

INTRODUCTION

Whiteflies are considered one of the world's major agricultural pests 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 on 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.

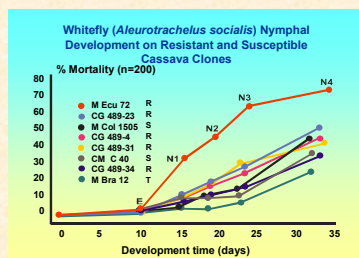


Fig. 2. A: Whitefly (*A. socialis*) nymphal mortality on resistant (R), tolerant (T) and susceptible (S) cassava clones.

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 ranging between 1-6, where 1 is an absence of damage and population and 6 is high population and damage (curling, chlorosis, sooty mold fungus, etc. Table 1). Three evaluations were performed in 2002 using the highest damage and population data for information processing.

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

Population scale (nymphs & pupae)	
1=	no whiteflies 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 target for map-based cloning using the BAC libraries as tools. We are using silver staining to visualize the allelic segregation of the markers.

RESULTS

FIELD EVALUATION

The whitefly resistant variety CG 489-31 (Fig. 2), a progeny from the Mecu 72 x MBra 12 cross, has been released to cassava farmers by CORPOICA, Colombia under the name Nataima-31 (Fig. 3).

Initial field evaluations showed that these materials (family CM 8996) had low levels of the pest, because test plants (materials very susceptible to *A. socialis*) did not present high levels of damage and populations (scale of 4 to 6 Table 1). The harvest evaluation showed that the root yield was between 4.5 and 86.5 ton/ha, and many materials presented desirable characteristics (high percentage of dry matter, palatability, etc.). Currently, the family is under a second sowing cycle at the same locality from Tolima, and high pressure exerted by the pest has been detected since test materials have high degrees of damage (from 4 to 5).

Preliminary evaluations have demonstrated that some materials from the family present low levels of damage and population (up to 2) (Fig. 4 and 5).

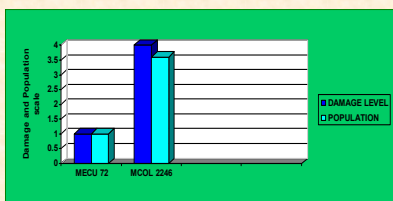


Fig. 4. Cassava damage and whitefly population ratings due to *A. socialis* feeding on parental genotypes Mecu-72 and MCol-2246 at CORPOICA, Nataima (Tolima, Colombia).

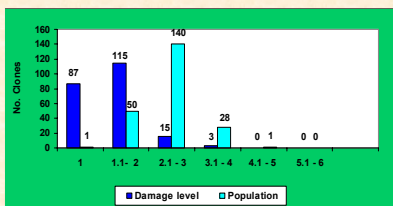


Fig. 5. Cassava damage and whitefly population ratings due to *A. socialis* feeding on clones from the family CM 8996 (Mecu-72 x MCol-2246) at CORPOICA, Nataima (Tolima, Colombia).

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 (Mba et al, by submitted). Approximately 155 of the SSRs were polymorphic in the parents and were evaluated in the F1 (Fig. 6).

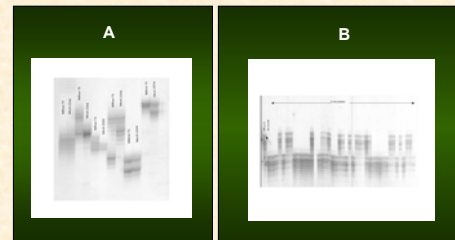


Fig. 6. Silver staining polyacrylamide gel showing: A: the parents Mecu-72 and MCol-2246 evaluated with six cassava SSRs. B: unique allele in Mecu-72 of cassava SSR 234. Forty-one F1 progenies show the inheritance of this allele.

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 X² 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).

CONCLUSIONS AND ONGOING WORK

- The field evaluations in the family CM 8996 and their parental show the resistance of the genotype Mecu-72 and the high susceptibility of the parental MCol-2246.
- Using SSR markers, putative association with the resistant lines were found. A linkage map is being constructed using the SSR data and the field phenotypic characterization.
- Based on going QTL analysis, the marker linked to the resistant gene(s) will be used as part of large scale screening of breeding lines and to accelerate the breeding cycle for whiteflies resistance. Fine mapping of the genes involved will be carried as a first step toward the cloning of the resistant genes and the study of their expression.

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