Positional Cloning of CMD2 the Gene that Confers High Level of Resistance to the Cassava Mosaic Disease (CMD).

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INTRODUCTION

Map-based or positional cloning is an important method for the isolation of genes known only by phenotype and the map position (Wicking & Williamson. 1991; Peterson & Wing. 1993). Positional cloning in plants has been demonstrated for different kinds of genes (Rommens et al. 1989; Arondel at al. 1992; Giraudat et al. 1992; Chang et al. 1993; Martin et al. 1993). We describe the development of molecular markers tightly linked to the CMD2 as the first step in positional cloning of the CMD2 gene that confers high level of resistance to the cassava mosaic disease (CMD). Previous work revealed that the SSR markers SSRY 28 and NS158 are the closest markers to the gene CMD2, and are located at distances of 9 and 3 cM respectively (Akano et al.2002; Zárate 2002, CIAT 2002). The experimental approach involves identification of recombinants between CMD2 and the above markers using a large full-sib population of 1690 individuals, followed by the use of several types of marker systems to find markers closer to CMD2 gene. These markers were then used to screen a BAC library for the construction of a BAC contig that transverses the region.

MATERIALS AND METHODS

The fine mapping population was 1690 individuals of a F1 progeny from a cross between TME3 that shows resistance to the African cassava mosaic disease (ACMD) as male parent, and the female parent TMS-30572 variety tolerant to CMD (Fig 1). The cross was evaluated in the 2002 growing season for CMD resistance in the field at IITA, Ibadan, under heavy natural pressure of the disease. DNA was isolated from the parents and individuals of the population. DNA extraction was carried out from fresh young leaves according to Dellaporta's method (1983) and diluted 10X in TE buffer without quantification for molecular marker analysis.The population was evaluated with the 2 SSR markers (SSRY-28 y NS-158) according as described by Mba et al.2001 and recombinants between the markers and CMD2 identified. DNA from 10 resistant recombinants and 10 susceptible recombinants were combined to form 2 bulks which were then evaluated with several markers system incluiding AFLPs, ISTRs, RAPDs and SSRs in a modified bulk segregant analysis (BSA) method (Michelmore et al.1991).

Evaluation with AFLP markers (Vos et al. 1995) was using a commercial AFLP kit (Invitrogen Life Technologies, Gaithersburg, MD) following the manufacturer's instructions, all 64 possible combinations were used in the evaluation. For ISTRs (Invert Sequence Tagged Repeat), the method described by Rohde et al. 1996 was used with all possible 64 combinations of the 8 F and 8 B universal retro-elements (retro-transposons) sequence primers. A set of 146 newly developed SSR markers (CIAT 2002) were used with PCR and PAGE electrophoresis conditions described by Mba et al.(2001). Evaluation with RAPDs markers was using 768 commercial primers (Operon Technologies Inc, CA) and a modified Williams et al. (1990) protocol. Markers that were polymorphic in the recombinants bulk were then analyzed in individuals of the bulks (Open Bulk). A polymorphic RAPD fragment in the individuals of the recombinant bulk was cloned using The PGEM-T easy vector system from Promega according to the supplier's instructions. Sequencing was carried out at the Iowa State University sequence facility. Primers were designed from sequence and the amplify product is being used as SCAR marker for MAS.

Figure 1. CMD resistance phenotypes of the F_1 fine mapping progeny (TME-3 x TMS30572)

RESULTS AND DISCUSSION

The evaluation of the fine-mapping population with the SSR markers SSRY28 and NS158 allowed the identification of 112 recombinant individuals (Fig.2). These individuals were then analyzed with additional molecular markers to identify those close to the target gene. The evaluation of the resistant and susceptible recombinant bulks with AFLP,SSR,ISTR produced several candidate markers that were polymorphic in the bulks but the polymorphism was not consistent when genotypes of the bulks were analyzed individually (opened bulks). However, analysis with RAPD markers produced 2 polymorphic candidate markers, AC-15 and RME-1 that remained consistent in the individuals of the bulk (Fig.3). Evaluation of the two markers in the entire fine map progeny and linkage analysis with MAPMAKER program (Lander et al. 1992) revealed that AC-15 is at least 2 cM from CMD2, while RME-1 is less than 1 cM from the gene.

The polymorphic fragment in RME-1, a 800bp band was cloned intopGEMT-easy and sequenced. Homology comparison between the sequence of the RAPD band and sequences in public database using BLAST (www.ncbi.nlm.nih.gov) revealed the sequence is similar to the minor caspid protein of bacteriophage T3 suggesting that the fragment is low copy gene. SCAR primers have been designed from the sequence for use in MAS since it is closer than to the gene than NS158, the closest marker to date to CMD2 (Fig.4). The SCAR marker linked to CMD2 was used to screen a Cassava BAC library constructed from TME3 having more than 70,000 clones and about 200 positive clones were identified, suggesting that the RAPD fragment might be a multiple copy number fragment. Additional effort is being made to screen the BAC library with higher stringency for the eventual construction of BAC contigs. Once contigs have been made, BAC ends will be mapped and candidate BAC clones sequenced (Fig5.)

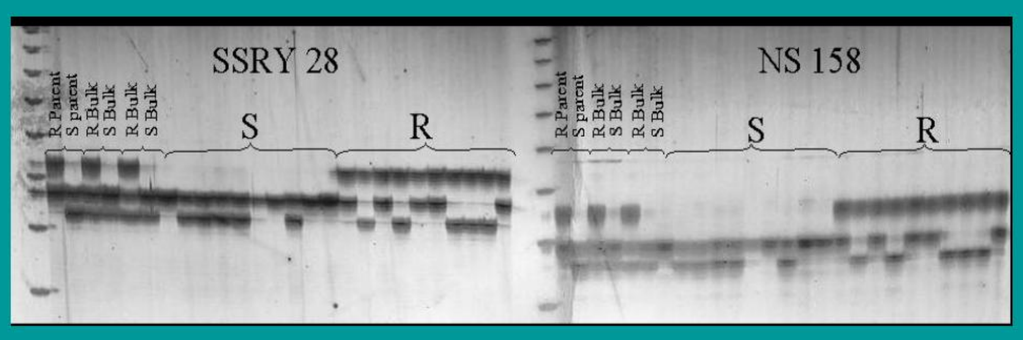

Fig.2. Identification of Susceptible and Resistance individuals of the fine mapping population by SSR evaluation with SSRY 28 and NS158. The resistant band appear in all resistant individuals (R).

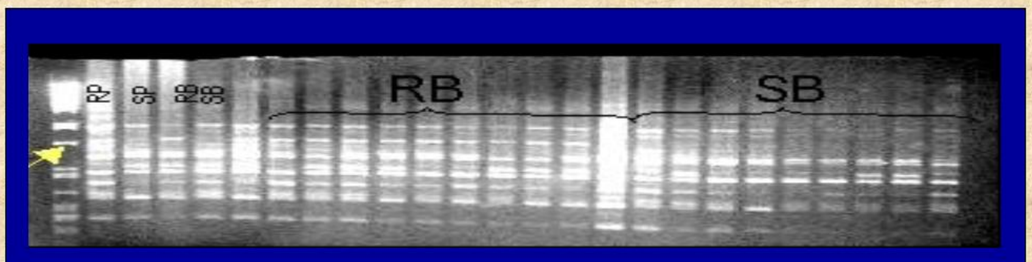

Fig.3. Ethidium bromide stained agarose gel of individuals from the recombinant bulks Evaluated with the RAPD marker RME-1.

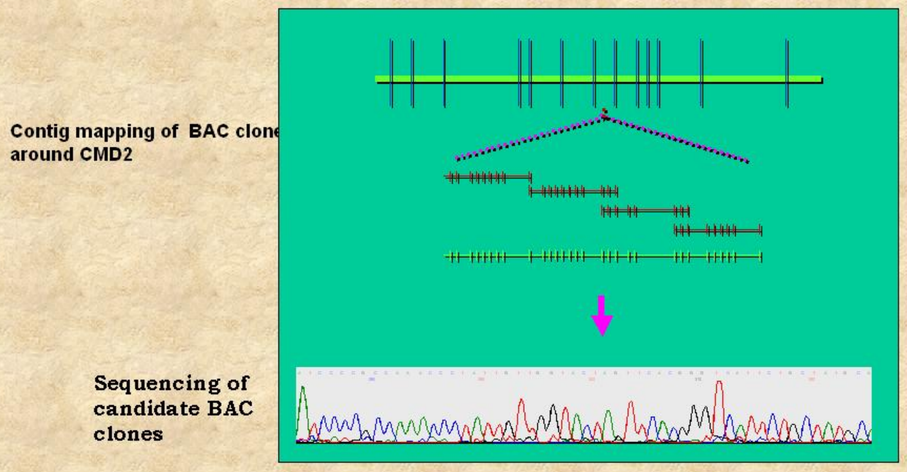

Fig. 5 Scheme for positional cloning of CMD2

CONCLUSIONS AND ONGOING WORK

- The use of different molecular marker systems to analyzed a target region allowed screening with a large number of random, unmapped molecular markers in a relatively short time and to select those few markers that reside in the vicinity of the target gene. RAPD markers have been successfully used to clone resistance genes, including the Pto gene and Mi gene in tomato, several Dm genes in lettuce.
- A high resolution map with 4 markers, one at less than 1 cM has been constructed around the genome region of CMD2. The cloning of this gene is now proceeding to the next stage of BAC library screening and the construction of a BAC contig around CMD2.

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