RECENT ADVANCES IN HOST PLANT RESISTANCE TO WHITEFLIES (*Aleurotrachelus socialis* Bondar) (Homoptera: Aleyrodidae) IN CASSAVA (*Manihot esculenta* Crantz)

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INTRODUCTION

Whiteflies are considered one of the world's major agricultural pest groups, attacking a wide range of plant hosts and causing considerable crop loss. There are nearly 1200 whitefly species with a host range that includes legumes, vegetables, fruit trees, ornamentals and root crops. As direct feeding pests and virus vectors, whiteflies cause major damage in agroecosystems based on cassava (Euphorbiaceae; *Manihot esculenta* Crantz) in the Americas, Africa and to a lesser extent, Asia. The most damaging species on cassava in northern South America is *Aleurotrachelus socialis.* Typical damage symptoms include curling of apical leaves, yellowing and necrosis of basal leaves and plant retardation (Fig. 1). Adult whiteflies are most frequently observed on the underside of apical leaves where they feed on plant fluids and oviposit. The "honeydew" excreted is a substrate for a sooty-mold fungus that interferes with photosynthesis (Fig. 1C). The combination of direct feeding and impaired photosynthetic rate reduces root yield by 4 to 79% depending on the duration of attack (Bellotti, 2002).

More than 5,000 cassava genotypes have been evaluated at CIAT and CORPOICA for whitefly resistance. At present, the major source of host resistance in cassava is the genotype MEcu-72 (Bellotti and Arias, 2001) (Fig. 1D). When feeding on MEcu-72 *A. socialis* had less oviposition, longer development periods, reduced size and higher mortality than when feeding on the susceptible genotype, (Fig, 2). Due to the importance of whiteflies as a pest and virus vector, it is important to understand the nature of genes that confer resistance in the resistant genotype, MEcu-72. To study the genetics of this resistance, a cross was made between MEcu-72 (resistance genotype) x MCol-2246 (a very susceptible genotype), to evaluate F1 segregation, using molecular markers. This will accelerate the selection of whitefly resistant germplasm and isolate resistant genes.

Fig.1. A: Nymphal Stages of *A. socialis*, on a cassava leaf. B: Leaf curling an a cassava plant with high populations of *A. socialis.* C: Presence of sooty mold fungus on a cassava leaves attacked by *A. socialis*. D: Resistant genotype MEcu-72 and a susceptible genotype.

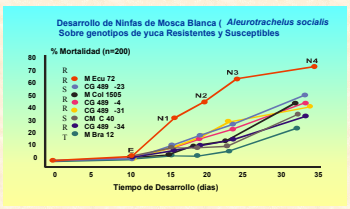


Fig. 2. A: Whitefly (*A. socialis)* nymphal mortality on resistant (R), tolerant (T) and susceptible (S) cassava clones.

MATERIALS AND METHODS

PLANT MATERIAL

For the present work we have used the cross MEcu-72 (as the resistant parent) x MCol-2246 (as the susceptible parent). A total F1 offspring of 286 genotypes (family CM8996) was produced from this cross. These materials were sowed and evaluated in the field during May 2001, March and August 2002 at two different locations: Espinal-Tolima, Colombia (CORPOICA-NATAIMA) at 350 m.a.s.l. and Santander de Quilichao, Cauca, Colombia, at 990 m.a.s.l. With this evaluation we will identify gene segregation in the offspring and we will be able to select the resistant and susceptible materials. The evaluation was performed in the field using population and damage scales (Table 1)

Table 1. Population and damage scales for evaluation cassava germplasm for resistance to whiteflies.*

Population scale (nymphs & pupae)
1= no whitefly stages present
2= 1-200 individuals per cassava leaf
3= 201-500 per leaf
4= 501-2000 per leaf
5= 2001-4000 per leaf
6= >4000 per leaf
Damage scale
1= no leaf damage
2= young leaves still green but slightly flaccid
3= some twisting of young leaves, slight leaf curling
4= apical leaves curled & twisted; yellow-green mottled appearance
5= same as 4, but with sooty mold & yellowing of leaves
6= considerable leaf necrosis & defoliation, sooty mold on mid & lower leaves and young stems

*Extracted of Bellotti & Arias, 2001 Crop Protection. 813-823.

MOLECULAR ANALYSIS

We are using Simple Sequences Repeat (SSR) to find markers associated with resistance for mapping the resistant gene(s). As part of a collaborative project with Clemson University funded by USAID a BAC library for cassava using the clone MEcu 72 was constructed. The library contains 73,728 clones with an average insert size of 93 kb. Based on a genome size of 760 Mb, library coverage is approximately 10 haploid genome equivalents. The whitefly resistance will be the target for map-based cloning using the BAC libraries as tools. We are using silver staining to visualize the allelic segregation of the markers. We are using RGAs sequences (isolated from cassava previously).

RESULTS

FIELD EVALUATION

Field evaluations carried out at Nataima (Tolima) demonstrate that there was considerable whitefly pressure as plant damage and pest populations were high (from 4 to 6 on the damage and population scales, Table 1). However, some genotypes, in spite of the high pressure, had low damage levels (less than 2.0). It can therefore be concluded that these genotypes have resistance levels similar to those of the resistant parent.

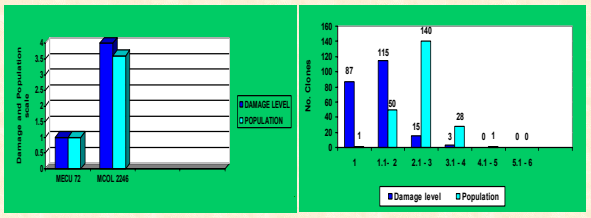


Fig. 3. Cassava damage and whitefly population ratings due to *A. socialis* feeding on parental genotypes MEcu-72, MCol-2246 and clones from the family CM 8996 at CORPOICA, Nataima (Tolima, Colombia).

MOLECULAR ANALYSIS

Both parents, MEcu-72 and MCol-2246, were evaluated with 343 cassava SSR markers (Mba et al, 2001), including 156 cDNA SSRs developed by Mba et al (submitted) (Fig. 4).

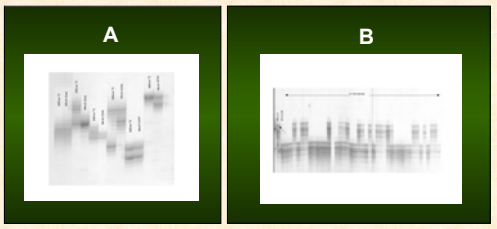


Fig. 4. Silver staining polyacrylamide gel showing: A: the parentals MEcu-72 and MCol-2246 evaluated with six cassava SSRs. B: unique allele in MEcu-72 of cassava SSR 234. Forty-one F1 progenies show the inheritance of this allele.

Approximately 155 of the SSRs were polymorphic in the parentals and were evaluated in the F1 (286 individuals) (Fig. 4). For the construction of the linkage map, 103 SSRs were analyzed, of which 71 were anchored and segregating from the heterozygous female parent (MEcu-72) of an interspecific cross. The map consist of 19 linkage groups; which represent the haploid genome of cassava (Fig. 6). These linkage groups span 550,2 cM and an average marker density of 1 per 7,9 cM. The position of the 71 SSRs markers is shown in figure 6 of the cassava molecular genetic map (LOD = 25 and tetha (θ) = 25). Map distances are shown in Kosambi map units. So far, 26 SSRs markers (shown in green, Fig. 1) have been previously placed on the cassava framework map (Fregene et al, 1997), the other 45 SSRs are new. Thirty one of the 71 SSRs were cDNA sequences (Mba, in preparation) and the others were genomic DNA.

AFLPs Analysis

An analysis was done of 128 combinations of primers with both parentals, MEcu-72 and MCol-2246, and both bulks of 10 whitefly resistant and 10 susceptible DNA. We obtained 53 polymorphic combinations, in which we found 425 polymorphic bands between the resistant and the susceptible (Fig. 5).

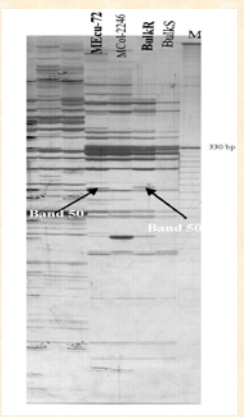
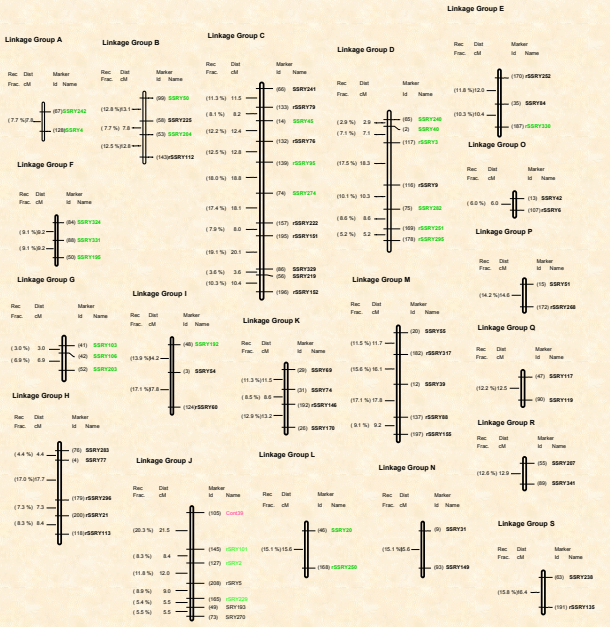


Fig. 5. Silver stained polyacrylamide gel showing: combination ACA-CTT of AFLP of both parents (R resistant, S susceptible) and Bulks resistants and susceptibles, show the polymorphic band # 50 unique in the resistants.

Fig. 6: Preliminary Cassava framework Map of MEcu-72 for Resistance to White Fly, consisting of SSRs. (Lod = 25 and theta = 25)



ASSOCIATION BETWEEN MOLECULAR MARKERS AND RESISTANCE

The molecular data are being analyzed using QTL packages (QTL cartographer Qgene) to determine linkages between the markers and the phenotypic characterization. As preliminary analysis X^2 at the 5% level was done using SAS. Putative associations were found between 43 SSRs markers and the field phenotypic characterization (score 1.0 to 2.0 of the levels of damage and populations Table 1).

CONCLUSIONS

- Field evaluations in the family CM 8996 and their parentals confirm resistance of the genotype MEcu-72 and susceptibility of the parental MCol-2246; this allow us to do preliminary selection of F1 genotypes.
- Using SSR markers, putative association with the parental lines were found.
- A linkage map is being constructed using the SSR data, a RGA and the field phenotypic characterization.

ON GOING WORK

- Saturation of Linkage map of Ecu-72, using AFLPs.

- Isolation, cloning, sequencing and mapping of AFLPs polymorphic bands between resistants and susceptibles genotypes and design of SCARs for marker assisted selection.

- QTLs analysis for resistance to whitefly.

- Mapping of cassava RGAs polymorphics (BACs Primers, Genes Resistance Primers) in F1 (276 genotypes).

- The whitefly resistance will be the target for map-based cloning using the BAC libraries as tools.

- Isolation of expressed sequences during the defense response of MEcu-72 to white fly attack.

- In order to identify differentially expressed sequences, a new technology known as DNA chips or microarray is available to scan a significant number of clones. Microarray expression profiling detailed experiments will be used to identify putative early-response regulatory and/or signaling genes and to test the function of selected candidate genes using reverse genetics.

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