that are similar to *Drosophila* Toll proteins and to the mammal interleuki­nas IL-1 (TIR domains) (McHale *et al.*, 2006). Another kind of R proteins is characterized by members with only one LRR extracelular do­main, as the Cf protein family of tomato that give resistance to the fungus *Cladosporium fulvum* (Stergiopoulos *et al.*, 2010). Other resistance proteins present, together with the LRR extracelular domain, a transmembrane domain and can present a Ser/Tre kinase (STK) domain, this is the case of Xa21 in rice that confers a high spectrum resistance to different strains of *Xanthomonas oryzae* (Song *et al.*, 1995). Taking into account that until now resistance is the only function assigned to the NBS-LLR proteins type (McHale *et al.*, 2006) and taking as base the permanence of conserved domains in resistance proteins, similar proteins can be identified in low stu­died plant species.

Availability of complete sequenced geno­mes allows, by bioinformatic strategies, the identification of candidate *R* genes that code for proteins with these conserved domains. However, functional studies are needed to determine the specificity of these candidate proteins. Alternatively, by mapping analysis is possible to locate these sequences in gene­tic maps to associate them with genetically identified loci associated with resistance to a particular pathogen or with QTLs (Quantita­tive Resistance Loci) (López *et al.*, 2007).

This work used the conserved nature of the R proteins to search, in the cassava ge­nome, for homologs of previously described proteins on other plant species. These se­quences were amplified and sequenced in the two parentals used in the construction of the genetic map to detect SNP (Single Nucleotide Polymorphism). It is hoped that the results substantiate the bases for strategy develop­ment to map these sequences in the cassava genetic map.

**Materials and methods**

**Plant material and DNA extraction**

Cassava cultivar used in the genome sequen­cing was AM560-2. In order to reduce the cassava heterogeneity and facilitate the ge­nome sequencing, it was cross with MCOL1505 variety for three generations originating AM560-2. The sequence of the genome of this variety represents the refe­rence sequence accessed from www.phytozome.com.

Cassava varieties, CM2177-2 and TMS30572, were supplied by the Cassava Germoplam Bank from CIAT in in vitro condi­tions. These varieties were propagated in the Molecular Biology Lab of the Department of Biology of the Universidad Nacional de Co­lombia. In vitro plant leaves were grinded in liquid nitrogen and DNA was extracted follo­wing the modified protocol previously des­cribed by Dellaporta *et al.* (1983). Both varie­ties are parentals of a F1 progeny composed of 150 individuals, which was previously de­veloped at CIAT and used to develop the first genetic map of cassava (Fregene *et al.*, 1997).

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| **Table 1.** Resistance proteins used for alignment in the cassava genome and selected genic regions to be amplified by PCR. | | | | |
| **Protein name** | **Plant** | **Pathogen** | **Structure** | **Primer design region** |
| L6 | Linen | *Melampsora lini* | TNL\* | UTR 5’ |
| M | Linen | *Melampsora lini* | TNL\* | UTR 5’ |
| RPP5 | *Arabidopsis* | *Hyaloperonospora arabidopsidis* | TNL\* | Intron |
| RPP1 | *Arabidopsis* | *Hyaloperonospora arabidopsidis* | TNL\* | Downstream |
| N | Tobacco | Tobacco mosaic virus (TMV) | TNL\* | Intron |
| Xa1 | Rice | *Xanthomonas oryzae* pv. *oryzae* | NL\*\* | Downstream |
| RPS5 | *Arabidopsis* | *Pseudomonas syringae* | NL\*\* | Downstream |
| RPM1 | *Arabidopsis* | *Pseudomonas syringae* | NL\*\* | Downstream |
| RPS2 | *Arabidopsis* | *Pseudomonas syringae* | NL\*\* | Downstream |
| RGC2 | Lectuce | *Bremia lactucae* | NL\*\* | Intron |
| I2 |  | *Fusarium oxysporum* sp licopersicum | NL\*\* | Downstream |
| Cf2 |  | *Cladosporium fulvum* | NL\*\* | Downstream |
| Pto | Tomato | *Pseudomonas syringae* pv tomato | STK\*\*\* | UTR 5’ and UTR 3’ |
| \*TNL: TIR-NBS-LRR. \*\*NL: NoTIR-NBS-LRR. \*\*\*STK: Serine/Treonine Kinase. | | | | |

**Identification of R genes**

A database was built including sequence in­formation and structure of previously repor­ted R proteins in different plant species. From this database some *R* genes were selec­ted that have been widely characterized (Table 1). From the sequences of the coding proteins of the selected *R* genes (Table 1) a TBLASTN was performed against the *Manihot esculenta* genome ([www.phytozome.com](http://www.phytozome.com)) in order to identify homolog proteins in cassava. TBLASTN standard parameters were used: expected E value threshold= -1, comparison matrix = Blosum62 and word size = 3. Based on the results obtained and from the respec­tive nucleotide sequences, primers were de­signed using Primer3 (Table 2) (Rozen and Skaletsky, 2000). Design parameters were modified to get amplification products from introns or UTRs (untranslated region) prefe­rably, by using the target function of the pro­gram. Other used parameters were: product size = 200 – 1000, primer size = min. = 18 bp, optimal = 20 bp, max. = 27 bp, primer Tm: min. = 57, optimal = 60, max. = 63 and, %GC primer: min. = 20, max. = 80. Primers were used in amplifications from TMS30572 and CM2177-2 varieties DNA.

**Amplificationa and sequencing**

PCR reactions were performed on 10 µl final volume containing DNA 10 ng, and final con­centrations of Buffer 1X (DreamTaq Buffer, Fermentas), MgCl2 2.5 mM, deoxynucleotides 0.2 mM each, primers 0.5 µM each and Taq polymerase 0.2 U (DreamTaq, Fermentas). Reactions were done on an iCycler thermocy­cler (BioRad). Reaction conditions were: ini­tial denaturation 95 °C for 3 min; 45 cycles of 94 °C for 45 sec, 45 sec of the calculated pri­mer annealing temperature for each primer set and 1 min of extension at 72 °C; and final extension at 72 °C for 5 min. It was nece­ssary to standardize the annealing tempera­ture for each primer set. PCR products were subjected to electroforesis on 1.2% agarose gel and dyed with ethidium bromide. The size of the amplified fragment was confirmed using the molecular weight marker 1Kb Plus Ladder ® (Invitrogen, Carlsbad, CA, U.S.A.)

Amplification products were sent to a lab of certified services (Macrogen) for sequen­cing. Sequences from both varieties were edited and inspected using the program Se­quencher (Genecodes, Inc) and aligned with the MUSCLE (MUltiple Sequence Comparison by Log- Expectation) program. From the alignments obtained SNPs (Single Nucleotide Polymorphisms) were manually identified.

**Results**

**Amplification of homologous R genes in cassava**

In total, 13 primer sets were designed that correspond to different *R* genes homologs (Ta­ble 2). For 12 primers (92.3%) amplification products were obtained in both cassava varieties CM2177-1 and TMS30572 after di­fferent changes in annealing temperatures were performed, which varied between 50 °C and 60 °C (Figure 1). For the gen with simi­larity to *Cf2* there was no amplification pro­duct. In the case of amplifications obtained with primers designed from the *RPP1* gen an expected size amplicon was obtained but only in the CM2177-2 variety. For the primers designed from the sequence with similarity to the *RPM1* gen, different size fragments were amplified, which suggests the presence of various copies of this gene in the cassava ge­nome.

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| **Table 2.** Primers designed from cassava genome sequences similar to resistance genes. | | | | |
| **Name** | **Right primer** | **Left primer** | **Product size** | **Annealing temperature** |
| S\_L6 | TTTCAGAGGTGGAGATACCCGCAA | AAGCTCGTCTAGGCACCATCTTGA | 211 | 52°C |
| S\_RPP5 | AAGGCCTAGAAAGGCACTAAGCGA | TGTCGACACGGTTAAGGTATGGCA | 493 | 50°C |
| S\_RPP1 | TTCCTTACCACAACACCTGGTGGA | ACGCCAACTCTCCATGATGTACCA | 390 | 52°C |
| S\_N | GAGCTAAGCACTTCGGAGCTTTCA | TCTGACGAGCTTGTTCGATATTGT | 428 | 52°C |
| S\_Xa1 | TGGGCCAAGATTTCTCACATCCCT | GCTCGTATATGCAGTGCTCCACTT | 666 | 54°C |
| S\_RPS5 | TGAAGCAGAGAAACACTGGTGGGA | AGGGTAGTGTAATGGGAGGAAATGGG | 304 | 58°C |
| S\_RPM1 | AAATTGTAGTCCGCGTTGCACCAG | GCTTCTGCTTCTGGCTTGCTTGAT | 420 |  |
| S\_RPS2 | AGACAGGCTTCCAACTCCAACTCA | TAAGCTCATTGGACATTGCCGTGC | 480 | 54°C |
| S\_RGC2 | TGATGTGTTGATGTGCTTCGTCCC | GGTATTTCTATGGACTAGCCGTGC | 253 | 50°C |
| S\_I2 | CTTTGCACAAGGCATGAGCAGGAT | TGACCATGCCAAGGCGACATGTAT | 635 | 52°C |
| S\_Cf2 | GCCTGCATGGTTTGTGATGATGGT | CTCCCTTTCTTGTGGATGTTGTGC | 772 | 60°C |
| S\_Pto 3´ | AGACTGTGGATCAAGCAGTGAAAC | AGGCGAATCCAATGTCTTCCAGGT | 257 | 54°C |
| S\_Pto 5´ | GTTTGGACAAACAAAGAGAAGGTGT | CATCCAAATCAAGCAAGGATTGCC | 222 | 54°C |

In this way, the amplicons obtained from these genes were not analyzed by sequencing and, in consequence, high quality sequences were obtained for the other 10 amplicons (Ta­ble 1).

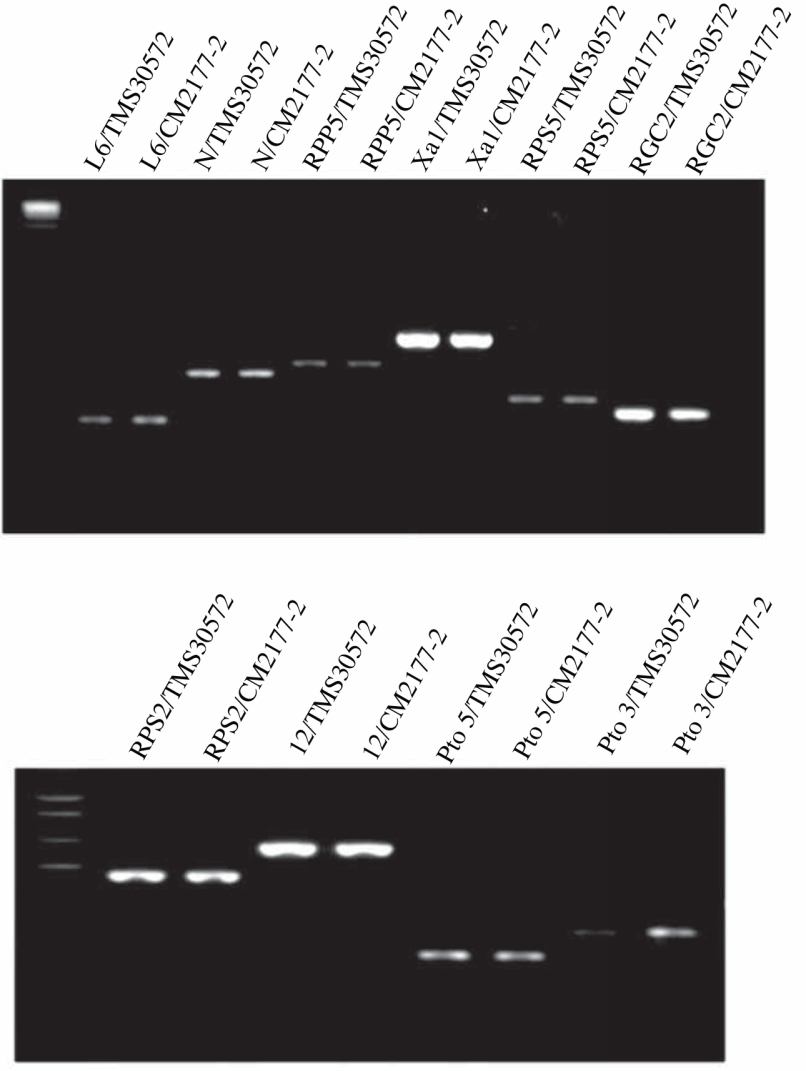
**SNPs and indels identification**

Comparison of obtained sequences for the 10 genes between the sequenced varieties and the reference genome, allowed the identifica­tion of a total of 50 SNPs present in four ca­ssava genes, which are similar to the *RPP5*, *RPS5*, *RPS2* and *Xa1* genes. In the alignment of the obtained sequences for the other genes there were no polymorphisms. From these SNPs, 24 correspond to transitions and 26 to transversions. Additionally, five insertions /deletions (indels) were detected only in the alignment of the *Xa1* and *RPP5* homolog genes.

**Intra-varietal SNPs**

As cassava is a tetraploid and heterozygous crop that can present up to four different alleles in some genes (Fregene *et al.*, 1997), therefore SNPs can be presented in a variety. In this study 4 SNPs were identified in the CM2177-2 variety and, 10 in the TMS30572 variety (Table 3). For both varieties it was found that 50% of the SNPs were transitions and the other 50% were transversions.

**Figure 1.** Amplification of homolog genes in CM2177-2 and TMS30572 cassava varieties.



**SNPs between varieties**

The number of SNPs for each gene between the three varieties is shown in Table 4. When the sequences of the varieties CM2177-2 and TMS30572 were compared there were 37 SNAps clearly identified, 48.6 % (18) corres­pond to transitions and 45.9% (19) are trans­versions. In Figure 2 there is an example of one of the SNPs detected in both varieties. Indels of one, two and four base pairs were also detected for the *RPP5* homolog gen and one indel of three base pairs for the *Xa1* ho­molog gene. These polymorphisms represent a valuable tool to be used in the strategy deve­lopment for mapping these sequences on the genetic map of cassava. In general, a higher number of SNPs was detected in the AM560-2 variety with respect to the CM2177-2 and TMS30572 varieties than between them. Most of the differences between the last two varieties and the variety which genome was sequenced is found in the *RPP5* homolog gene where 71.4% of polymorphism were obtained.

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| **Table 3.** Intravarietal SNPs determination in cassava varieties used in this study. | | | | |
| **Protein** | **Cultivar** | **Total**  **(no.)** | **Transicions** | **Transversions** |
| RPP5 | TMS30572 | 1 | 1 | 0 |
| CM2177-2 | 0 | 0 | 0 |
| RPS2 | TMS30572 | 4 | 2 | 2 |
| CM2177-2 | 0 | 0 | 0 |
| RPS5 | TMS30572 | 5 | 2 | 3 |
| CM2177-2 | 0 | 0 | 0 |
| Xa1 | TMS30572 | 0 | 0 | 0 |
| CM2177-2 | 4 | 2 | 2 |
| Total |  | 14 | 7 | 7 |

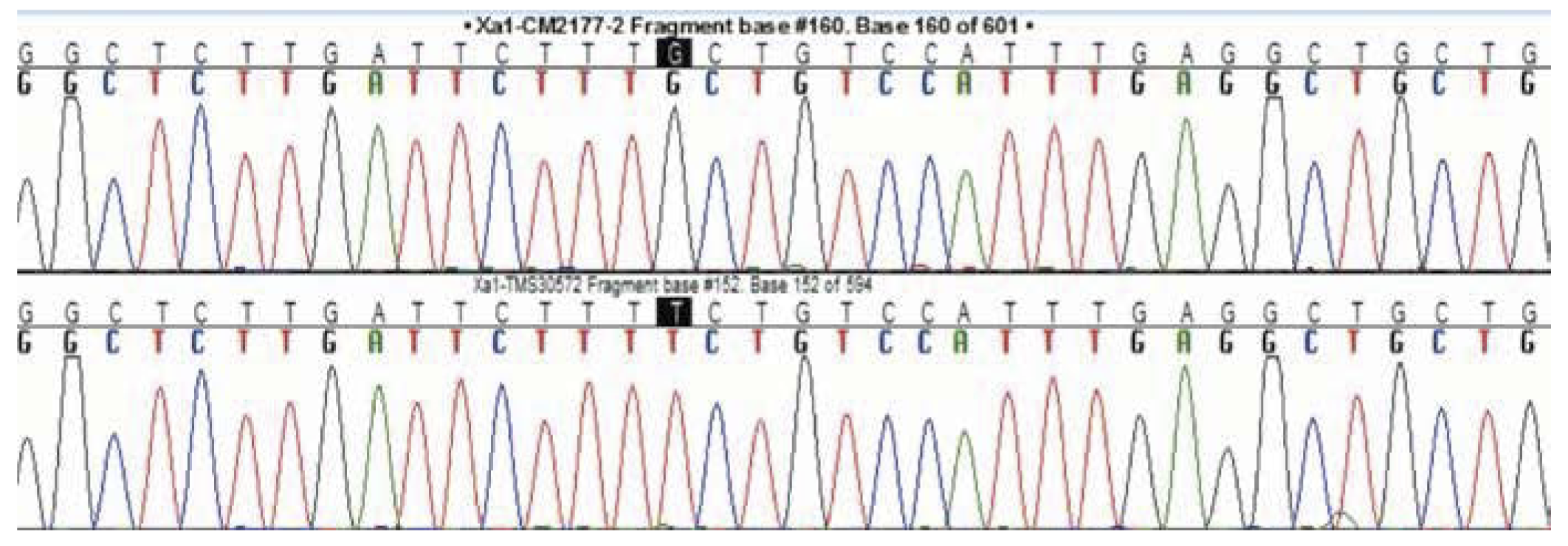
**Discussion**

Genetic map development has been very useful to isolate genes (Keller *et al.*, 2005; Nagamura *et al.*, 1997). Most of the genetic maps has been build using molecular mar­kers of anonymous genomic regions, which are regions where it is unknown if sequences belong to coding or non-coding regions (Fregene *et al.*, 1997; Mba *et al.*, 2001; van Os *et al.*, 2006). However in the last years, with the expressed sequences collections and the availability of full genomes, it has been inclu­ded the use of markers developed in coding sequences (Blair *et al.*, 2011; Gujaria *et al.*, 2011). A strategy that has been used for identification and mapping of genes of interest is the approximation to candidate genes, which is based on conserved sequences pre­sent between species (Hu *et al.*, 2008; Muchero *et al.*, 2010). Conserved domain sequences in R proteins allow, through the candidate gene strategy, the identification of coding sequences for those domains in other plant species. However, their presence alone does not establish a partnership between a gene and the plant resistance to one species or particular strain of a pathogen. This could be possible through mapping of the candidate gene and their association with resistance loci or previously identified QTLs.

This work achieved the identification of cassava genes with high similarity to resis­tance proteins of different plant species. These genes were amplified and sequenced in both parentals used for the cassava genetic map development; this allows the identifica­tion of SNPs for some genes. This information will enable designing of strategies to map them. Direct sequencing of amplicons allows the direct polymorphism identification wi­thout the need of previous cloning and se­quencing of a number of clones. This is a significant fact, since cassava has high hete­rozygocity and it is tetraploid it requires the sequencing of a relatively high number of clones to increase the probability to cover all the possible allelic variants in a particular cassava variety. Genetic maps of *M. esculenta* are primarly based in anonymous markers like

RFLPs, RAPDs, SSRs (Fregene *et al.*, 1997; Mba *et al.*, 2001; López *et al.*, 2007). In this way, the inclusion of markers generated from candidate genes in genetic maps will contri­bute to associate markers with phenotypes. Additionally, since they are genes it is more probable to find their associations with parti­cular phenotypes and, can be a starting point to develop markers for breeding programs assisted by markers. However, these kinds of strategies should be complemented with phe­notyping in response to different pathogens strains or species to increase the probability to associate genes with phenotype.

**Figure 2.** Example of a chromatogram for the Xa1 homologous gene amplified in CM2177-2 and TMS30572 cassava varieties. The highlighted position corresponds to one of the SNPs found between these varieties.



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| **Table 4.** Polymorphism determination in the different cassava varieties used in the study. | | | | | | | | | | | |
| **Protein** | **Variety** | **Variety** | | | | | | | | | |
| **TMS30572** | | | | |  | **AM560-2** | | | |
| **Total**  **(no.)** | **Transicions** | **Transversions** | **Indels** | **Total**  **(no.)** | | | **Transicions** | **Transversions** | **Indels** |
| RPP5 | CM2177-2 | 9 | 3 | 6 | 0 | 12 | | | 4 | 5 | 3 |
| TMS30572 | - | - | - | - | 13 | | | 4 | 6 | 3 |
| RPS2 | CM2177-2 | 1 | 0 | 1 | 0 | 1 | | | 0 | 1 | 0 |
| TMS30572 | - | - | - | - | 0 | | | 0 | 0 | 0 |
| RPS5 | CM2177-2 | 5 | 0 | 0 | 0 | 1 | | | 0 | 1 | 0 |
| TMS30572 | - | - | - | - | 0 | | | 0 | 0 | 0 |
| Xa1 | CM2177-2 | 5 | 2 | 3 | 0 | 7 | | | 3 | 3 | 1 |
| TMS30572 | - | - | - | - | 1 | | | 0 | 0 | 1 |

As an alternative to genetic mapping, strategies based on association mapping have been developed in the last years, from poly­morphisms in candidate genes among a group of particular individuals –not necessarily po­pulations from targeted crosses- an associa­tion with phenotype can be established (Hall *et al.*, 2010). This kind of alternative is valuable in plants like cassava that has a long life cycle, low seed production and, in consequence, any cross demands long periods of time. In this way, cassava genes with si­milarity to *RPP5*, *RPS2*, *RPP5* and *Xa*, for which a good number of candidate polymor­phism was obtained, comprise good candi­dates to start genotyping on a large group of varieties in order to perform association ma­pping.

Although there are characteristics contro­lled by various genes, it is possible to find SNP type markers associated with a pheno­type of interest. For example, fragrance phe­notype which is highly complex was associa­ted to a unique SNP (Jin, *et al.*, 2003). Plant pathogens resistance is not an exception, and although there are many cases in which this phenotype is controlled by numerous genes, there are examples evidencing sequence changes of some genes that can explain diffe­rences between resistant and susceptible cul­tivars (Bryan *et al.*, 2000; Krattinger *et al.*, 2009). It is also important to note that other kind of polymorphisms, like indels, can ex­plain phenotypical differences. For species like *M. esculenta* a low SNPs frequency is ex­pected because of its endogamy and propaga­tion, mainly asexual, by cuttings.

In order to increase the probability to find SNPs introns or downstream regions, proba­bly 3’UTRs, of the selected genes were se­quenced. These regions have shown that the polymorphism frequency could be up to three times higher than in other regions (Ganal *et al.*, 2009; Rafalski, 2002). The intron of the cassava gene similar to *RPP5* happened to be the sequence with more polymorphism, demonstrating that these regions have a high potential to search for markers.

Cassava SNPs frequency estimated in this work was 1 SNP each 164.14 bp. In *Ara­bidopsis*, for instance, there is an estimate of 1 SNP each 2.2 kb for intronic regions; while in exons it is 1 SNP each 3.1 kb (The Arabidop­sis Genome Iniciative, 2000). In maize has been reported higher SNPs frequencies: 1 each 31 bp in no-coding sequences and 1 each 124 bp in coding sequences (Ching *et al.*, 2002). In soybean the approximate frequency is 1 SNP each 270 bp (Zhu *et al.*, 2003). Ob­viously SNP frequency changes among species and it is not homogeneous along the genome. In this sense, it is important to consider re­gions close to *R* genes that can have low se­lective pressures and consequently higher probabilities to find polymorphisms. Nowa­days this possibility is a reality since the ca­ssava genome is available. Identification of these kinds of markers, though not directly associated with *R* genes, will allow the reduc­tion on the range where previously identified QTLs or resistance loci have been localized.

The methodology used in this work is proposed as a strategy to identify SNPs in a higher number of candidate genes and/or genomic regions closely located to homologs of resistance genes. Polymorphisms obtained in this work are a valuable resource, since from them, CAPs markers can be designed to ge­notype the F1 from CM2177-2 and TMS30572 crossing, in order to locate these genes in the cassava genetic map to establish associations with the regions imply in resistance.

**Conclusions**

Diverse cassava genes with similarity to pre­viously reported *R* genes from plants were amplified. The sequences obtained from the cassava CM2177-2 and TMS30572 varieties allowed the detection of various SNPs and indels, which can be used to genotype the F1 population of this cross and to map them in the cassava genetic map.

**Acknowledgments**

The Project of this work was financed by the Banco de la Republica (Project 2747) and Col­ciencias (projecto No 110152128403), the authors express their gratitude to those di­rectors.

**References**

Blair, M.; Hurtado, C.; Chavarro, M.; Munoz-Torres, M.; Giraldo, F.; Pedraza, J.; Tomkinsy, R.; and Wing, R. 2011. Gene-based SSR markers for common bean (*Phaseolus vulgaris* L.) derived from root and leaf tissue ESTs. BMCPlant Biol.*.*11:50.

Bryan, G. T.; Wu, K. S.; Farrall, L.; Jia, Y.; Hershey, H. P.; McAdams, S. A.; Faulk, K. N.; Donaldson, G. K.; Tarchiniy, R.; and Valent, B. 2000. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene Pi-ta. Plant Cell.12:2033 - 2046.

Ceballos, H. 2002. La yuca en Colombia y el mundo: nuevas perspectivas para un cultivo milenario. En: CIAT (eds.). La yuca en el Tercer Milenio: Sistemas modernos de producción, procesamiento, utilización y comercialización. 586 p.

Ching, A.; Caldwell, K. S.; Jung, M.; Dolan, M.; Smith, O. S.; Tingey, S.; Morgantey,M.; and Rafalski, A. J. 2002. SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. BMC Genet*.* 3:19.

Dellaporta, S.; Woody, J.; and Hicks, J. 1983. A plant DNA minipreparation: version II. Plant Mol. Biol. Rep. 1:19 - 21.

Fregene, M.; Angel, F.; Gomez, R.; Rodriguez, F.; Chavarriaga, P.; Roca, W.; Tohmey J.; and Bonierbale, M. 1997. A molecular genetic map of cassava (*Manihot esculenta* Crantz). Theor. Appl. Genet. 95:431 - 441.

Ganal, M. W.; Altmanny, T.; and Roder, M. S. 2009. SNP identification in crop plants. Curr. Opi. Plant Biol*.* 12:211 - 217.

Gujaria, N.; Kumar, A.; Dauthal, P.; Dubey, A.; Hiremath, P.; Bhanu, A.; Farmer, A.; Bhide, M.; Shah, T.; Gaur, P.; Upadhyaya, H.; Bhatia, S.; Cook, D.; Mayy,G.; and Varshney, R. 2011. Development and use of genic molecular markers (GMMs) for construction of a transcript map of chickpea (*Cicer arietinum* L.). Theor. Appl. Genet. 122:1577 - 1589.

Hall, D.; Tegstromy,C.; e Ingvarsson, P. K. 2010. Using association mapping to dissect the genetic basis of complex traits in plants. Brief. Funct. Genom. .9:157 - 165.

Hammond-Kosack, K. E.; y Kanyuka, K. 2007. Resistance genes (R genes) in plants. En: Encyclopedia of Life Sciences, J.W. Sons, ed. (Londres). p. 1 - 21.

Hu, K. M.; Qiu, D. Y.; Shen, X. L.; Liy, X. H.; and Wang, S. P. 2008. Isolation and manipulation of quantitative trait loci for disease resistance in rice using a candidate gene approach. Mol. Plant. 1:786 - 793.

Jin, Q.; Waters, D.; Cordeiro, G. M.; Henry, R. J.; and Reinke, R. F. 2003. A single nucleotide polymorphism (SNP) marker linked to the fragrance gene in rice (*Oryza sativa* L.). Plant Sci.165:359 - 364.

Jones, J. D. and Dangl, J. L. 2006. The plant immune system. Nature*.*444:323 - 329.

Keller, B.; Feuillet, C.; and Yahiaoui, N. 2005. Map-based isolation of disease resistance genes from bread wheat: cloning in a supersize genome. Genet. Res.*.*85:93 - 100.

Krattinger, S. G.; Lagudah, E. S.; Spielmeyer, W.; SinghR. P.; Huerta-Espino, J.; McFadden, H.; Bossolini, E.; Seltery,L. L.; and Keller, B. 2009. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science*.*323:1360 - 1363.

López, C. E.; Restrepo, S.; and Verdier, V. 2006. Limitations of cassava bacterial blight: New advances. Acta Biol. Col. 11:21 - 45.

López, C. E.; Quesada-Ocampo, L. M.; Bohorquez, A.; Duque, M. C.; Vargas, J.; Tohme, J.; and Verdier, V. 2007. Mapping EST-derived SSRs and ESTs involved in resistance to bacterial blight in *Manihot esculenta*. Genome 50:1078 - 1088.

Mba, R. E.; Stephenson, P.; Edwards, K.; Melzer, S.; NkumbiraJ.; Gullberg, U.; Apel, K.; Gale, M.; Tohme, J.; and Fregene, M. 2001. Simple sequence repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: towards an SSR-based molecular genetic map of cassava. Theor. Appl. Genet. 102:21 - 31.

McHale, L.; Tan, X.; Koehly, P.; and Michelmore, R. W.2006. Plant NBS-LRR proteins: adaptable guards. Genet. biol.*.*7:212.

Meyers, B. C.; Kozik, A.; Griego, A.; Kuangy, H.; and Michelmore, R. W. 2003. Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. Plant Cell.15:809 - 834.

Muchero, W.; Ehlersy J. D.; and Roberts, P. A. 2010. Restriction site polymorphism-based candidate gene mapping for seedling drought tolerance in cowpea [*Vigna unguiculata* (L.) Walp.]. Theor. Appl. Genet. 120:509 -518.

Nagamura, Y.; Antonioy, B. A.; and Sasaki, T. 1997. Rice molecular genetic map using RFLPs and its applications. Plant Mol. Biol. 35:79 - 87.

Rafalski, A. 2002. Applications of single nucleotide polymorphisms in crop genetics. Curr. Opin. Plant Biol*.* 5:94 - 100.

Rozen, S.; and Skaletsky, H. 2000. Primer3 on the WWW for general users and for biologist programmers. Meth. Mol. Biol. 132:365 - 386.

Song,W. Y.; Wang, G. L.; Chen, L. L.; Kim, H. S.; Pi, L. Y.; Holsten, T.; Gardner, J.; Wang, B.; Zhai, W. X.; Zhu, L. H.; Fauquet, C.; and Ronald, P. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. Science*.* 270:1804 - 1806.

Stergiopoulos, I.; van den Burg, H. A.; Okmen, B.; Beenen, H. G.; van Liere, S.; Kemay, G. H.; and de Wit, P. J.2010. Tomato Cf resistance proteins mediate recognition of cognate homologous effectors from fungi pathogenic on dicots and monocots. Procc. Nat. Acad. Sci. 107:7610 - 7615.

The genome Arabidopsis Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature*.*408:796 - 815.

van Os, H.; Andrzejewski, S.; Bakker, E.; Barrena, I.; Bryan, G.J.; Caromel, B.; Ghareeb, B.; and Isidore, E. *et al.* 2006. Construction of a 10,000-Marker ultradense genetic recombination map of potato: providing a framework for accelerated gene isolation and a genomewide physical map. Genetics.173:1075 - 1087.

Zhu,Y. L.; Song, Q. J.; Hyten, D. L.; Van Tassell, C. P.; Matukumalli, L. K.; Grimm, D. R.; Hyatt, S. M.; Fickus, E. W.; Youngy, N.D.; and Cregan, P. B. 2003. Single-nucleotide polymorphisms in soybean. Genetics*.*163:1123 - 1134.

Zipfel, P. F. 2009. Complement and immune defense: from innate immunity to human diseases. Immunologyletters*.*126:1 - 7.