



African Cassava Whitefly Project: towards whitefly resistance in cassava: Discovery, development, and strategy

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Cassava Globally

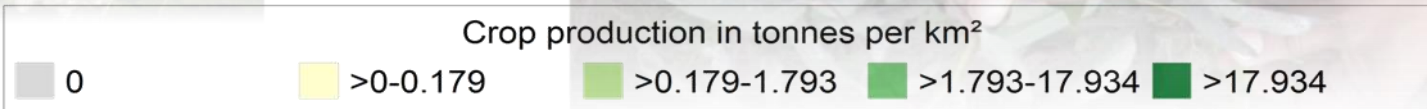
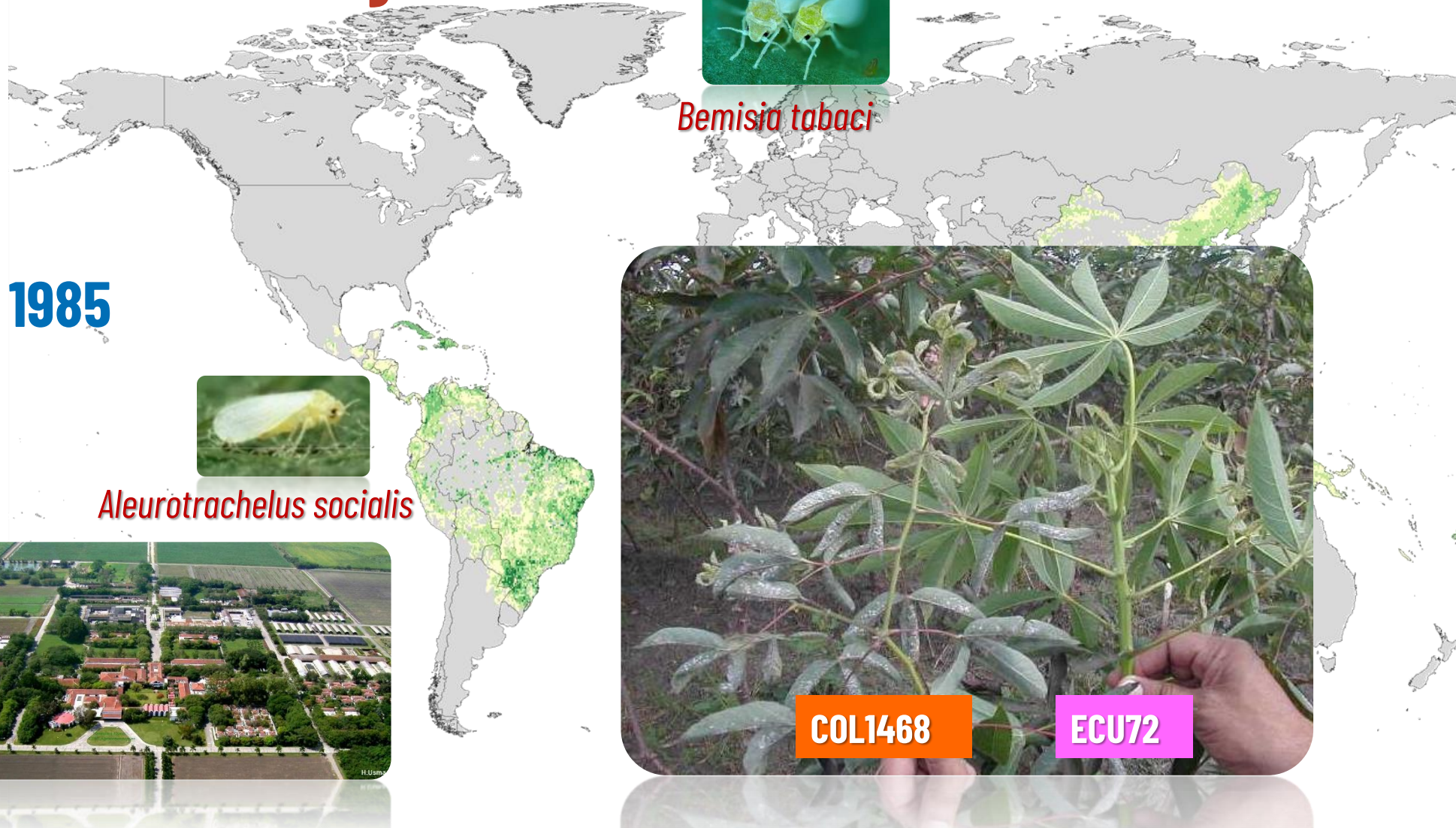
1985



Bemisia tabaci



Aleurotrachelus socialis



95% quantile (q_{95}) of the world presence area=17.93425

Source: Monfreda et al. (2008)

African Cassava Whitefly Project (ACWP)

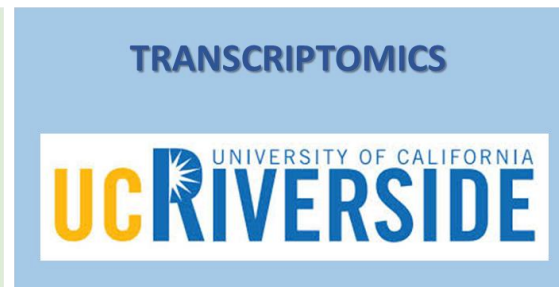
Phase I & II



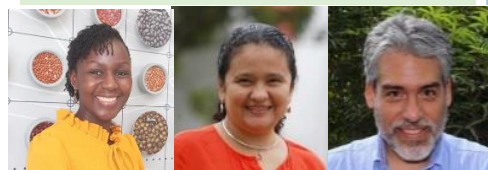
Solutions for the African cassava whitefly (ACW) problem in the East African countries, Uganda, Tanzania and Malawi and achieve substantially reduced ACW populations and increased cassava yields in the farmers' fields, a strategic partnership was created in 2013 by Gates Foundation, NRI, including global partners.

We are developing, validate and pass on whitefly-control technologies including:

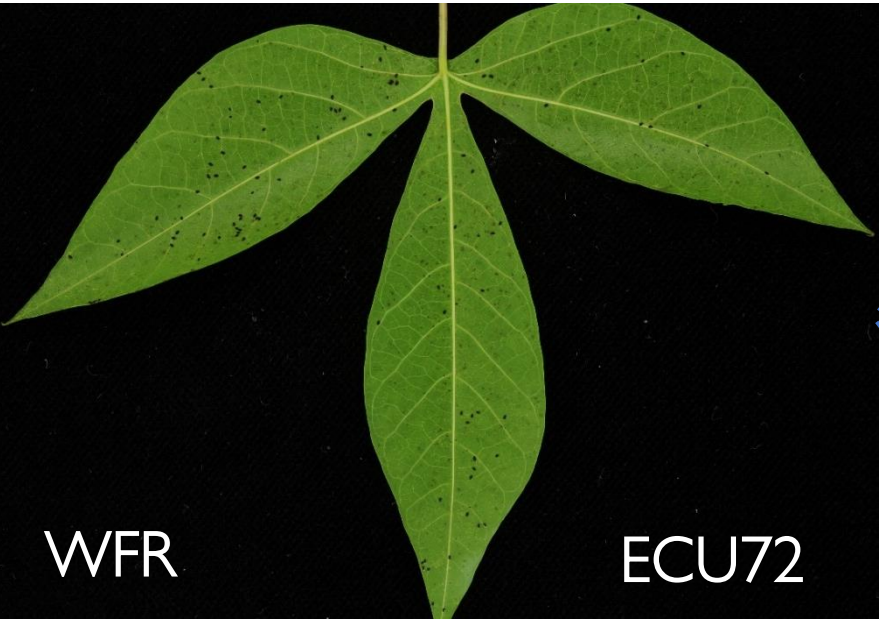
- (i) whitefly resistant (WFR) varieties, unravel the mechanism of action
- (ii) pre-breeding genotypes with Latin American (LA) WFR genes,
- (iii) molecular markers WFR (both genetic and biochemical).



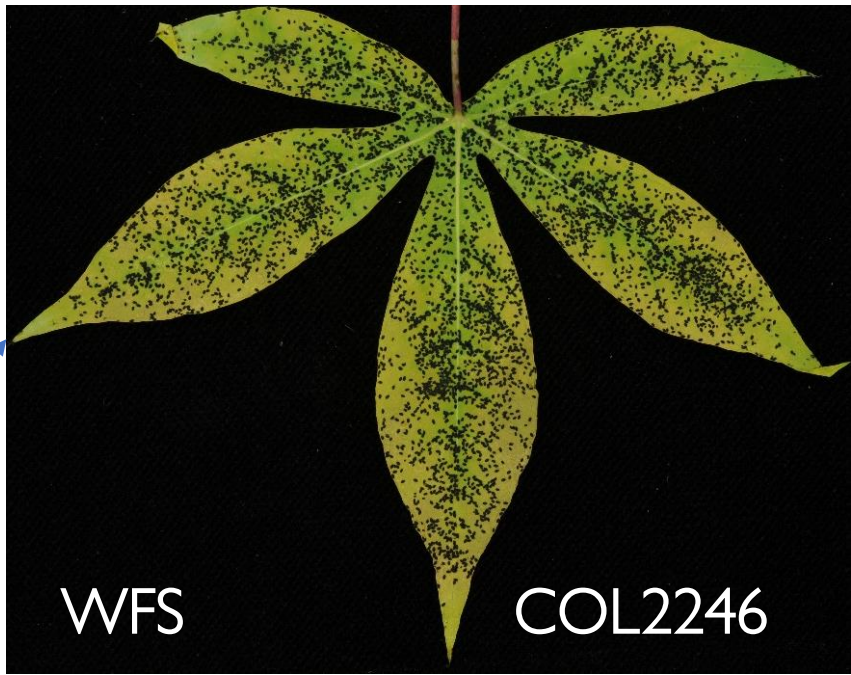
BILL & MELINDA
GATES foundation



Whitefly Resistant Variability in Cassava



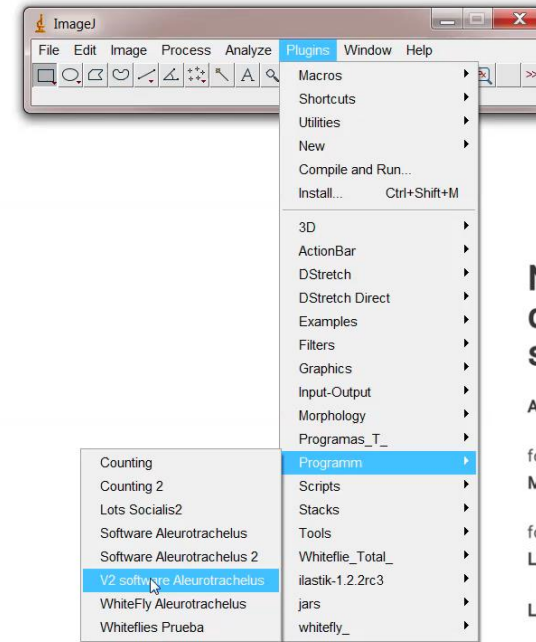
X



X



Phenotyping using: Plugin “Nymphstar” for *Aleurotrachelus socialis* on Cassava



Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

NYMPHSTAR: an accurate high-throughput quantitative method for whitefly (*Aleurotrachelus socialis* Bondar) resistance phenotyping in cassava

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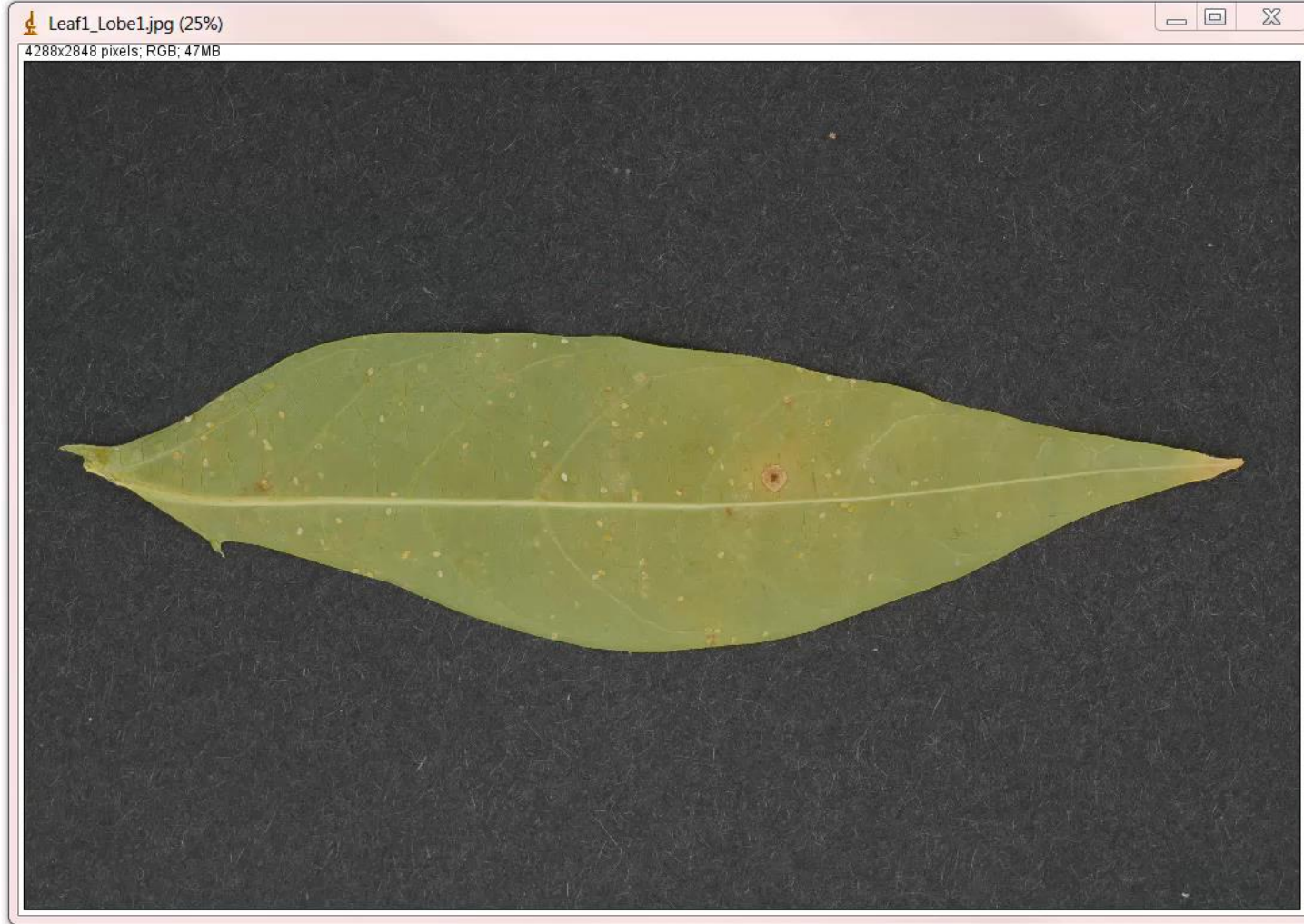
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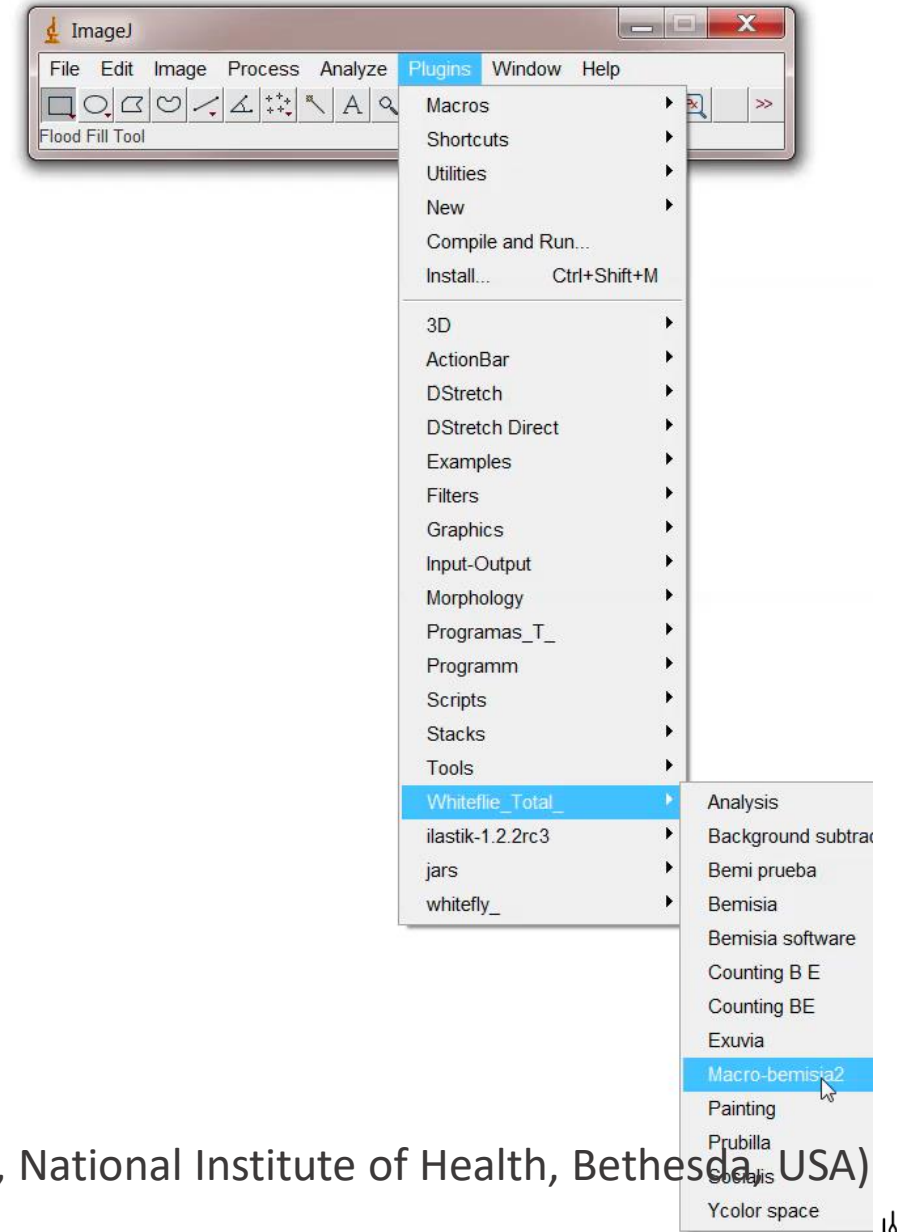
ImageJ (ImageJ), National Institute of Health, Bethesda, USA)

Preprint: [NYMPHSTAR: an accurate high-throughput quantitative method for whitefly \(*Aleurotrachelus socialis* Bondar\) resistance phenotyping in cassava | Research Square](#). This preprint had been accepted in *The Plant Phenome Journal*

CIAT's approach phenotyping *Nymphstar*

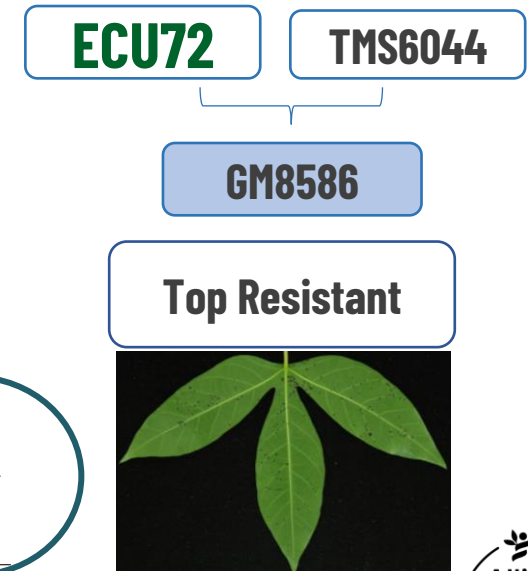
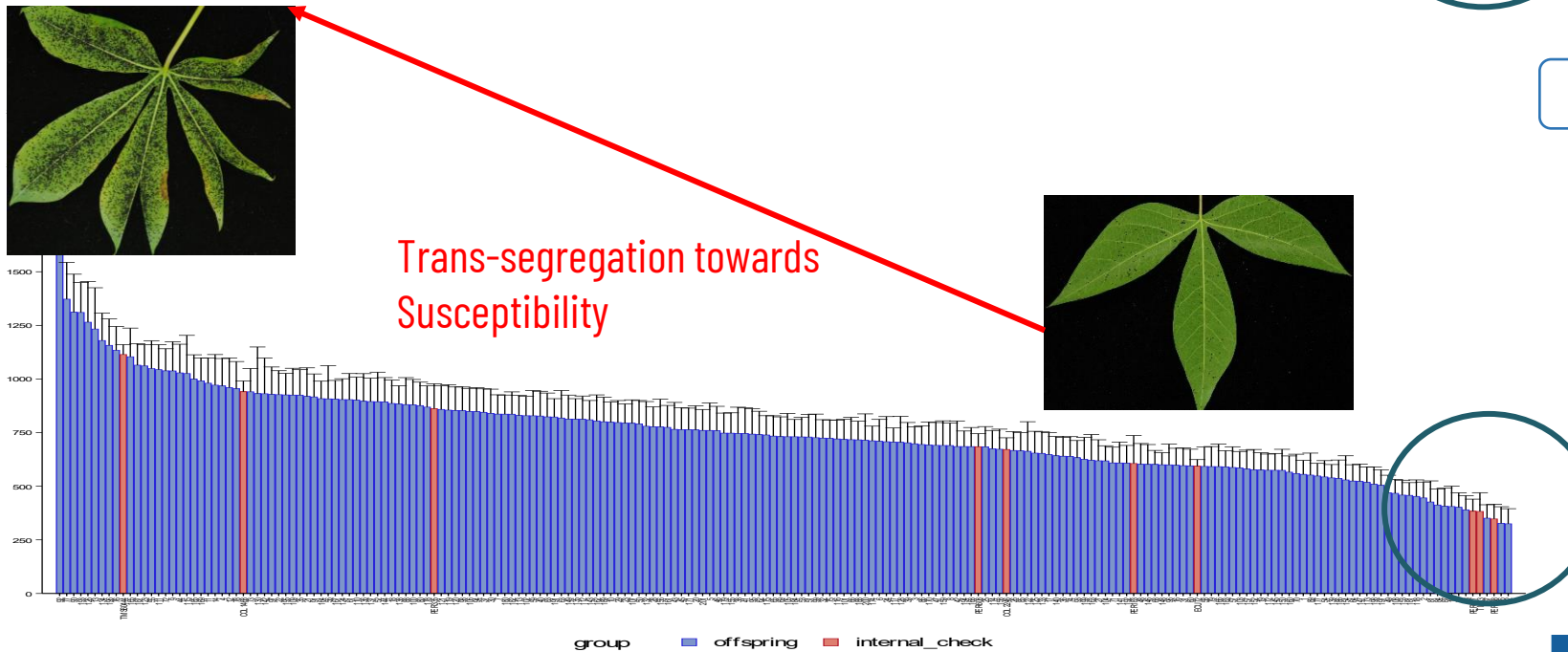
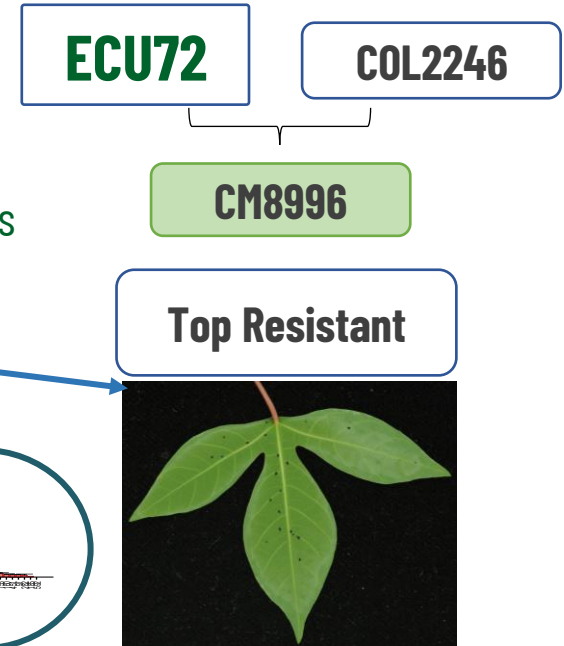
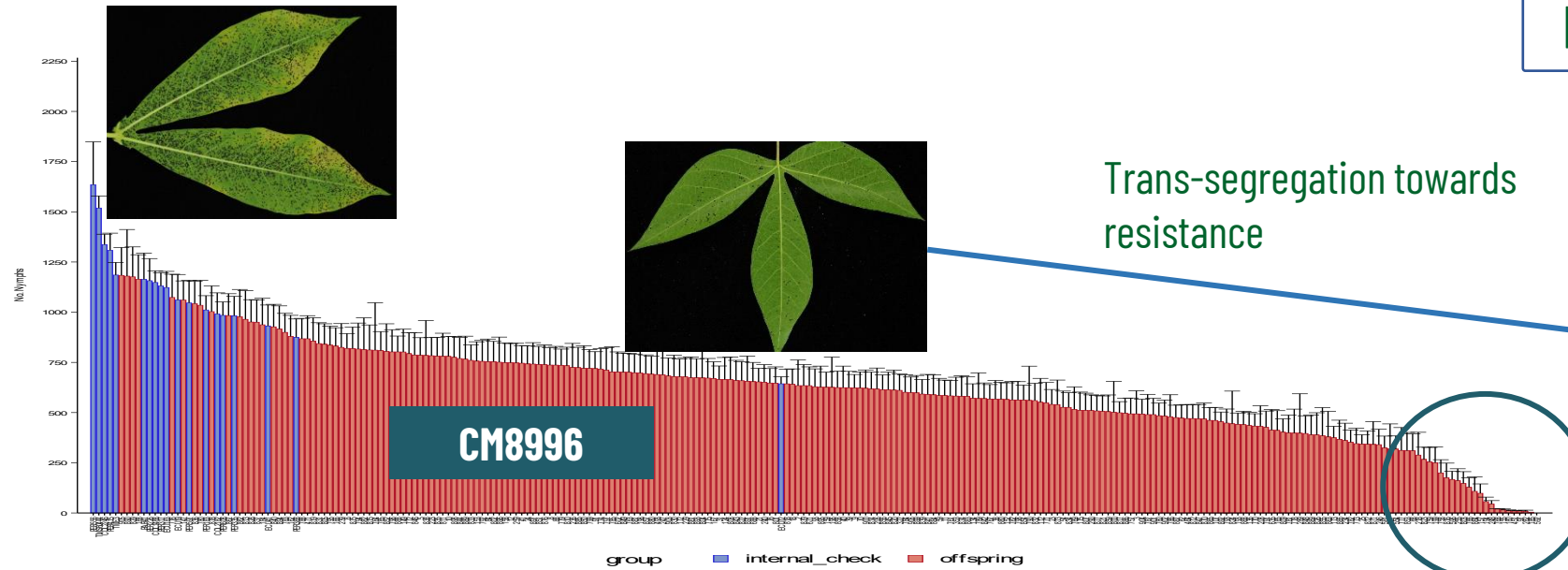


B. tabaci



ImageJ (ImageJ, National Institute of Health, Bethesda, USA)

Whitefly phenotyping on two F1 pops.: CM8996 & GM8586



Population development

F1s, advanced intercrossing F2, F3

**Top WFR F2s:
Advanced
Intercrossing
(16 progenies)
to produce F3**

F	Female	Male	Family/Cross	# clones	Conservation Status	Wf Phenotyping	Genomic approach
F1	ECU72	COL2246	CM8996	220	In vitro	Complete	Rad-Seq/Map/QTLs/GWAS
F1	ECU72	TMS60444/NGA11	GM8586	200	In vitro	Complete	WGS/Map/QTLs
F2	CM8996-199	CM8996-199	AM1588	210	In vitro/ greenhouse/field	Complete	RAD-Seq/Map/QTLs in process/GWAS complete
F2	GM8586-64	GM8586-64	AM1620	110	Greenhouse	Complete	—
F2	GM8586-103	GM8586-103	AM1621	86	Greenhouse	Complete	—
F2	CM8996-581	CM8996-199	GM12200	193	Greenhouse	Complete	—
F2	CM8996-596	CM8996-199	GM12201	360	Greenhouse	Complete	—
F2	CM8996-758	CM8996-199	GM12202	450	Greenhouse	Complete	—
F2	CM8996-193	CM8996-199	GM12198	53	Greenhouse	Complete	—
F2	CM8996-246	CM8996-199	GM12199	560	Greenhouse	Complete	—
F2	GM8586-198	GM8586-64	GM13464	253	Greenhouse	Complete	—
F2	GM8586-198	GM8586-103	GM13465	152	Greenhouse	Complete	—
F2	GM8586-198	GM8586-153	GM13466	93	Greenhouse	Complete	—
F2	GM8586-49	GM8586-64	GM13463	142	Greenhouse	Complete	—
F3	AM1588-83	AM1588-83	—	107	In vitro/ greenhouse/field	In process	—
F3	AM1588-178	AM1588-83	—	43	In vitro/ Greenhouse	In process	—
F3	AM1588-15	AM1588-15	—	59	In vitro/ greenhouse/field	In process	—
F3	AM1588-136	AM1588-129	—	26	In vitro/ Greenhouse	In process	—
F3	AM1588-136	AM1588-83	—	14	In vitro/ Greenhouse	In process	—



AM1588-83



AM1588-24

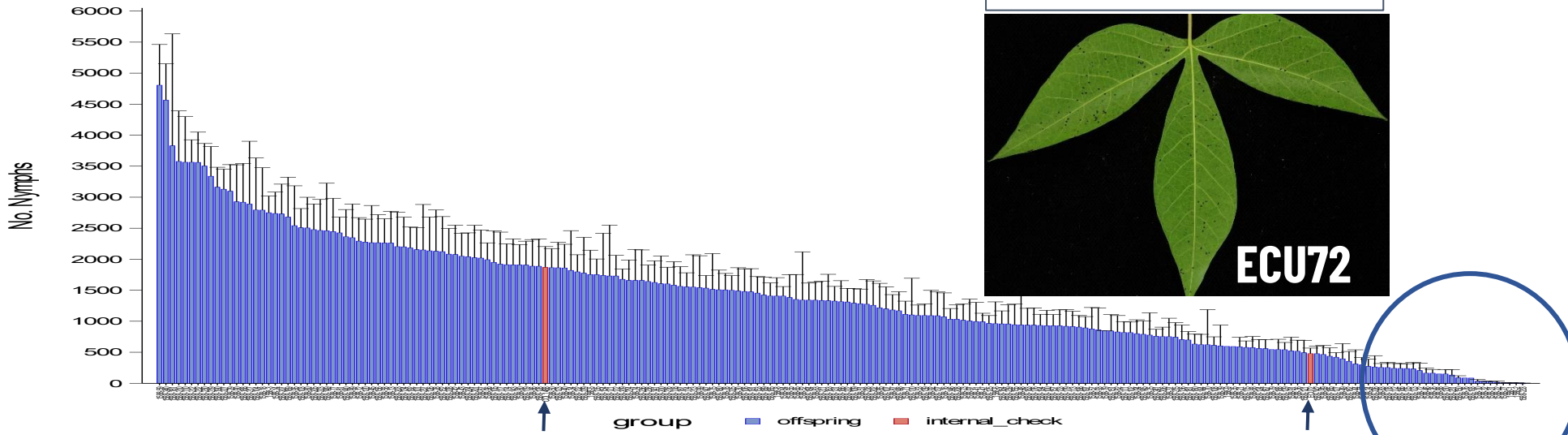


AM1588-121

Whitefly phenotyping in true F2 AM1588

AM1588 Nymphs counts

Total offspring evaluated: 211



Top 16 S	Clone
1	AM1588-212
2	AM1588-190
3	AM1588-29
4	AM1588-90
5	AM1588-18
6	AM1588-228
7	AM1588-169
8	AM1588-168
9	AM1588-205
10	AM1588-207
11	AM1588-140
12	AM1588-89
13	AM1588-45
14	AM1588-13
15	AM1588-214
16	AM1588-134

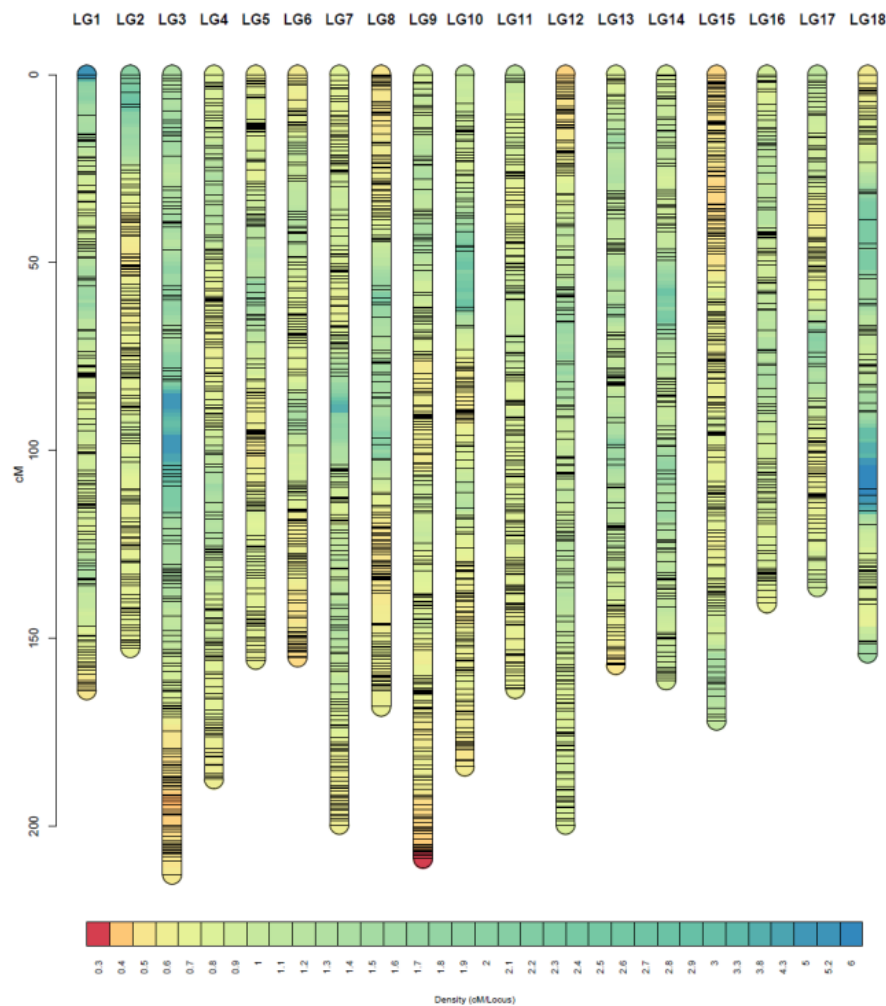


Top 16 R	Clone
1	AM1588-83
2	AM1588-24
3	AM1588-44
4	AM1588-15
5	AM1588-175
6	AM1588-127
7	AM1588-69
8	AM1588-142
9	AM1588-121
10	AM1588-68
11	AM1588-36
12	AM1588-178
13	AM1588-21
14	AM1588-129
15	AM1588-136
16	AM1588-186

Top Resistant F2 AM1588 for crossing to advance to F3

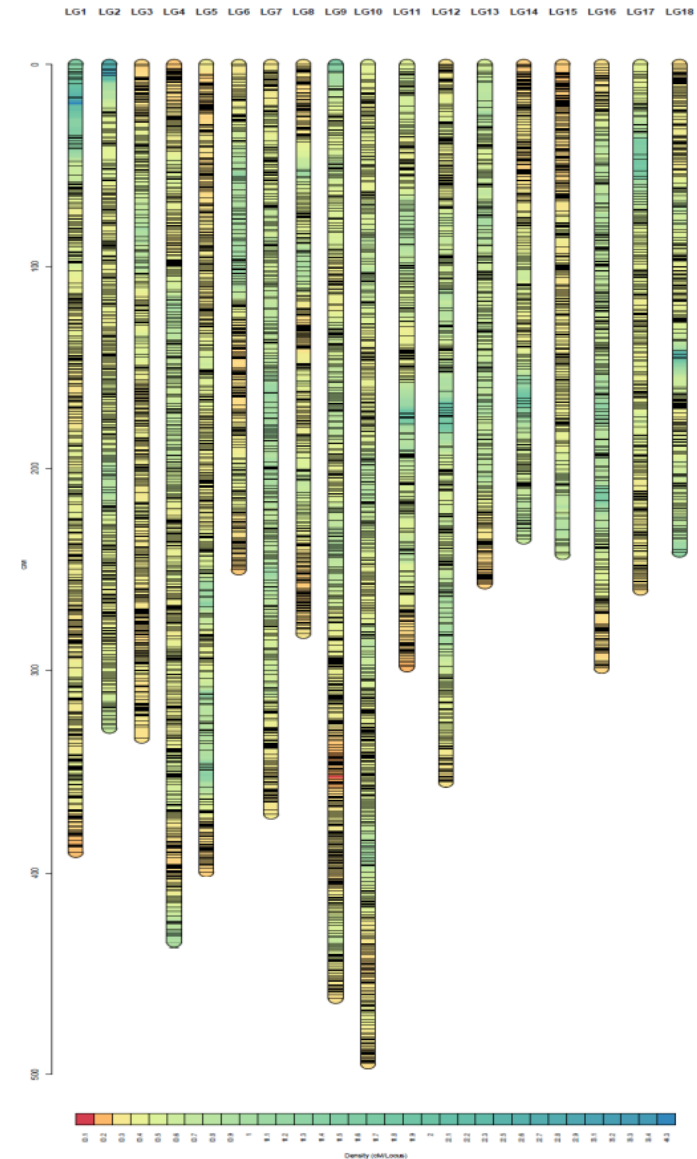
Linkage Map – CM8996

STAT	VALUE
Cross	ECU72 x COL2246
Sequencing method	RADseq EcoRI
Raw Variants	2.5M
GATK Hard Filtering	400K
Additional Filtering and preprocessing	5110
Mapped	5039
Total Length (cM)	3060
Average Length (cM)	170
Average Markers per LG	280



Linkage Map – GM8586

STAT	VALUE
Cross	ECU72 x TMS60444
Sequencing method	WGS
Raw Variants	19M
GATK Hard Filtering	2.7M
Additional Filtering and preprocessing	16081
Mapped	15883
Total Length (cM)	5931
Average Length (cM)	330
Average Markers per LG	882



F1 CM8996: QTLs (R/QTL, Phytom)

QTL Analysis – CM8996 Results

Multiple QTL Mapping												
phenotype	chr	start position bp	start position cM	end position bp	End position bp	size Mbp	size cM	Interaction	%var MQM model	additive	dominance	ratio
CM_are2017_Exp3_GLM	LG5	8548020	68.51	11496606	80.87	2.95	12.36	x	10.2	0.829	-0.146	-0.176
CM_cnt2013_Exp1_BLUP	LG18	12084126	131.27	14716362	135.54	2.63	4.27	LG10 Interacting with LG18	60.67	-19.836	-1.265	-0.064
	<u>LG10</u>	<u>19100530</u>	<u>126.22</u>	<u>20181275</u>	<u>129.80</u>	<u>1.08</u>	<u>3.58</u>			19.337	-29.053	-1.502
CM_cnt2016_Exp2_BLUP	LG3	15667640	109.79	18254280	118.32	2.59	8.53	There are interactions between all three LG	66.57	-6.556	33.847	5.163
	LG18	9475888	91.20	11375033	151.20	1.90	60.00			6.368	-8.885	-1.395
	LG7	8347077	86.43	9264789	108.36	0.92	21.93			40.406	-42.650	-1.056
CM_cnt2016_Exp2_GLM	LG3	15667640	109.79	18254280	118.32	2.59	8.53	There are interactions between all three LG.	56.45	-8.653	41.169	4.758
	LG18	9475888	91.20	11491469	151.48	2.02	60.27			7.952	-11.303	-1.421
	LG7	8347077	86.43	9264789	108.36	0.92	21.93			45.427	-51.492	-1.134
CM_are2017_exp4_lf1	LG14	9969524	84.96	17523207	103.87	7.55	18.91	x	11.94	0.036	-1.214	-33.999
CM_cnt2016_exp2_lf1	LG7	8347077	86.43	9264789	108.36	0.92	21.93	LG08 Interacting with LG07	59.99	54.674	-53.171	-0.973
	<u>LG8</u>	<u>5155647</u>	<u>49.33</u>	<u>6618934</u>	<u>59.30</u>	<u>1.46</u>	<u>9.97</u>			34.908	-38.220	-1.095
CM_cnt2016_exp2_lf2	LG3	15667640	109.79	22416819	130.50	6.75	20.71	LG07 interacting with LG03	55.96	-6.370	54.080	8.490
	<u>LG7</u>	<u>8347077</u>	<u>86.43</u>	<u>9264789</u>	<u>108.36</u>	<u>0.92</u>	<u>21.93</u>			53.667	-66.541	-1.240
CM_cnt2017_exp4_lf1	LG14	9969524	84.96	17523207	103.87	7.55	18.91	x	13.16	45.071	-386.427	-8.574

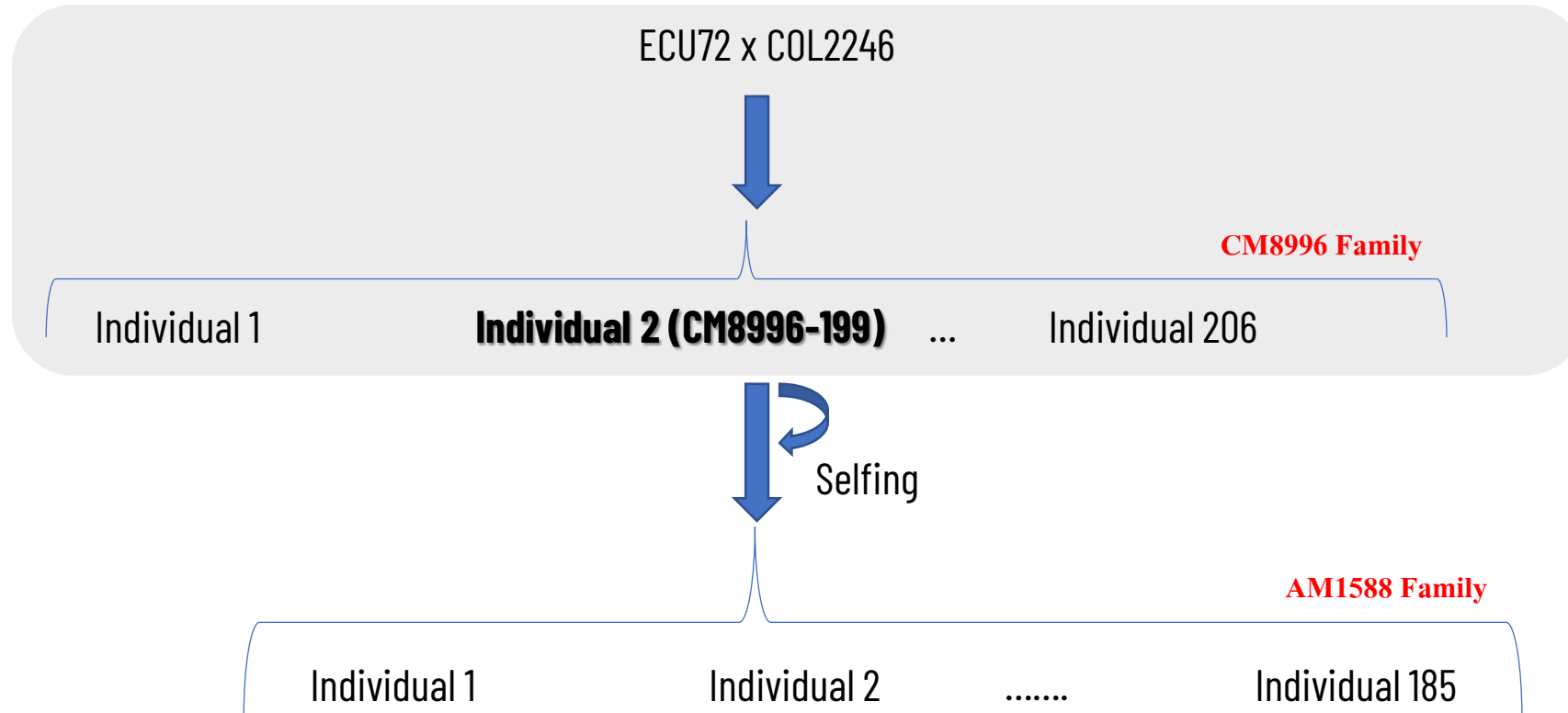
F1 GM8586: QTLs (R/QTL, Phytom)

QTL Analysis – GM8586 Results

Single QTL Mapping												
phenotype	chr	lod peak	start position bp	start position cM	End position bp	End position cM	size Mbp	size cM	%var - H	additive	dominance	ratio
GM_cnt2016_Exp1_BLUP	LG18	5.15	12846653	207.49	21929250	239.70	9.08	32.21	1.44	98.842	-38.340	-0.388
GM_cnt2016_Exp1_GLM	LG18	5.15	10151464	159.67	21929250	239.70	11.78	80.04	15.21	123.496	-47.753	-0.387
Multiple QTL Analysis												
phenotype	chr	start position bp	start position cM	End position bp	End position cM	size Mbp	size cM	Interaction	%var MQM model	additive	dominance	ratio
GM_cnt2016_Exp1_BLUP	LG18	7507784	97.07	8657091	125.67	1.15	28.61	LG1 is interacting with LG18	25.73	81.870	30.295	0.370
	LG1	32535720	373.73	34657641	387.06	2.12	13.33			-39.300	71.977	1.831
GM_cnt2016_Exp1_GLM	LG18	10151464	145.65	21929250	239.70	11.78	94.05	x	15.21	125.161	-47.927	-0.383

Pedigree

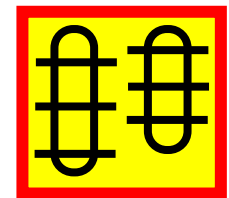
CP population



Linkage Mapping Methodology

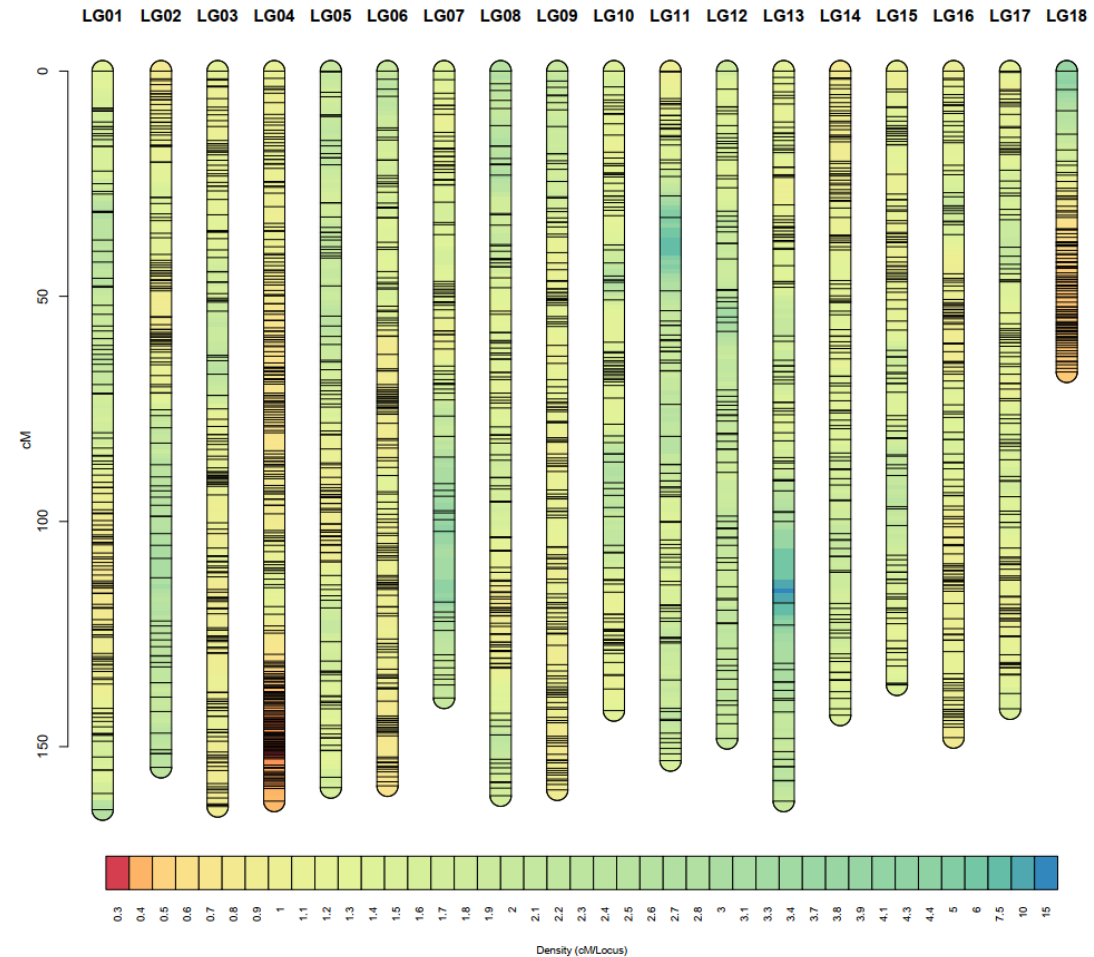
- Alignment and Filtering of RAD seq data
- Using the CP coding scheme (with all loci <hkxhk>)
- Detecting the linkage phases {00} and {11}.
- Re-coding towards F2, with translations depending on the phase (combination):
- Regression mapping in JoinMap 5.0 (Van Ooijen 2006)
- Kosambi mapping function
- Segregation Distortion : Chi-square test χ^2 at $p = 0.001$
- Identical loci excluded
- Final number of loci on genetic map: **2,017**
- Number of accessions: 185

{00}:	{11}:
hh - A	hh - B
hk - H	hk - H
kk - B	kk - A



Linkage groups F2 AM1588

Chromosome	No. of markers	Length (cM)
1	127	164.1
2	110	154.6
3	150	163.3
4	97	157.9
5	111	159.1
6	153	158.7
7	83	139.2
8	115	160.9
9	148	159.6
10	99	142
11	85	153
12	79	148
13	91	162.1
14	115	143
15	102	136.3
16	140	148
17	111	141.6
18	101	66.8

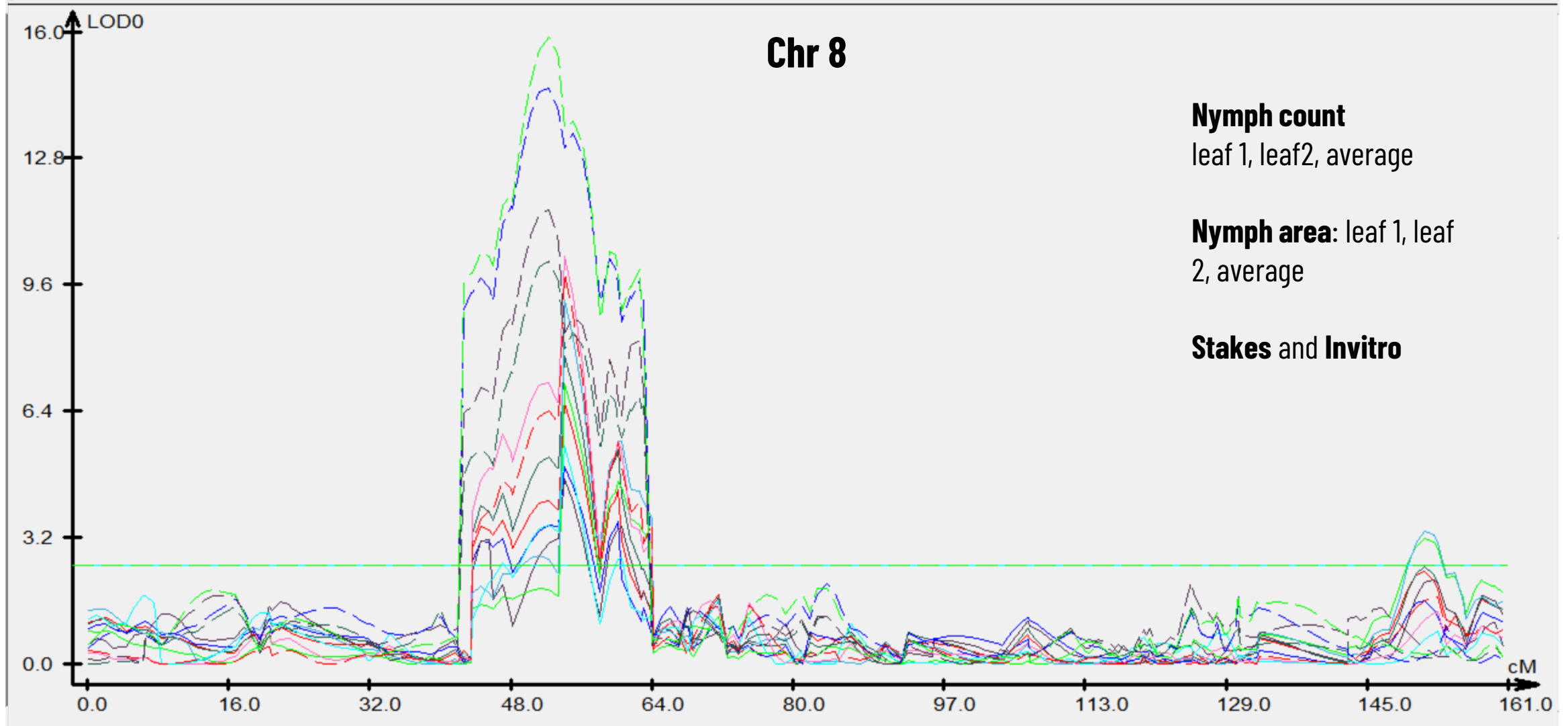


~100 markers per LG
 Average length = 147.7 cM
 Total length = 2658 cM

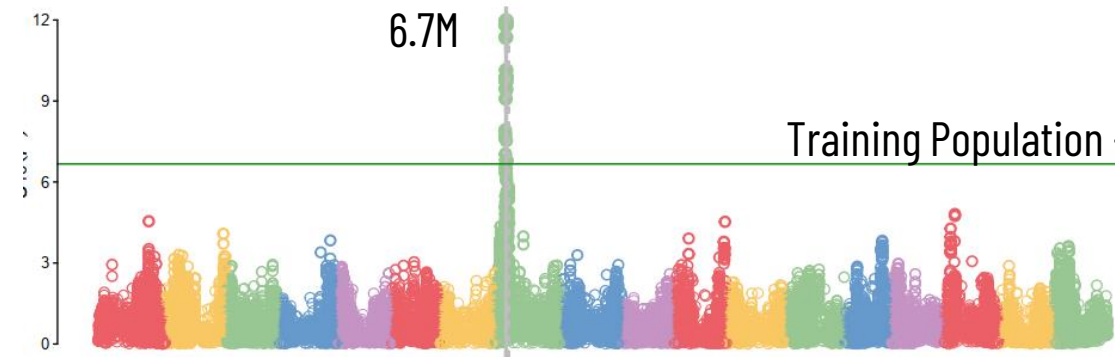
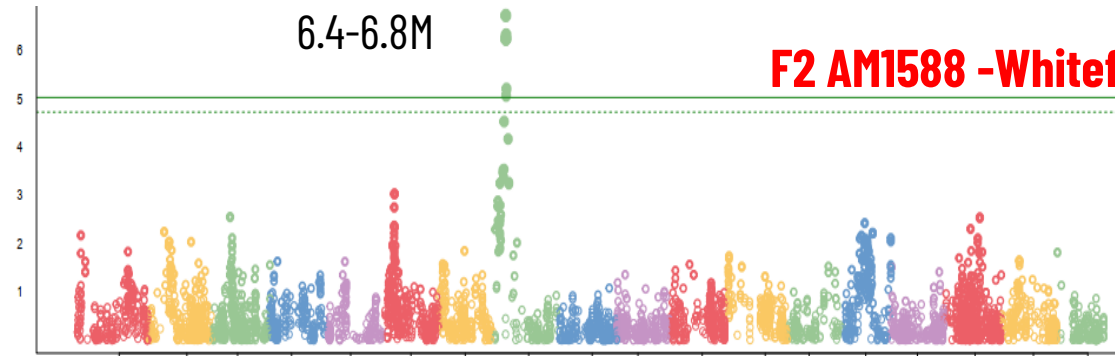
QTL mapping results, F2 AM1588

- WinQTL Cartographer, R/QTL and MapQTL used for mapping (for comparison purposes)
- Composite Interval Mapping method, with 1000 permutations
- QTL identified on **Chr 8** in the **5.3-8.0Mb** region (2 LOD interval), peak around **6.4-6.6Mb** region
- R² value between **11-58%** depending on 'trait'
- Potential candidate genes identified (wound-induced genes and associated with cell wall), especially around the QTL peak region, further examination of the candidate genes ongoing

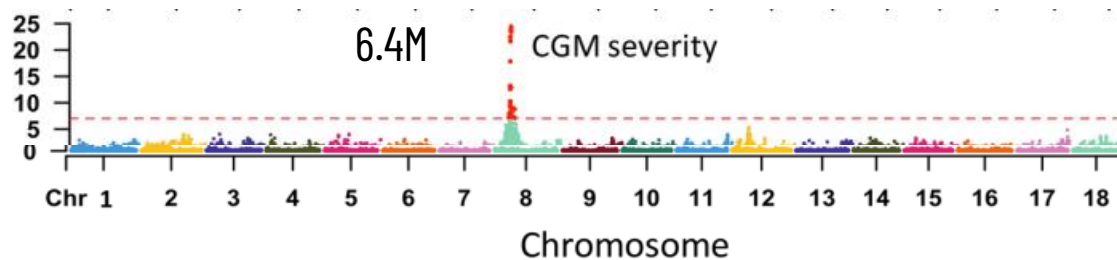
QTL mapping results, F2 AM1588



Potential resistance hot spot to sucking pests in Chromosome 8

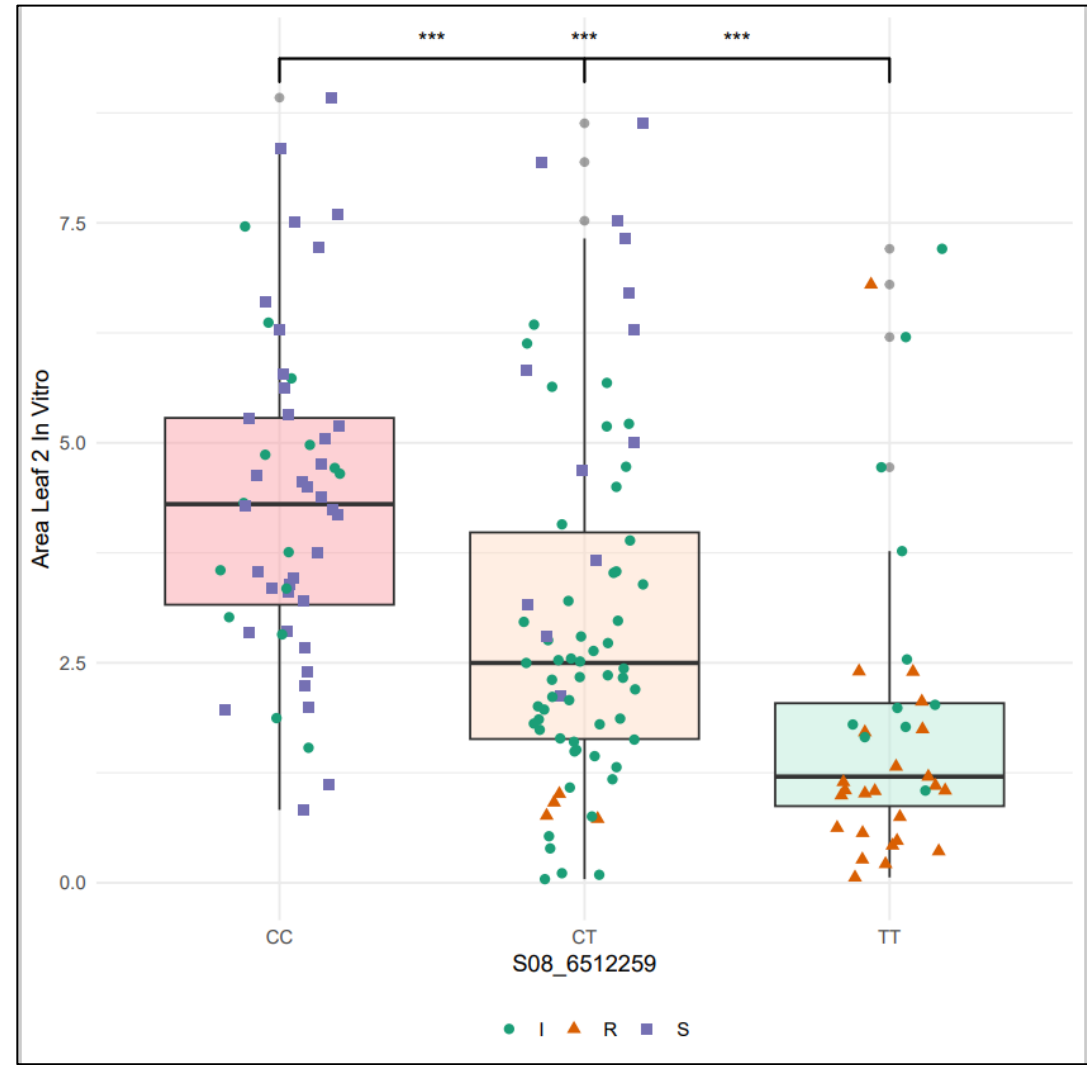
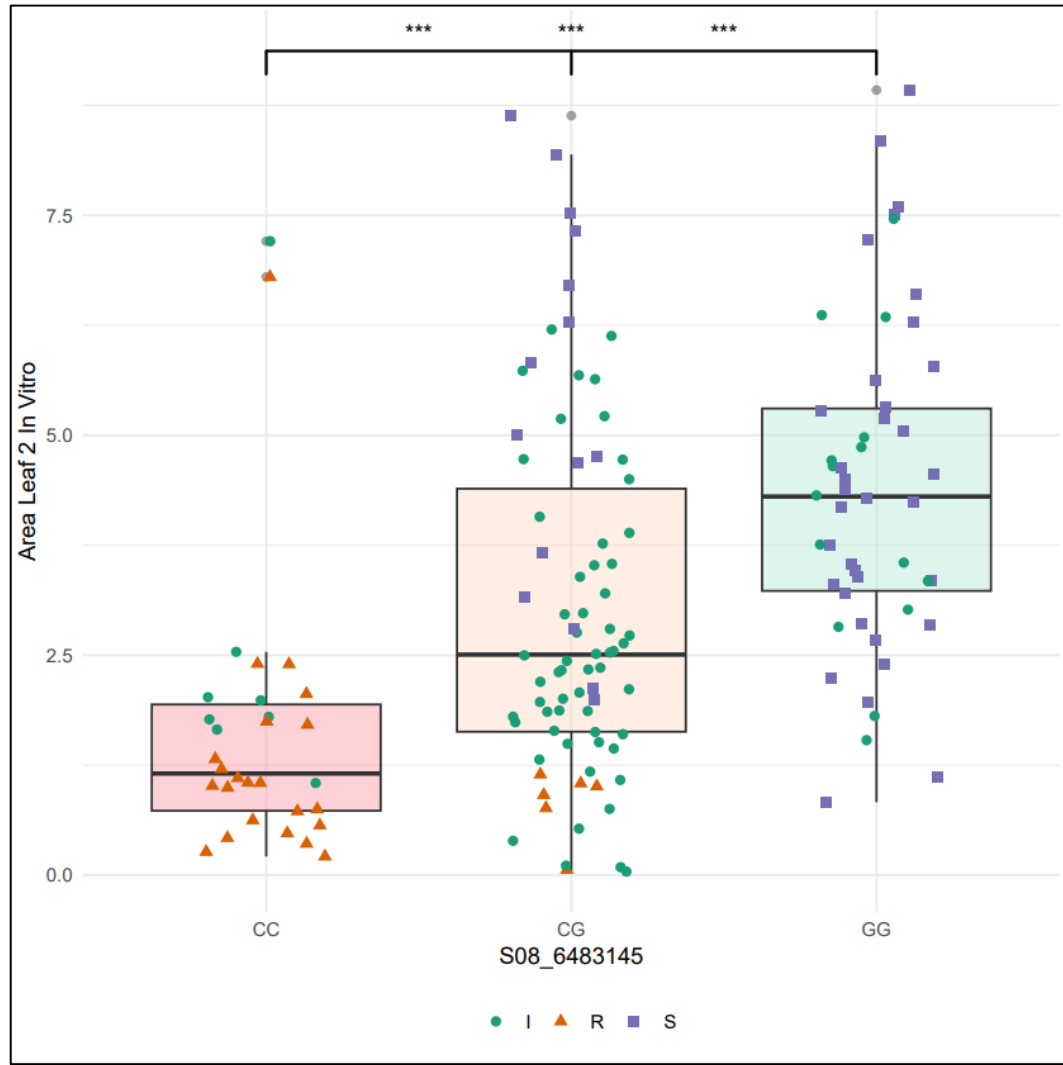


(Thrips Manhattan plot): Xiaofei Zhang

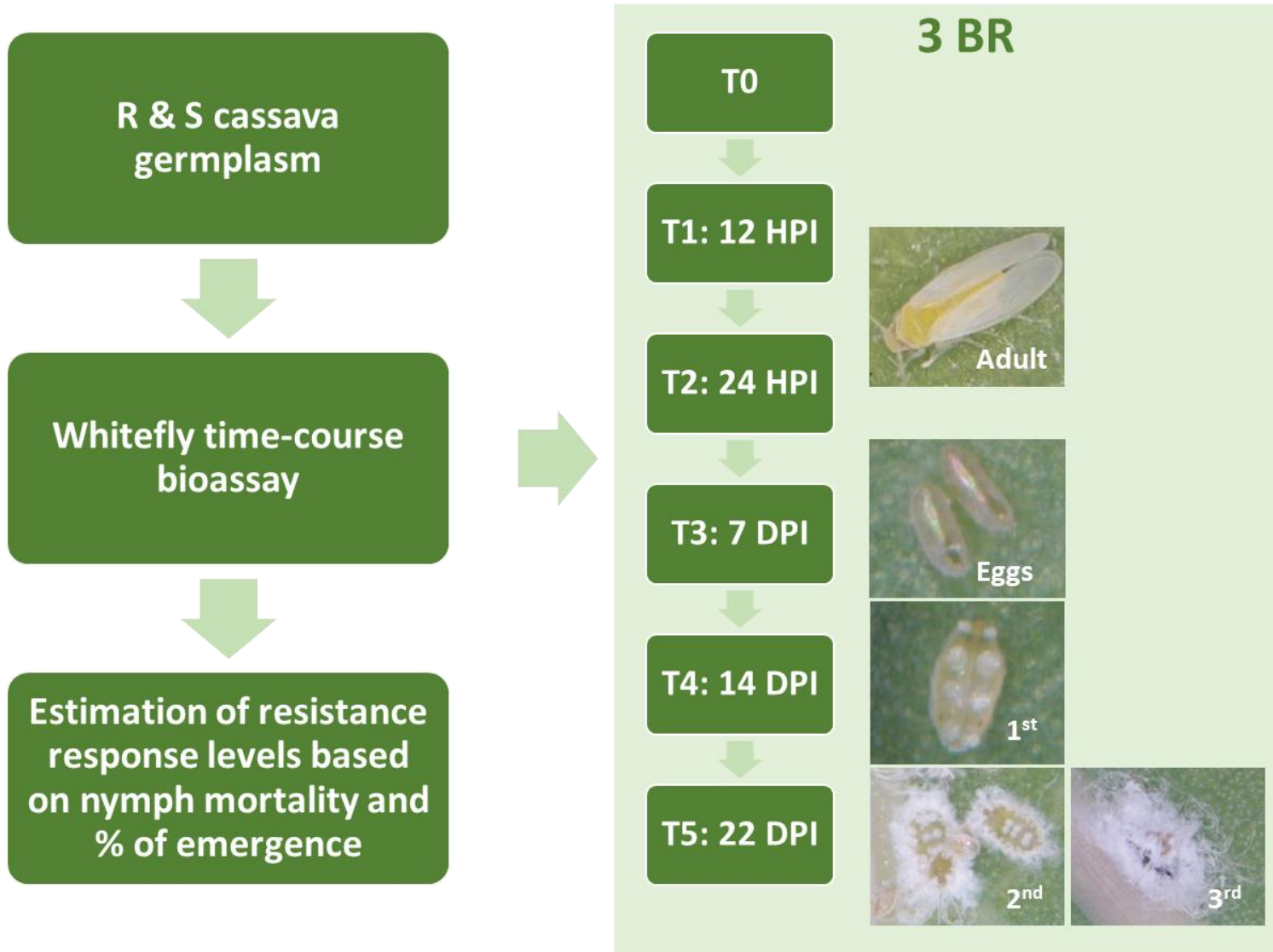


Rabbi et al., 2020, Plant Molecular Biology

Box plots show the estimated genotypic effect on the **F2 AM1588**. The p-value of the t-test comparison ($p < 0.05$) is plotted for each comparison.



Multi-Omic's strategy to improve markers/gene discovery



RNA material
Transcriptomics analysis

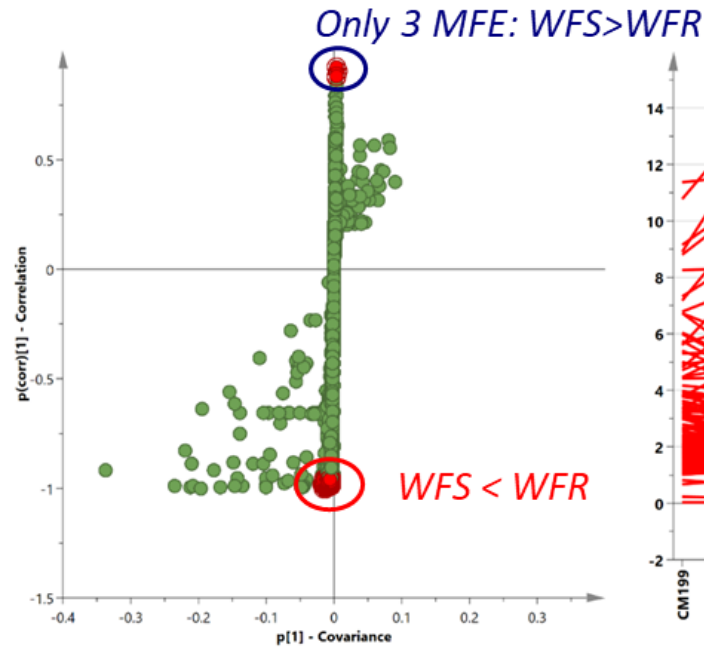


Lyophilized leave material
Metabolomics profiling

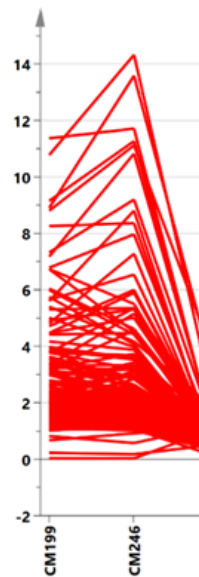


Irigoyen ML; Garceau DC; Bohorquez-Chaux A; Becerra López-Lavalle LA; Perez-Fons L; Fraser PD; Walling LL. 2020. Genome-wide analyses of cassava Pathogenesis-related (PR) gene families reveal core transcriptome responses to whitefly infestation, salicylic acid and jasmonic acid. BMC Genomics 21:93

Unraveling potential Biomarkers that will improve our understanding of genome mutations that control WFR



225 potential biomarkers
($p < |0.05|$; $pcorr > |0.95|$)

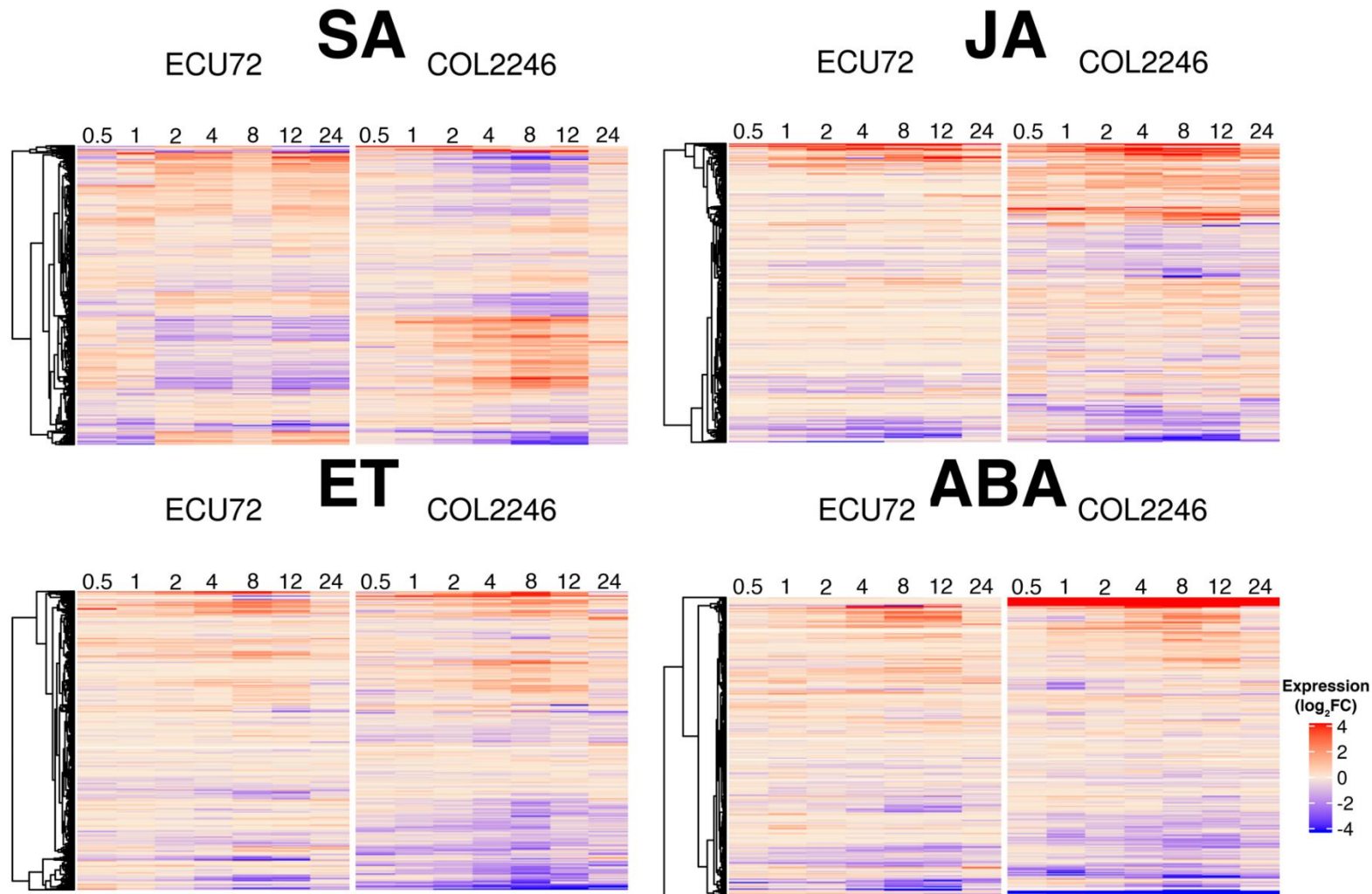


Alkaloid	Australine (=7- α -epialexine) [a polyhydroxy pyrrolizidine alkaloid]; Diethylaspartate, etc...
Phenylethanoid	Dimethoxyphenyl glucoside (=taxicatin) Hydroxytyrosol glucoside Vanillyl alcohol glucoside Cornoside (phenylethanoid gluc) etc...
Phenylpropanoids	Dihydroxycoumarine (e.g. esculetin, daphnetin aglycones) Carboxy-hydroxybenzofurane Caffeoylquinone Coumarin glucoside (e.g.: esculetin-gluc (esculin), cichoriin or daphnin) Sinapoyl malate p-Coumaric acid glucuronide 3,4-Dimethoxyhydrocinnamic acid Trimethylacetophenone Sinapyl alcohol p-propoxy mandelic acid, etc..
Flavonoids/tannins	Delphinidin 3-O-(6''-O-malonyl)- β -D-glucoside tri-galloyl-glycerol (=1,2,3-Propanetriyl tris(3,4,5-trihydroxybenzoate) [hydrolysable gallotannins] Myricetin-hexoside Galloylbergenin Myricetin-hexoside Galloylbergenin Myricetin-hexoside Galloylbergenin

Cyanogenic-Gluc	<i>Lotaustralin anitrile-6'-apioside (I)</i> CyG: Didemethylsimmondsin =(2E)-[(2S,3S,4S,6S)-6-(β -D-Glucopyranosyloxy)-2,3,4-trihydroxycyclohexylidene]acetonitrile; Tetra-O-acetyl-amino-deoxy-hexose
Sesquiterpenoid	Geranyl-disaccharide cichorioside B (sesquiterpene glyc) Artemisin (sesquiterpenoid) Octyl-gallate Hymenoxone (sesquiterpenoid) etc...
Apocar-gluc/Sesquiterp-gluc	Dihydrophaseic acid-4-O- β -glucoside Ebuloside (terpenoid iridoid) Dendromonilside C Icariside B3 (apocar-gluc) Laurosides B (=apocar-gluc, megastigmane-gluc) Euphorbioside A (sesquiterpenoid-gluc) Apocarotenoid-gluc (blumenol-like) Euphorbioside B (a bisnossesquiterpenoids glycoside found in latex)

Perez-Fons L; Bohorquez-Chaux A; Irigoyen ML; Garceau DC; Morreel K; Boerjan W; Walling LL; Becerra LópezLavalley LA; Fraser PD. 2019. A metabolomics characterization of natural variation in the resistance of cassava to whitefly. BMC Plant Biology 19:518

UCR contribution to unravel the WFR mechanism



- Ecu72 (R) and Col2446 (S) have divergent and reciprocal **SA** responses, while overall **JA**, **ABA**, and **ET** are more similar.
- This suggests that there a **master regulator (switch)** that controls **SA** signaling is altered in R vs S.

"Integrative transcriptomics reveals association of abscisic acid and lignin pathways with cassava whitefly resistance" BMC Plant Biology (Accepted)

Plant samples infested with *A. socialis* are clustering according to genotype and sampling time.

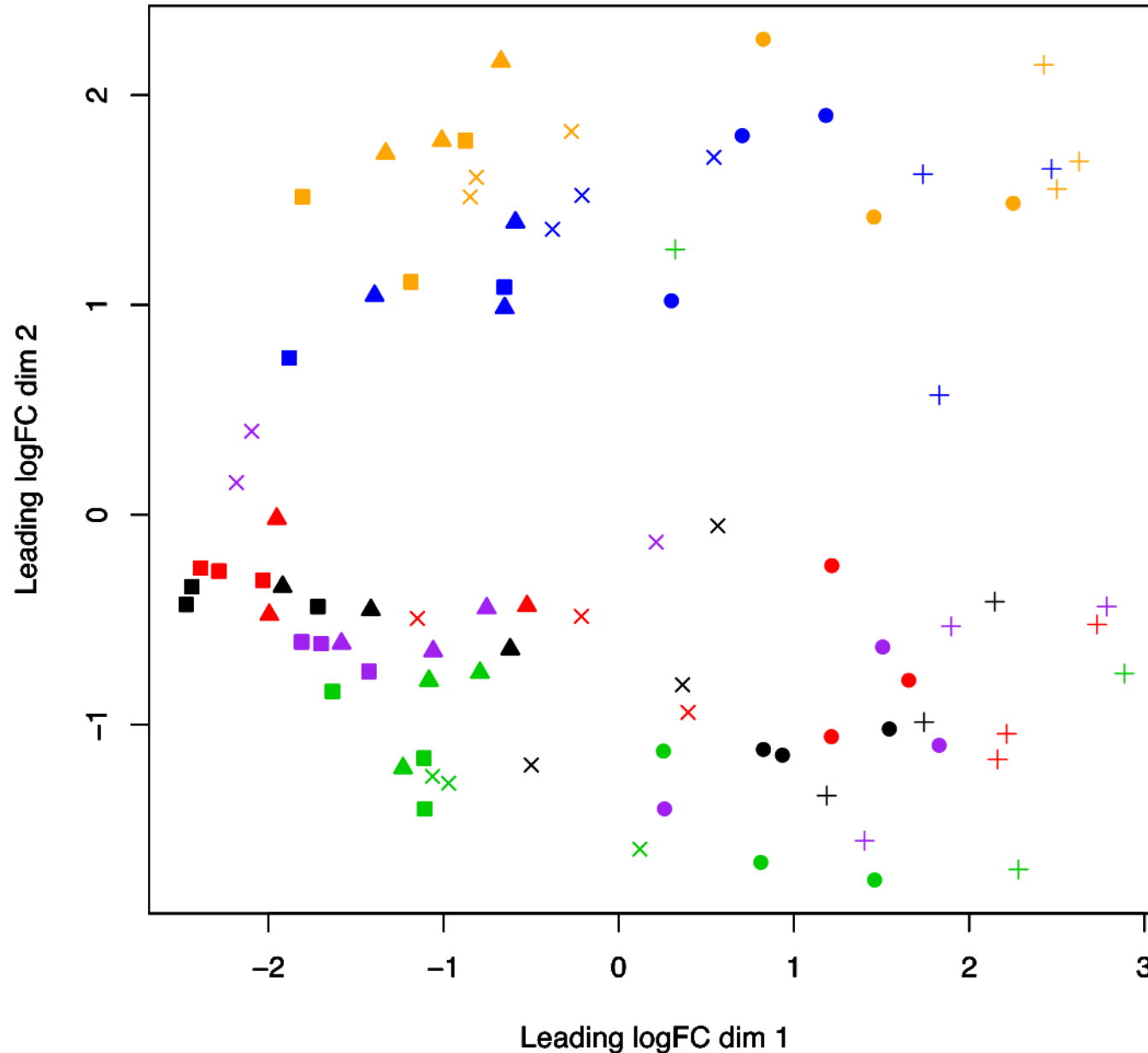


Summary:

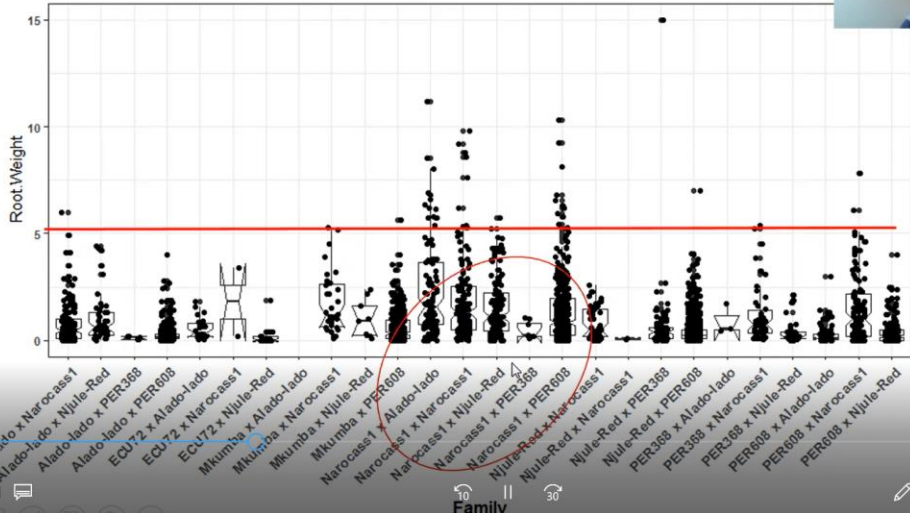
1. In general, resistant and susceptible genotypes cluster apart, indicating a genotype effect.

PER368 and PER608 show contrasting response to PER183.

2. Subtle progression of clusters from left to right indicates a temporal effect.



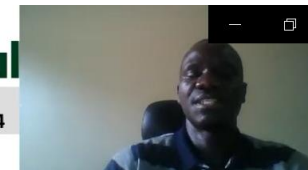
Highest yielding crosses of NAROCASS



Outstanding results of partners in Uganda (NaCRRI). Dr. Alfred Ozimati

Top 20 Seedlings Selected (Independent cul

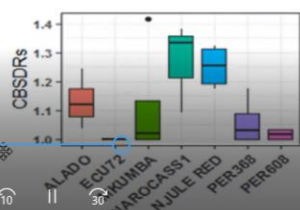
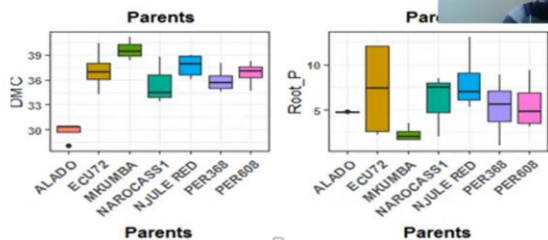
Family	WFC3	CGM3	NC3	CMD3	CBSD3	Vigor	WFC4	WFD4	CGM4	NC4			
Narocass1 x PER608	14	1	24	1	1	7	23	1	1				
Mkumba x PER608	15	1	24	1	1	7	5	1	1				
Narocass1 x Njule-Red	4	1	3	1	1	7	13	1	1	32	1	1	
Narocass1 x Alado-lado	21	1	12	1	1	7	21	1	1	0	1	1	
Narocass1 x Alado-lado	0	1	9	1	1	7	4	1	1	0	1	1	
PER608 x Narocass1	3	1	15	1	1	7	4	1	1	9	1	1	
PER608 x Narocass1	4	1	4	1	1	7	14	1	1	11	1	1	
PER608 x Narocass1	11	1	12	1	1	7	5	1	1	25	1	1	
Mkumba x Narocass1	17	1	11	1	1	7	8	1	1	7	1	1	
ECU72 x Alado-lado	3	1	1	1	1	7	2	1	1	3	1	1	
ECU72 x Alado-lado	8	1	0	1	1	7	3	1	1	11	1	1	
Mkumba	11	1	10	1	1	7	16	1	1	0	1	1	
Mkumba	3	1	6	1	1	7	3	1	1	0	1	1	
Mkumba	16	1	6	1	1	7	5	1	1	1	1	1	
Mkumba	8	1	0	1	1	7	0	1	1	0	1	1	
Mkumba	15	1	5	1	1	7	2	1	1	5	1	1	
Mkumba	16	1	2	1	1	7	5	1	1	0	1	1	
Mkumba	3	1	6	1	1	7	21	1	1	17	1	1	
Mkumba	8	1	6	1	1	7	4	1	1	11	1	1	
Mkumba	8	1	14	1	1	7	4	1	1	3	1	1	
Mkumba	7	1	15	1	1	7	15	1	1	0	1	1	
Mkumba	8	1	7	1	1	7	6	1	1	0	1	1	
Narocass1	7	1	1	1	1	7	6	1	1	5	1	1	
Narocass1	8	1	0	1	1	7	8	1	1	7	1	1	
Narocass1	3	1	0	1	1	7	15	1	1	15	1	1	



Agronomic performance of parental ge

2-years of parental evaluations

- ❖ Cassava brown streak
- ❖ Fresh root yield
- ❖ Dry Matter Content



Cassava Lab, DNA extraction and GBS seq analysis of 750 samples of partners in Uganda (NaCRRI).

Outstanding results of partners in Tanzania (TARI). Dr. Esther Masumba

INTROGRESSION OF WHITEFLY RESISTANCE INTO AFRICAN VARIETIES

Reciprocal crossing between:

- 1) LA x Improved varieties; 2) LA x Landraces; 3) Improved varieties x Landraces

Parental Genotypes and Crossing combinations

IMPROVED VARIETIES

- Mkumba
- TARI CASS4 (TZ130)
- Mkuranga 1
- Kiroba

LATIN AMERICAN GENOTYPES

- ECU 72
- PER 368
- PER 608

LANDRACES

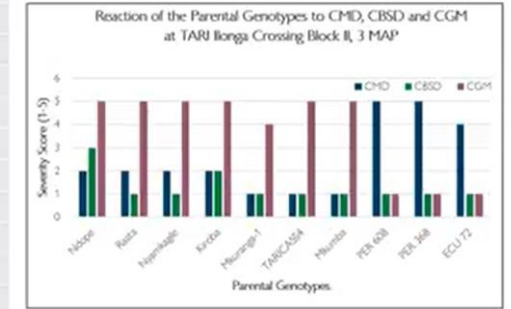
- Ndope
- Nyamkagile
- Rasta
- Rwakitangaza

Cassava Lab, DNA extraction, and GBS seq analysis of 475 samples of partners in Tanzania (TARI), and Malawi (DARS).

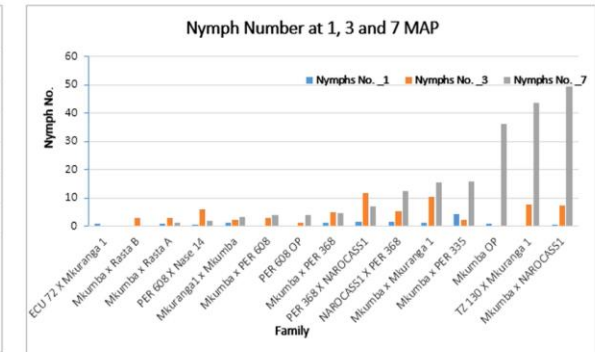
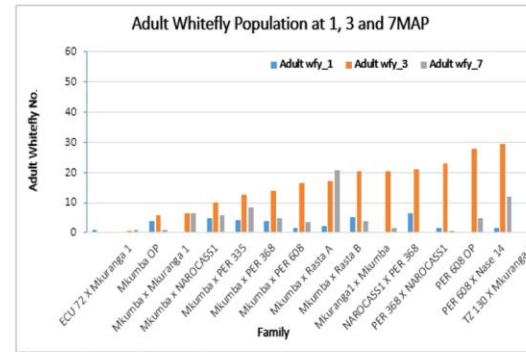
Field performance of the Parental Genotypes at TARI Ilonga Crossing Block II, eastern Tanzania

CMD, CBSD and CGM severity expressed by the parental genotypes at 3 MAP

Variety	CMDs3	CBSDs3	CGMs3
Ndope	2.0	3.0	5.0
Rasta	2.0	1.0	5.0
Nyamkagile	2.0	1.0	5.0
Kiroba	2.0	2.0	5.0
Mkuranga-I	1.0	1.0	4.0
TARI CASS4	1.0	1.0	5.0
Mkumba	1.0	1.0	5.0
PER608	5.0	1.0	1.0
PER368	5.0	1.0	1.0
ECU72	4.0	1.0	1.0
Mean	2.5	1.3	3.7
LSD	0.4	0.3	0.6
CV%	26.4	27.9	17.1
SE	0.5	0.3	0.6
P-value	<.001***	0.024*	<.001***



Mean Adult Whitefly Population and Nymph Number, Seedling Nursery I, Chambezi Experimental Station



Observation:

- Low whitefly population at 1 and 7 MAP
- Increased whitefly population at 3MAP
- Consistently low WF and nymph counts on ECU72 x Mkuranga and Mkumba associated families
- Generally, low nymph counts compared to whitefly population
- Higher nymph counts at 7 MAP (mostly Mkumba associated families)

Currently in progress

- Genotyping of F2 with selected WF markers to confirm association with the trait
- Marker validation using different genetic backgrounds (Malawi, Tanzania, and other populations). We already received the results of the Intertek about WF markers. We are in the process of analysis.
- Phenotyping of F3 families, is in process. We observed very good results and high levels of resistance.

Next steps:

- Selection of best marker(s) for application in MAS for the trait

CIAT Cassava team



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Thanks!