Unraveling whitefly resistance in cassava (*Manihot esculenta*)

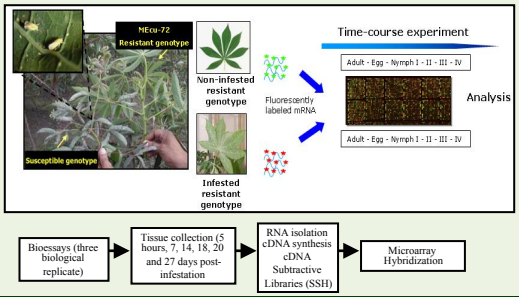
Bohorquez, Adriana[1], Becerra Lopez-Lavalle, Luis[1]., Bellotti, Anthony C.[1] and Joe Tohme,[1].

[1]CIAT, Agrobiodiversity and Biotechnology Project, AA6713, Cali, Colombia

INTRODUCTION

Whiteflies (WF) are the major biotic stress that threatens the sustainability of staple crop, including cassava, causing direct damage due to feeding and can obliterate the entire cassava crop. Twelve WF species are serious pests of cassava being the most important *Aleurotrachelus socialis* (LAC) and *Bemisia tabaci* (Africa). One of the most potent resistance mechanisms to *A. socialis* was discovered at CIAT. On the resistance line, MEcu 72 WF deposit fewer eggs, establish fewer feeding sites, nymph development is delayed, and WF mortality is increased. One approach was proposed to unravel the genetic mechanism of white fly resistance (WFR). Gene expression profiling, using microarray technology, coupled the subtractive libraries approach. The aim was to capture genes that were differentially induced during WF attack. Microarrays technology on challenged and non-challenged WF resistant/susceptible subtractive libraries, as well as, on the 5000 cassava unigene microarray (Lopez et al, 2004), were used to identify differentially expressed genes in cassava during *A. socialis* attack. Our results suggest that WFR is a complex trait, in which more than one genetic region may be involved. This WFR can be used in molecular breeding to accelerate the development of WFR cassava cultivars with field attributes valued by smallholder farmers in Latin America. By understanding the mechanism of resistance to *A. socialis*, our studies may lead to strategies that will confer resistance to other WF species that today decimate cassava in Africa and Asia.

METODOLOGY



RESULTS

In this study, changes in the Cassava transcriptome profile were examined throughout the life cycle of the whitefly, as changes in the plant defense gene RNAs occur in crop plants in response to adult and nymphal stages. Significant Analysis of Microarray (SAM) software identified 550 genes as significantly regulated in the six collect times and the two comparisons (resistant infested vs resistant non-infested & resistant infested vs susceptible infested), which 310 Up-regulated and 240 down-regulated. Functional categories were defined using the GO classification scheme. Twenty-one percent were of unknown function, no match or "expressed proteins". GO identified genes involved in Defense response, cell wall modification, oxidative stress, photosynthesis (Table 1), transport, response to stimulus, proteolysis, carbohydrate metabolism, etc. (Figure 1). Some of these sequences are part of the signaling pathways regulated by jasmonic acid (JA) and ethylene (ET), which are implicated in the defense response during pathogens and herbivores attack to plants (Figure 2). The application of functional genomics approach in the study of cassava defense responses, opens a wide range of future applications at different levels, both *in silico* and experimentally. Gene expression analysis, construction of physical and genetic maps, genomic sequence analysis, gene silencing and production of genetically modified organisms are some of the projects will be developed in the future.

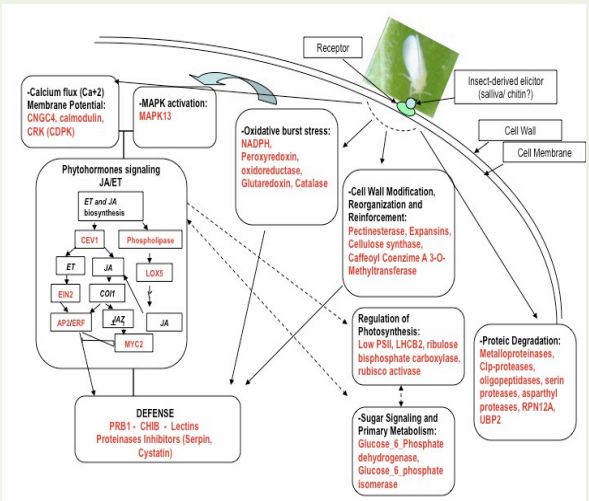


Figure 2: Graphic representation of the hypothetical model of the defense response triggered by the whitefly *A. socialis*, when feeding on the cassava genotype MEcu 72, based on the sequences isolated in the subtractive libraries and differentially expressed in microarrays hybridization (genes shown in red). The dashed arrows indicated that the Photosynthesis and Primary Metabolism are down-regulated.

1- When *A. socialis* feeding on leaves of genotype MEcu 72, introduce their stylet, and insect-derived elicitor are recognized by a plant receptor. In the case of chitin, it is proven that the family of transcription factors (TF) *AP2/ERF* are induced by chitin.

2- *AP2/ERF* TF is induced by the signaling cascade that begins with plasma membrane depolarization and Ca^{2+} flux, then are activated MAPK signaling cascades and subsequent induction of phytohormones pathways such as JA/ET.

3- *AP2/ERF* TF are potential mediators of the synergistically induction process between JA and ET and induce defense genes such as basic vacuolar proteins *PRB1, CHIB (PR-3)*, as well as lectins and proteinase inhibitors. RT-PCR analyses showed that *PRB1* and *LOX5* RNAs are accumulated when *A. socialis* feeding on the resistant genotype MEcu 72 (Figure 3).

4- The defense response is complex and involves all the processes of cellular metabolism, some of which themselves can be effector mechanisms that are controlling the attacker. Among these are, the generation of ROS.

5- Cell wall modification, which may make it difficult to insect feeding and may also mediate the defense response regulated by JA/ET, and finally the protein degradation machinery. At the same time the plant represses its primary metabolism and photosynthesis, reallocating C and N resources to the defense.

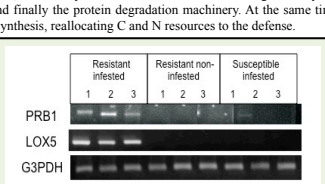


Figure 3: RT-PCR analyses of RNA isolated from cassava resistant Ecu72 and susceptible CMC40. Total RNA was extracted from both infested and non-infested leaves at time1 (adult & egg), time 2 (nymph I & II), and time 3 (nymph III & IV). Genes involved in defense PRB1 (ATPRB1) and LOX5. G3PDH (endogenous gen).

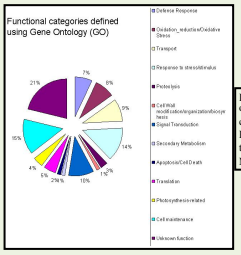


Figure 1: Functional categories of regulated genes during cassava-whitefly interaction. Hybridizations were done with the Cassava Unigene Microarray, 5000 unigenes)

Table 1: Genes regulated by *A. socialis* in Cassava, obtained from subtractive libraries and Microarray hybridization. Data are shown for 4 functional categories. These sequences differentially expressed were compared to known protein sequences (The Arabidopsis Information Resource, TAIR) and mapped to Gene Ontology (GO) terms and KEGG pathways using BLASTX. Sequences were mapped in cassava genome (http://www.phytozome.net/cassava) and were designed specific primers for RT-PCR analyses.

Accession No.	Cassava genome ubicati	Putative function/ homology species
		Defense Response
AT3G01500.3	cassava4.1_011848m	CA1 (CARBONIC ANHYDRASE 1); carbonate dehydratase [Arabidopsis thaliana]
AT3G12490.1	cassava4.1_019957m	cysteine protease inhibitor, putative / cystatin,
AT3G12500.1	cassava4.1_011797m*	ATHCHIB (ARABIDOPSIS THALIANA BASIC CHITINASE); chitinase;
AT3G22400.1	cassava4.1_001506m*	LOX5; electron carrier/ iron ion binding / lipoxygenase/ metal ion binding [Arabidopsis thaliana]
AT4G26850.1	cassava4.1_007450m	VTC2 (vitamin c defective 2); GDP-D-glucose phosphorylase/
ATG14580.1	cassava4.1_017950m	ATPRB1;
AT4G13040.1	cassava4.1_015207m	AP2 domain-containing transcription factor family protein [Arabidopsis thaliana]
AT5G10530.1	cassava4.1_021217m	lectin protein kinase, putative; KOG3839;
ATG47710.1	cassava4.1_009293m	serpin, putative / serine protease inhibitor, putative;
CK643217	cassava4030.m1	HSP81-2 (HEAT SHOCK PROTEIN 81-2); ATP binding
CK643135	cassava29272.valid.m1	Glucan endo-1,3-beta-glucosidase precursor, putative [Ricinus communis]
CK643633	cassava37548.valid.m1	acid phosphatase class B family protein
CK652060	cassava43261.valid.m1	VTC2 (vitamin c defective 2); GDP-D-glucose phosphorylase/ GDP-galactose:glucose-1-phosphate guanyltrans
CK641370	cassava18171.valid.m1	Pathogenesis_related Thaumatin
CK644117	cassava18448.valid.m1	CEV1 (CONSTITUTIVE EXPRESSION OF VSP 1);
CK644893	cassava11124.m1	ATP-dependent Clp protease
CK650618	cassava45529.m1	DCL2 (DICER-LIKE 2); ATP binding / ATP-dependent helicase/ RNA binding / double-stranded RNA binding /
CK645537	cassava42330.valid.m1	ATCNGC4 (CYCLIC NUCLEOTIDE-GATED CATION CHANNEL 4); calmodulin binding / cation channel/ c
CK644758	cassava23234.valid.m1	galactolipase/ phospholipase [Arabidopsis thaliana]
CK643796	cassava22662.valid.m1	CB5-D (CYTOCHROME B5 ISOFORM D);
CK652623	cassava36300.valid.m1	ATNSI (NUCLEAR SHUTTLE INTERACTING); N-acetyltransferase (interaction host-virus)
CK643504	cassava18071.valid.m1	PAP3 (PURPLE ACID PHOSPHATASE 3); acid phosphatase/ protein serine/threonine phosphatase
CK641344	cassava11809.valid.m1	EIN2 (ETHYLENE INSENSITIVE 2); transporter
		Oxidation_reduction/Oxidative Stress
BAD30454.1		putative calreticulin interacted protein [Oryza sativa subsp. Japonica]
AT4G35090.1	cassava4.1_006302m	CAT2 (CATALASE 2); catalase; CAT2; [Arabidopsis thaliana]
AAM65092.1		protein-methionine-S-oxide reductase [Arabidopsis thaliana]
ABZ88803.1		glutaredoxin [Hevea brasiliensis]
AT4G33010.1	cassava4.1_000718m	AtGLDP1 (Arabidopsis thaliana glycine decarboxylase P-protein 1)
AT1G12900.1	cassava4.1_008965m	GAPA-2 (GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE A SUBUNIT 2)
AT4G25130.1	cassava4.1_014167m	peptide methionine sulfoxide reductase, putative;
CK643827	cassava19234.valid.m1	peroxiredoxin type 2, putative [Arabidopsis thaliana]
CK647962	cassava30467.valid.m1	oxidoreductase, zinc-binding dehydrogenase family protein
CK641770	cassava37533.valid.m1	VPS2.3
CK652338	cassava43363.valid.m1	Peroxiredoxin. PRX like-2 [Arabidopsis thaliana]
CK645026	cassava23389.valid.m1	NDA1 (ALTERNATIVE NADPH DEHYDROGENASE 1); NADH dehydrogenase [Arabidopsis thaliana]
CK644215	cassava13813.valid.m1	2-oxoglutarate-dependent dioxygenase, putative [Arabidopsis thaliana]
CK643734	cassava3953.m1	CYP714A1 (cytochrome P450, family 714, subfamily A, polypeptide 1); oxygen binding [Arabidopsis thaliana]
CK643531	cassava29807.valid.m1	NADPH putative Ricinus
CK643412	cassava12748.valid.m1	ATY2 (Arabidopsis thioredoxin y2); thiol-disulfide exchange intermediate [Arabidopsis thaliana]
CK644331	cassava35874.valid.m1	BR6OX2 (BRASSINOSTEROID-6-OXIDASE 2) monooxygenase/ oxygen binding [Arabidopsis thaliana]
CK650853	cassava37297.valid.m1	oxidoreductase, 2OG-Fe(II) oxygenase family protein
CK641577	cassava43725.valid.m1	short-chain dehydrogenase/reductase (SDR) family protein
		Cell Wall modification/organization/biosynthesis
AT3G59010.1	cassava4.1_004607m	pectinesterase family protein;
AT3G21140.1	cassava4.1_008259m	ATPRP2 (PROLINE-RICH PROTEIN 2); ATPRP2;
AT3G06260.1	cassava4.1_027013m	GATL4 (Galactomonosyltransferase-like 4);
CK643857	cassava19459.valid.m1	ATEXPA1 (ARABIDOPSIS THALIANA EXPANSIN A4) [Arabidopsis thaliana]
CK647714	cassava42239.valid.m1	ATPME1; pectinesterase
CK652711	cassava42803.valid.m1	UGE2 (UDP-D-glucose/UDP-D-galactose 4-epimerase 2) [Arabidopsis thaliana]
CK644988	cassava6940.valid.m1	caffeoyl-CoA 3-O-methyltransferase, putative
		Photosynthesis
ATIG61520.1	cassava4.1_013684m	LHCA3; chlorophyll binding; LHCA3; K08909;
AT3G61470.1	cassava4.1_013916m	LHCA2; chlorophyll binding; LHCA2; K08908;
ATSG54270.1	cassava4.1_014117m	LHCB3 (LIGHT-HARVESTING CHLOROPHYLL B-BINDING PROTEIN 3);
AT5G38430.1	cassava4.1_017243m	ribulose bisphosphate carboxylase small chain 1B /
AT5G38410.2	cassava4.1_018735m	ribulose bisphosphate carboxylase small chain 3B /
ATIG79040.1	cassava4.1_018756m	PSBR (photosystem II subunit R); PSBR;
AT2G30570.1	cassava4.1_019040m	PSBW (PHOTOSYSTEM II REACTION CENTER W); PSBW;
ATIG54780.1	cassava4.1_012916m	thylakoid lumen 18.3 kDa protein; PF04536;
AT2G39730.2	cassava4.1_007872m	RCA (RUBISCO ACTIVASE); ADP binding / ATP binding /
ATSG66570.1	cassava4.1_013817m	PSBO1 (PS II OXYGEN-EVOLVING COMPLEX 1)[Arabidopsis thaliana]
ATIG67090.1	cassava4.1_017170m	RBCS1A (RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL CHAIN
AT2G34430.1	cassava4.1_014040m	LHB1B1; chlorophyll binding; LHB1B1;
AT2G39730.2	cassava4.1_006803m	RCA (RUBISCO ACTIVASE); ADP binding / ATP binding / enzyme
AT2G20260.1	cassava4.1_018492m	PSAE-2 (photosystem I subunit E-2); catalytic; PSAE-2;
AT4G05180.1	cassava4.1_015521m	PSBQ-2; calcium ion binding; PSBQ-2;
ATIG52230.1	cassava4.1_018595m	PSAH2 (PHOTOSYSTEM I SUBUNIT H2); PSAH2;

ONGOING WORK

-QTL mapping approach to identify the genetic basis for cassava's quantitative resistance to *A. socialis*.

-Real-Time PCR validation of candidate genes.

ACKNOWLEDGMENT

-We acknowledge Gines-Mera fellowship for support the Ph.D studies and Fausto Rodriguez for his technical support.