Outbreak Causes and Sustainable Solutions for African Cassava Whitefly in Malawi, Tanzania and Uganda

CIAT - Period 7

Start October-01-2017, End March-31-2018

1-Phenotypic characterization data (8 evaluation cycles) of CM8996 and GM8586 families for whitefly (*Aleurotrachelus socialis*) resistance in greenhouse available (CIAT)

Genotypes: CM8996 progeny ECU72♀ (Whitefly Resistant, WFR) x COL2246 ♂ (Whitefly Susceptible WFS) (240 F1s). GM8586 progeny ECU72♀ (WFR) x TMS60444 ♂ (WFS) (201 F1s). Other checks: PER368, PER415, PER317, PER608 (WFR), PER335 (WFR), COL1468 (WFS), TME3 (WFS) PER183 (WFS) and ECU183 (WFS).

We used these materials that came from stakes, in vitro plants, and micro-stakes, in order to know if there are differences in the response depending on the propagation method.

Experimental procedure:

Bioassay using cassava plants stakes and *in vitro*, were planted for 8-10 weeks in sterile "rich" soil in a ratio of 3:1 black soil (no clay topsoil): sand, and sterile "poor" soil in in a ratio of 3:1 sand: black soil (no clay topsoil). Black plastic bags were using for sowing (10 cm W x 15 cm H). Also large white mesh cages (18 m L x 3 m W x 3 m H) located in the greenhouse, were used in this study. One month later, plants infested with adult whiteflies of *A. socialis* from colony, were used for infest the trial. Infested leaves 1 and 2 of each treatment are collected to the 34-36 days post-infestation. Response: Total number of nymphs III and IV, and percentage of affected leaf (Figure 1).

Experimental Design: For phenotyping of CM8996 and GM8586 families, for resistance to *A. socialis* in greenhouse, the choice experiments and randomized complete block, were used (Table 1).

Table 1. Summary of Whitefly phenotyping choice experiments

Families + checks	Year	Propagation method	Substrate	#replicas (offspring)	#replicas (checks)
CM8996	2013	in vitro	2:1 sand:soil + peatmoss	2	8
CM8996	2016	Stake	3:1 sand:soil	6	24
CM8996	2017	Stake	1:3 sand:soil	6	24
CM8996	2017	in vitro	2:1 sand:soil + peatmoss	6	24
GM8586	2015	Micro-stakes	Peatmoss	6	24
GM8586	2017	Stake	1:3 sand:soil	6	24
GM8586	2018	in vitro	2:1 sand:soil + peatmoss	6	24
CM8996	2018	in vitro	2:1 sand:soil + peatmoss	6	24

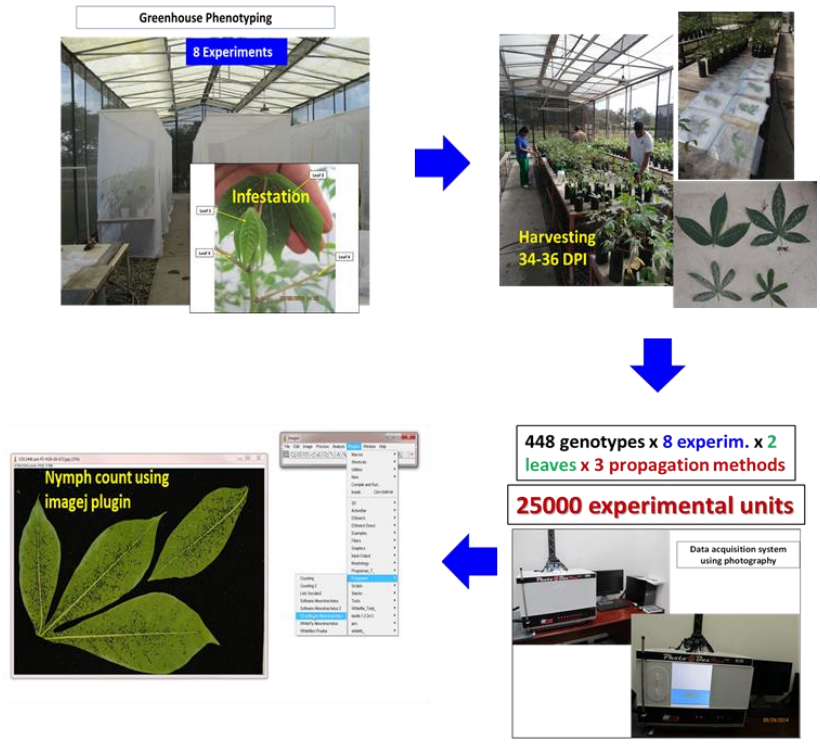

Figure 1. Workflow of Phenotyping methodology of whitefly resistance in cassava

Acquisition data:

We developed an automated system of assessment of plant resistance to *A. socialis* whitefly based on image processing techniques. Images are taken by using an acquisition protocol and processed in ImageJ (ImageJ, National Health Institute, Bethesda, USA), by a method of nymph counting developed as a plugin.

The method includes three main steps, pre-processing, processing and data acquisition which are applied to each image. Preprocessing step is used to two tasks one to filter noise and delete background aiming to remove undesired data and ease the processing. And two to obtain a duplicate image with a completely clean leaf. The processing step identify the objects desired (nymphs) which are then filtered by shape and size, as some nymphs are connected to adjacent ones, we used an algorithm to separate and segment them. Afterwards, the preprocessed image on the second task is merged with the processed one. Finally, in the data acquisition is obtained the number, area in pixels, and percentage of nymphs, as well as the percentage of the leaf. All of this using the same final image which is saved in JPG format.

Images could be assessed individual or a series of images (batch). For images in batch is necessary to choose two different folders, the source folder which contain original images and the destination folder which processed images and data analysis will be stored. The processed of the images is automatic, once it is chosen the two folders, it works by itself processing and saving the images one by one, at the same time that is storing the information of the count in an excel file. The time consumption of image processing in batch, depends of the number of pictures contain on the source folder, to analyze one picture, it takes 35 seconds, according to that, to analyze 450 images, it can takes, about 15750 seconds it means 4,38 hours. Using this system we evaluated a total of ~ 25000 photos from all phenotyping trials in ~ 14 days (Table 2).

Aiming to improve the system of evaluation of whiteflies resistance around the world, we are testing a new method for assessing the resistance *Bemisia tabaci* whiteflies using the same photographic integration of the system for *A. socialis*. These methodologies were shared with project partners and students, in the Stakeholder and Technical Training Meeting (Esella Country Hotel, Kampala, Uganda 14th to 19th June 2015) and, in the Technical Training Meeting (MARI, Dar es Salaam, Tanzania, 14th to 19th June 2017).

The adjusted means of nymphs and percentage of affected leaf, obtained previously with our methodology, are quantitative measures of the characteristic of resistance to whitefly in families CM8996 and GM8586, their parental, and the other checks. A manuscript is being prepared with the Phenotyping methodology including the plugin developed for the counting of nymphs of *A. socialis*.

Table 2. Consolidated phenotypic data

STAT	N
Insects	22,114,188
Pictures	25000
Leaves	23,798
Plants	8,852
Experiments	5 / 3
Crosses	CM8996 / GM8586

Statistical Analysis:

For the statistical analysis of the data is proposed at first place the behavior analysis of the response variable which are the counts of the whitefly nymphs on the leaves. Given the nature of these variables (counts), it is important to emphasize that this discrete variable is usually associated with probability models such as the Poisson and Negative Binomial ideal for counts. As part of the fit assessment process, four models were analyzed and compared with respect to the frequencies observed as shown in Figure 2. We can highlight that the model with the best graphic fit is the Negative Binomial. Additionally, the statistical goodness of fit test (Kolmogorov-Smirnoff test) corroborates how well this result fits.

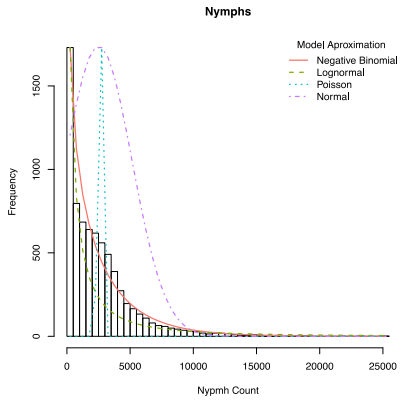

Figure 2. Histogram of frequency for variable number of nymphs and fit of probabilistic models

On the other hand, it is important to emphasize that the data we are taking comes from counts at spatial level of the affected leaves and we found that spatial aggregation patterns are very associated with some

types of probability models such as Poisson or Negative Binomial. In our case, most of the evaluated leaves presented spatial patterns of aggregation in the appearance of the nymphs on the leaf and, therefore, is another reason for the use of the Negative Binomial model (Figure 3).

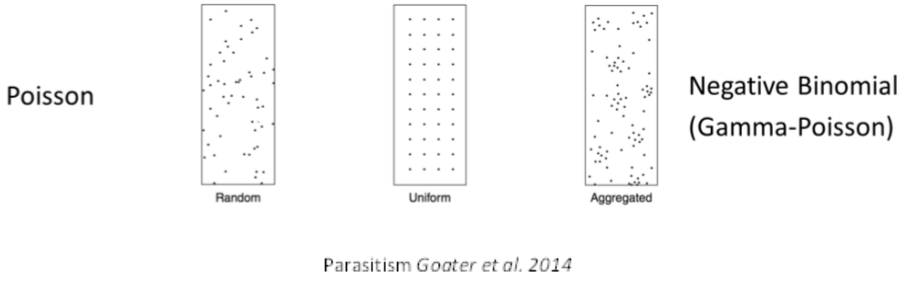

Figure 3. Patterns of spatial aggregation of species

Once identified that our response variable has a good fit to the Negative Binomial probability model, and considering that part of our objectives are to evaluate the differences between genotypes according to the counts of nymphs to identify the materials with greater resistance to the white fly. Hence, is proposed the use of generalized linear models (GLM for its acronym in English) which look upon this type of characteristics in statistical modeling.

After fit the generalized linear model with Negative Binomial function, we used the adjusted means in the comparisons of the genotypes for each essay handed in the different eras and types of the soil substrate used. The comparisons of the adjusted means are shown below:

Figure 4 shows the correlation and dispersion matrices for the two families CM8996 (left) and GM8586 (right). From here we can emphasize that the correlations between the results of the tests by period and type of substrate are generally low. This indicates that the assays have a low level of reproducibility and this can be associated mainly with the use of different types of substrate which generate significant differences in the levels of infestation (Figure 6). It is also important to note that in this case all the genotypes of the families are compared and it is possible that some of them present a better performance in terms of heritability separately. However, it should be noted that heritability tends to be less than 30% in fitness traits, as is the case of resistance to pests and diseases (Visscher et al, 2008).

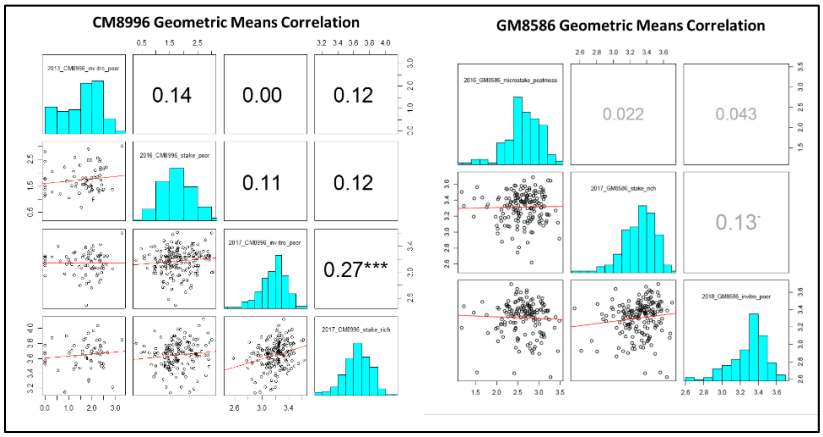

Figure 4. Offspring reproducibility and Heritability

Continuing with the analysis of reproducibility and heritability we perform an analysis only for the subset of data that corresponds to the control genotypes (resistant and susceptible). In Figure 5 we can highlight that the Pearson correlation indicators and the scatter diagrams now show stronger positive correlations between trials, that is to say that the adjusted means between genotype tests are conserved or have the same hierarchical order. It is important to note that the "N" of the checks is larger than the "N" of the progenies. These correlations are higher in cases where the type of substrate is the same, which shows us that the control genotypes have greater heritability and that this condition is even maintained between the periods and types of propagation: stake, micro-stake and in vitro, but it is altered a little when the substrates change.

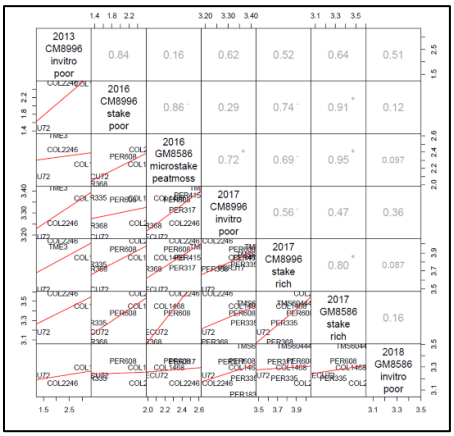

Figure 5. Checks reproducibility and Heritability

In order to contrast the levels of infestation between substrates (rich and poor) we conducted an analysis of all the trials (Figure 6) where we found that the levels of infestation for trials with poor substrate are significantly lower (better at 3000 counts per leaf) while in the rich substrate the levels of infestation far exceed the threshold of 3000 and even come to present twice as many genotypes. Low infestations have the data more normally distributed than high infestations.

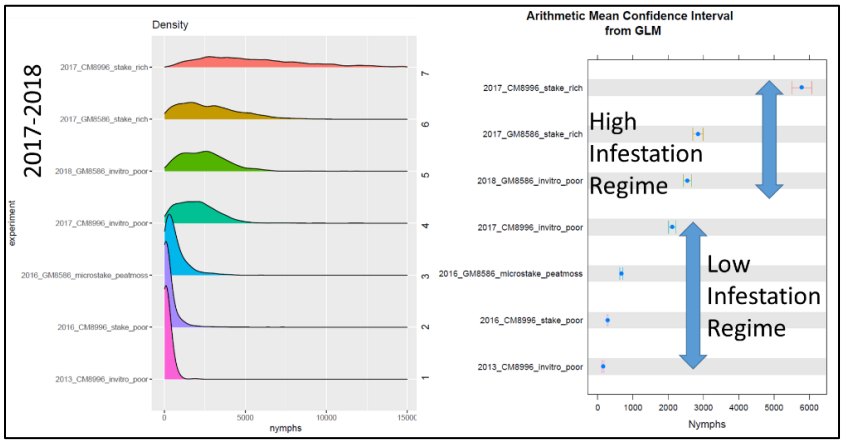

Figure 6. Offspring Density and Arithmetic Mean confidence interval from GLM

We can also observe in Figure 7 through the comparative analysis of adjusted means only for control genotypes that present significant differences but their levels do not reach as wide as those of the types of substrates. However, it is possible to verify how genotypes that are resistant have lower levels of counts than susceptible ones.

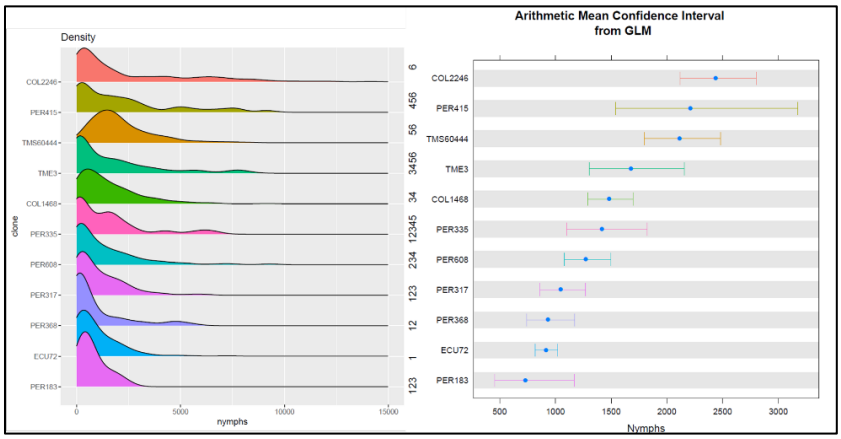

Figure 7. Checks Density and Arithmetic Mean confidence interval from GLM

On the other hand, comparisons of adjusted means were performed for each genotype in the low and high infestation scenarios using a general linear model ANOVA (under normality) and a generalized lineal one (the one proposed with a negative binomial) with the purpose of identifying the benefits that It brings the use of a more appropriate model for the type of data as we have explained previously. In Figure 8 we can show that the model under normality (ANOVA left side) has very little resolution to differentiate the genotypes according to their level of infestation while the negative binomial model gives us a wide resolution and allows to identify greater differences between the resistant and susceptible genotypes. Also the levels of infestation influence the resolution of the different resistance categories, as can be seen in figure 8A, at low levels of infestation (<2000 nymphs / leaf) the resolution of the resistance categories is higher compared to high levels of infestation (> 2000 nymphs / leaf), shown in Figure 8B.

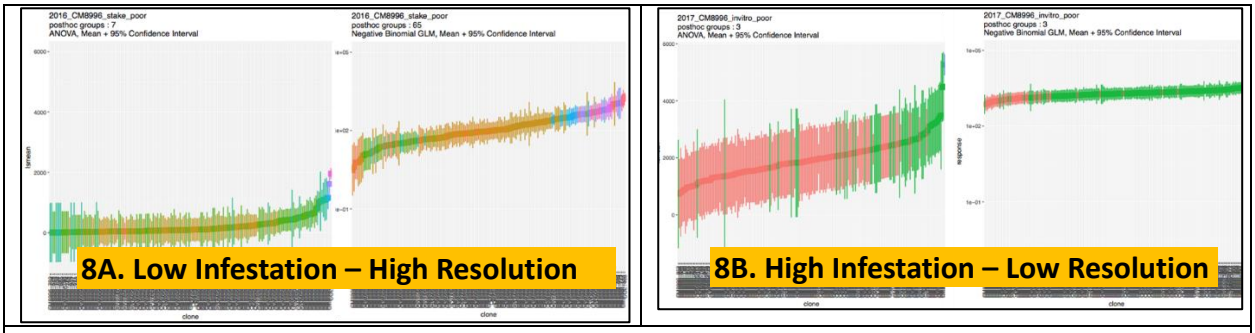

Figure 8A and 8B. Effect of the level of infestation on the resolution and separation of statistically significant groups (shown in different colors) and, differences in resolution using ANOVA vs. negative binomial GLM

Finally, in Figure 9 the results of the analyzes are presented using a BLUPs (Best Linear Unbiased Prediction) estimator which has advantages from the statistical point of view when transforming the data, guaranteeing the normality in the values of the BLUPs, which is something desired for the subsequent

analysis of QTLs since this essentially runs a series of linear regressions which have greater statistical power under normality.

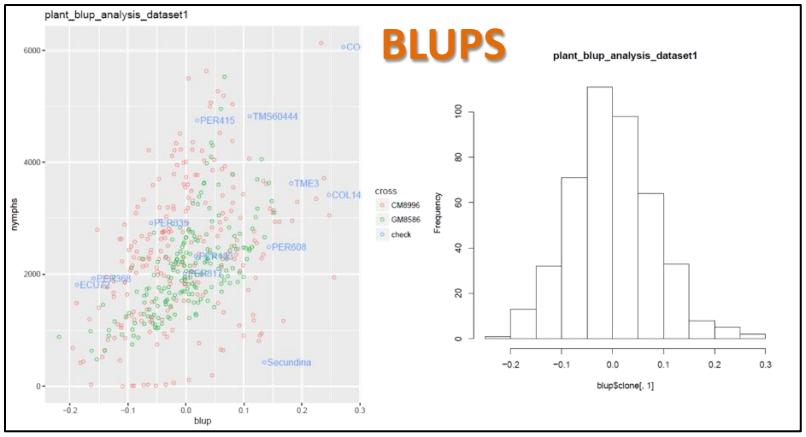

Figure 9. BLUPs Analysis (Best Linear Unbiased Prediction) in CM8996 and GM8586 segregation families

2- CM8996 and GM8586 high resolution linkage map (CIAT)

Linkage Map of Cassava CM8996 Whitefly Resistance segregating family: Illumina sequences obtained from an EcoRI RAD-seq libraries of 218 F1 individuals plus parental lines ECU72 and COL2246 were used for implementing a variant discovery pipeline based upon the Broad Institute Genomic Analysis Toolkit (GATK) best practices. The pipeline included preprocessing, base recalibration and joint genotyping of the variants found. Additionally to read quality (>10), mapping quality (>40), genotype quality (>30) and missing genotypes filters (< 0.2), a mendelian segregation (Chi squared p<0.05) and a spacing filter (no SNPs nearer than 50kb in the Phytozome v6.1 assembled sequence) were applied. This lead to 2376 high quality, > 50kb spaced, markers. A kinship coefficient analysis on these markers allowed us to discard an individual whose maternal origin is not ECU72, thus having a final segregating population of 217 individuals. In the previous version (March 2016) of the linkage map a linkage group remained a chimeric union of Chr01 and Chr02 markers. The grouping of these markers was resolved into the expected Chr01 and Chr02 linkage groups using a minimal spanning tree algorithm (LOD> 12, rf <0.4) implemented with OneMap and the R igraph and visnetwork libraries requiring minimal user intervention compared to the JoinMap semi-automated hierarchical LOD/rf method. 2373 makers were placed in 18 linkage groups and this grouping was used as the basis for a multilocus maximum likelihood estimation of marker order using JoinMap 4.1 with Haldane distance and default search parameters. The resulting map has 245 cM average length per linkage group, excluding the Chr02 group (87 markers) that has an extremely inflated length of 5329 cM. This length inflation is due to a cluster of 14 COL2246 markers with genetic distances around 10k cM in the parental map, while the maternal ECU72 marker map has a length of 250 cM. These problematic markers along with the previous chimeric group found in March, might be evidence of a real cytogenetic anomaly in COL2246, like a telomeric translocation from Chr02 to Chr01, but further analysis is needed to discard some kind of artifact. Overall this map is congruent with the pseudomolecule order

based on the consensus genetic map from the International Cassava Genetic Map Consortium and integrates previous unlinked scaffolds.

Table 3. CM8996 map statistics

STAT	Value
Cross	ECU72 x COL2256
Seq	RAD EcorI
GATK Strict Filter SNPs	2,898,303
Mendelian	11,803
Segregating	10,659
LD filtered (clusters r^2>0.95)	2,326
Mapped	2,320

Linkage Map of Cassava GM8586 Whitefly Resistance segregating family

Sequencing and GATK based SNP calling: Illumina based whole genome shotgun sequencing of ECU72 and TMS60444, plus 196 offspring was carried by Novogene, California, USA. Reads were trimmed 3 bp from the start. Preprocessing of WGS clean and trimmed reads were mapped to the JGI Cassava v6.1 genome, duplicates marked with Picard. No variant recalibration was attempted as no reference variant set was available at the time. Haplotype Caller was run on each sample and single sample vcfs were obtained and split into chromosome and scaffolds files for improved computational time. A joint genotyping with the whole family set was made with genotype GVCF, adding Allele Balance vcf annotation. A hard filter was applied to the vcf files at the variant level as recommended by the GATK development team for non-model species. Only biallelic SNPs were taken into account from this point on. After this a genotype level filter for minimum depth (DP > 6) and quality (GQ >21) was applied, filtered genotypes set to missing, and finally a set of variants with missing data below 20% was picked using Select Variants.

Linkage analysis: Kinship analysis showed no need to discard any samples due to being unrelated to the parental lines. Markers with undistorted mendelian segregations (Xi^2 p>0.05) were selected. From this set markers at least 50 kb apart were used for mapping. Once the set of 50 kb separated mendelian segregating markers for both GM8586 family was established, the data was encoded as cross-pollination (cp) population for JoinMap linkage mapping via perl script. As the hierarchical grouping of markers in the JoinMap software was a labor intensive and manually iterative process we decided to use more advanced grouping approaches.

First the grouping of the markers was carried out using the minimal spanning tree of the two point recombination fraction graph between all markers using the igraph R package, specifying recombination fraction (0.4) and LOD (12) threshold as filters for selecting graph edges. This approach resulted in identical results with the grouping algorithm of OneMap. However the use of igraph in R allowed us to manually inspect the resulting minimal spanning tee of the recombination graph through visNetwork generated graphics. Linkage groups and markers were coded into .loc and .map files respectively. From this input, marker order and phases were determined with JoinMap 4.1 map function using default parameters.

Table 4. GM8586 map statistics

STAT	Value
Cross	ECU72 x TMS60444
Seq	WGS
GATK Strict Filter SNPs	26,959,292
Mendelian	531,114
Segregating	435,873
LD filtered (clusters r^2>0.95)	2,945
Mapped	2,943

Table 5. GM8586 WGS based linkage map and pseudomolecule lengths by linkage group. n markers takes into account just the SNPs that mapped to the expected chromosome

Group	n markers	Map length (cM)	Intermarker mean map distance (cM)	Intermarker mean pseudomolecule	Map vs Pseudomolecule correlation coefficient
Chr01	971	724	0.75	786630	0.93
Chr02	646	581	0.9	935975	0.96
Chr03	724	468	0.65	817283	0.92
Chr04	1022	725	0.71	1163374	0.96
Chr05	978	583	0.58	1248323	0.92
Chr06	810	620	0.77	555937	0.68
Chr07	901	766	0.81	835712	0.98
Chr08	970	558	0.58	1023133	0.98
Chr09	1055	785	0.75	675211	0.98
Chr10	920	837	0.91	606253	0.96
Chr11	666	744	1.07	574904	0.97
Chr12	980	751	0.77	934320	0.98
Chr13	862	659	0.77	1128788	0.96
Chr14	817	729	0.89	746365	0.92
Chr15	769	426	0.52	676561	0.93
Chr16	587	358	0.61	630651	0.98
Chr17	862	537	0.62	1064501	0.97
Chr18	805	536	0.67	1593783	0.7
Total	15345	11389	0.74	899850	

3-CM8996 and GM8586 segregation families QTL mapping of white fly resistance (CIAT)

The high resolution linkage map of CM8996 and GM8586 were the basis for QTL mapping for whitefly resistance trait: nymph count, with resistance data (Negative binomial GLM and BLUPs analysis) collected for both families. For the CM8996 progeny an interval mapping analysis with default parameters in MAPQTL 6, followed by permutation tests for a significance of $p < 0.05$ resulted in the detection of a QTL in Chr10 for nymph count with a LOD of 5.1. The interval surrounding the QTL spans 733 kb between the markers Chr10_24788995 and Chr10_25522305, and contains 87 genes, some with biological process related to resistance like a NBS-LRR cluster and different transcription factors. Other candidates of note just below statistical significance can be located in Chr14 and could be confirmed when complete genotype data (around 220 individuals) is collected.

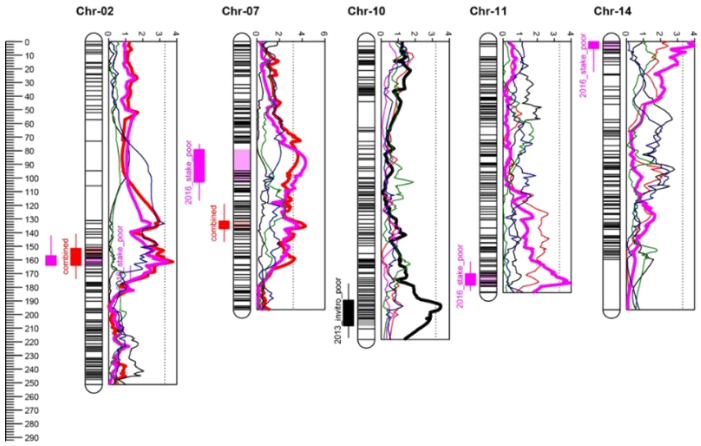

Figure 10. Linkage Map of Cassava CM8996 Whitefly Resistance segregating family and QTL analysis. Linkage group numbers assigned according to JGI assembly pseudomolecules (version 6.1)

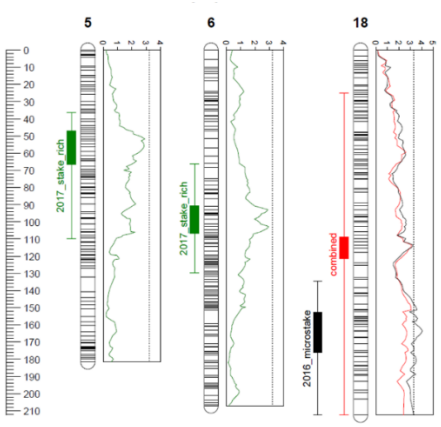

Figure 11. Linkage Map of Cassava GM8586 Whitefly Resistance segregating family and QTL analysis. Linkage group numbers assigned according to JGI assembly pseudomolecules (version 6.1)

Table 6. Significance QTLs summary

Column1	Significant peal	Column2	Chromosome wide Nymph Count QTL (Interval Mapping single (	Column3	Column4	Column5	LOD	alpha 0.05	Column6	Column7	Column8
QTL	Boundary	Cross	Experiment	Group	Position	Locus	marker	threshold	type	r2	Trait Model
1	start	CM8996	combined evidence	Chr02	161.413	Chr02_11524926	3.76	3.3	CW	8.2	BLUP
2	start	CM8996	combined evidence	Chr07	135.296	Chr07_21079613	4.3	3.1	CW	9.4	BLUP
2	end	CM8996	combined evidence	Chr07	134.305	Chr07_20625417	4.17	3.1	CW	8.8	BLUP
3	start	CM8996	2013_invitro_poor	Chr10	193.701	Chr10_25037934	3.49	3.2	CW	18.9	Marginal BLUP
3	end	CM8996	2013_invitro_poor	Chr10	192.707	Chr10_25019060	3.47	3.2	CW	19	Marginal BLUP
4	start	CM8996	2016_stake_poor	Chr02	161.413	Chr02_11524926	3.45	3.3	CW	9.5	Marginal BLUP
5	start	CM8996	2016_stake_poor	Chr07	94.573	Chr07_8996867	4.01	3.2	CW	11.2	Marginal BLUP
5	end	CM8996	2016_stake_poor	Chr07	96.326	Chr04_10204535	3.94	3.2	CW	10.5	Marginal BLUP
6	start	CM8996	2016_stake_poor	Chr11	176.834	Chr11_26204672	3.89	3.3	CW	10.5	Marginal BLUP
6	end	CM8996	2016_stake_poor	Chr11	177.106	Chr11_26167473	3.85	3.3	CW	10.4	Marginal BLUP
7	start	CM8996	2016_stake_poor	Chr14	3.149	Chr14_1191580	3.98	3.3	CW	10.7	Marginal BLUP
7	end	CM8996	2016_stake_poor	Chr14	3.149	Chr14_1577523	3.98	3.3	CW	10.7	Marginal BLUP
8	start	GM8586	2016_microstake	Chr18	163.524	Chr18_13208277	4.08	3.3	CW	11.2	Marginal BLUP
8	end	GM8586	2016_microstake	Chr18	163.837	Chr18_23355991	4.06	3.3	CW	11.2	Marginal BLUP
10	start	CM8996	2017_stake_rich	Chr15	69.252	Chr15_8642404	3.35	3.3	CW	9.3	GLM (linear predictor)
11	start	CM8996	2017_stake_rich	Chr18	89.813	Chr18_11137154	3.47	2.8	CW	9.6	GLM (linear predictor)
11	end	CM8996	2017_stake_rich	Chr18	89.667	Chr18_11081517	3.35	2.8	CW	9.3	GLM (linear predictor)
12	start	GM8586	2017_stake_rich	Chr05	51.728	Chr05_8538681	3.43	3.3	CW	8.3	GLM (linear predictor)

4-Transcriptome and Metabolome data-base established (UCR, RHUL, CIAT).

Time course bioassays for 12 genotypes (0 hr, 8 hr, 24 hr, 7 d, 14 d, and 21 d) were performed with three biological replicates, 216 RNAs were extracted and sent to UCR for transcriptome profiling using RNA-seq (Figure 10, Table 7). During this study, we have developed an RNA extraction methodology, and we have submitted the manuscript: "An optimized isolation protocol produces high functioning RNA from cassava tissues (*Manihot esculenta* Crantz)" to FEBS Open Bio Journal. Data analysis is being performed at UCR and results from this were shared at the annual meeting. Also, the same tissues were lyophilized and sent to Royal Holloway to undertake metabolomics profiling that will complement the information from the transcriptomic data.

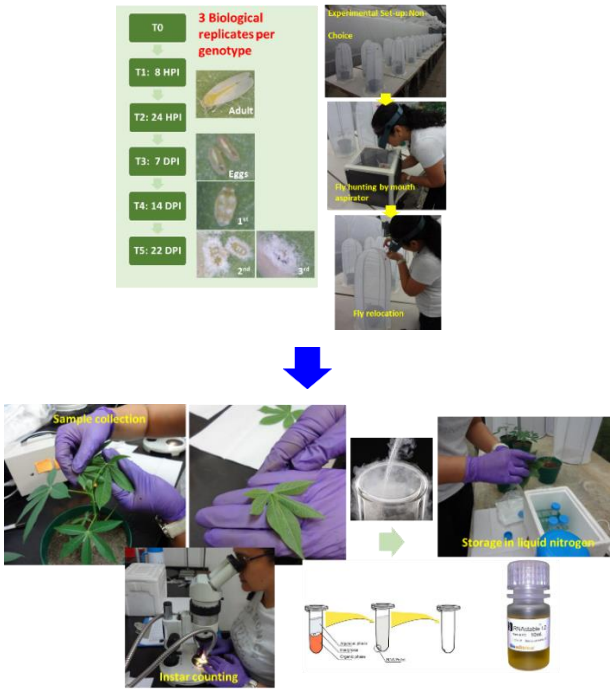

Figure 12. Schematic outline of experimental work for Transcriptomic and Metabolomics analysis

Table 7. Summary of the RNAs sent to UCR from the non-choice experiments for the establishment of the transcriptome of the resistance to whitefly in cassava

Genotype	WF Resistance status	#RNAs/3 Biological
ECU72	WFR	18
COL2246	WFS	18
TMS60444	WFS	18
PER368	WFR	18
PER335	WFR	18
PER415	WFR	18
PER317	WFR	18
PER608	WFR	18
COL1468	WFS	18
TME3	WFS	18
PER183	WFS	18
ECU183	WFS	18
TOTAL RNAs		216

Time-course bioassays for CM8996 and GM8586 segregation families (0 hr, 24 hr, and 14 d) with two biological replicates were performed in greenhouse and were sent to Royal Holloway for metabolomics studies. These activities are up to 100% completion.

Leaves tissues from ECU72 and COL2246 treated separately with jasmonic acid (JA) and salicylic acid (SA) (CIAT-UCR):

Based on preliminary transcriptome data analysis conducted on CIAT's WF bioassays by UCR, it indicated that the effect SA and JA have on resistant ECU72 and susceptible COL2246 basal transcriptome profile could help to make full use of the data generated by the time-course bioassays with *A. socialis* (0 hr., 0.5 hr., 1 hr., 2 hr., 8hr, 12 hr. and 24 hr.). Leaves tissues from ECU72 and COL2246 treated separately with jasmonic acid (JA) and salicylic acid (SA) were collected for each biological replicate (3X). Ninety RNAs were sent to UCR. This activity is 100% completion.

5-Collaboration with NRI

Latin-American genotypes whitefly resistant and susceptible were multiplied *in vitro* and sent to NRI for bioassays with *Bemisia tabaci*. This activity is 100% completion. (Table 8).

Table 8. Summary of Latin-American genotypes sent to NRI

item	Accession	Synonyms	Common names	Genus	Species	Biological status	Country	Containers
1	ECU72	072	Injerta	*Manihot*	*esculenta*	Landrace	Ecuador	70
2	COL2246	COL2246	Lengua De Pisco	*Manihot*	*esculenta*	Landrace	Colombia	70
3	NGA11	TMS60444	Tms60444	*Manihot*	*esculenta*	Improved-Line	Nigeria	10
4	PER368	UNPRG50	Imacita T.4	*Manihot*	*esculenta*	Landrace	Perú	10
5	PER335	UNPRG8	Amaril.D Tejedores	*Manihot*	*esculenta*	Landrace	Perú	10
6	PER415	UNPRG112	Imacita T.3	*Manihot*	*esculenta*	Landrace	Perú	10
7	PER317	/	/	*Manihot*	*esculenta*	Landrace	Perú	10
8	PER608	UNPRG186	Poblana	*Manihot*	*esculenta*	Landrace	Perú	10
9	ECU19	019	Amarilla	*Manihot*	*esculenta*	Landrace	Ecuador	11
10	ECU64	064	/	*Manihot*	*esculenta*	Landrace	Ecuador	14
11	ECU145	145	Taureña	*Manihot*	*esculenta*	Landrace	Ecuador	11
12	PER273	/	Montañera de Tumbes	*Manihot*	*esculenta*	Landrace	Perú	11
13	PER330	UNPRG3	Bandurria	*Manihot*	*esculenta*	Landrace	Perú	13
14	PER334	UNPRG7	Conga de Piura	*Manihot*	*esculenta*	Landrace	Perú	11
15	BRA375	BRA-061433	Sutinguinha	*Manihot*	*esculenta*	Landrace	Brasil	12
16	PER183	/	Eeat 1	*Manihot*	*esculenta*	Landrace	Perú	36
17	ECU183	/	/	*Manihot*	*esculenta*	Landrace	Ecuador	41

Key Milestone Deviation: No applicable

Course Correction:

Plans for Next Reporting Period: Accordingly to the agree plan

Risks:

Sustainability: Our current work is progressing as expected.

Scalability: We anticipate that by the end of this reporting cycle we will be in position to offer new avenues toward strengthening potential product development as split over of our work. As we approach the identification of candidate genes the transgenic approach for profiling gene expression.

Lessons Learned: Our monthly meeting between UCR, RHUL, NRI and CIAT has facilitated progress towards milestones