➢ Cotton Breeding for Fusarium wilt race 4 (FOV4) Resistance in Cotton

with additional molecular approach

- ➢ FOV4 Genetics and QTL Mapping
- ➢ FOV4 Transcriptomics / Gene Expression

USDA-ARS, University Cooperators, CA Cotton Growers, and Cotton Incorporated
– Cooperative Project - Prepared by Dr. Mauricio Ulloa

Breeding Efforts Supported by California Cotton Grower's Organizations, including CCGGA and CI

2006

California FOV4 Nursery 2017

University of California,

Dr. Robert Hutmacher, Mark Keeley, Steve Wright with many other staff and students, and more recently Tarilee Frigulti have performed the breeding activities since 2012 of this collaborative effort.

Continue Breeding Support Effort 2012-Present of Dr. Ulloa, USDA-ARS, Lubbock, TX

2017

Prepared by Dr. Mauricio Ulloa, USDA, ARS

Cotton Germplasm Collections Evaluated by USDA-ARS and Univ. of CA. for FOV4 Resistance

✓ **New Mexico State University – Dr. Roy Cantrell**

- NM 59-851, ACALA 1517-75, ACALA 1517-88, ACALA 1517-91, ACALA 1517-95, ACALA 1517-99, etc., Dr. Ulloa/Research Associate -1996 - 1998, Pima lines provided MS. Cindy Waddell – 2003

✓ **USDA-ARS – Maricopa AZ – Dr. Percy 2003**

- Pima S-series, PS1-PS32, Pima lines from NMSU, Pima population developed for Genetics/QTLs mapping 1999 -2000 F_1 and F_2. (around 186 F6 lines - collaborative project with Dr. Ulloa).

✓ **USDA-ARS Shafter, CA - & College Station, TX Collection**

- Acala FBCX-2, Shorty, Acala Shafter Station, Del Cerro SS227, UCD368 Hopi M5-11, Acala 8, Acala 4-42 1958-A , Acala 4-42 1966-A, Acala SJ-2, SJ-3SJ-5, etc., 2003 – 2006.

✓ **Regional Breeding Testing Network (RBTN) germplasm**

- from more than 12 breeding cotton programs across the belt. Since 2004 - Present.

International Cotton Germplasm Programs/Collections Evaluated for FOV4 Resistance

✓ **Australia CSIRO – Fiber Max germplasm collection**

2004 visited and exchanged germplasm.

- Tested germplasm for this program which also included tolerant Upland Sicot 189 and resistance source MCU-5 germplasm lines to **FOV Australian races**.

- **Australian germplasm lines were susceptible to the California FOV4.**

✓ **Republic of Uzbekistan germplasm collection FOV races**

Since 2006 and visited several times since 2009, 2011, 2103.

Tested several germplasm lines.

- **Discovered new potential source of FOV4 resistance.**

✓ **Country of Peru**

- Visited in 2009, 2016 and had the opportunity to visit cotton fields and evaluate germplasm known as Tanguis.
- **2016 started new Agreement with IPA Peru to exchange and develop germplasm. In 2017, Seven breeding lines ongoing evaluation.**

FIRST TIMELY RESPONSE FROM FOV4 RESISTANCE EVALUATIONS

- Greenhouse Evaluations
- Field Evaluations

- Identified Pima Germplasm resistant to FOV4

  - Some USDA-ARS Pima (e.g., Pima-S6) – Original Pima-S6 (PS6) release was a variable/heterozygous-pool, but it is a good source for FOV4 resistance from USDA-ARS compared to other PS1,2… releases. PS6 resistance source has been confirmed by independent labs (UC, Davis, UC Riverside, USDA-ARS College Station, and Phytogen Seed Co.)

  - An experimental, later known as commercial cultivar Phytogen 800

- This research identified potentially new improved resistant germplasm for release

Prepared by Dr. Mauricio Ulloa, USDA, ARS

2007/2008

- **PUBLIC PIMA GERMPLASM LINES JONTLY RELEASED BY USDA-ARS, UNIV. OF CALIFORNIA, & UNV. OF NEW MEXICO**

- **SJ-07P-FR01, SJ-07P-FR02, SJ-07P-FR03, and SJ-07P-FR04**

Pedigrees: 8810 (P72xP73 Pima Series AZ) and NMSI-1601 originated from a Sea Island cotton

2015/2016

- **PUBLIC PIMA GERMPLASM LINES JONTLY RELEASED BY USDA-ARS & UNIV. OF CALIFORNIA**

- **SJ-FR05, SJ-FR06, SJ--FR07, SJ-FR08, and SJ-FR09**

Pedigrees: Pima S6 (Series AZ) and 89590 Line (P62 x Sea Island St. Vicent)

Projected New Pima Releases

2018 Pedigree: Pima-S6 x Pima-S7

Field Site with Fusarium wilt - FOV4

Fusarium wilt RACE 4 (FOV4)

ROOT DIP GREENHOUSE FOV4 TEST

FOV race 4 Seedling Inoculation

Disease severity index (DSI) of leaves, (scale 0 – 5)

Vascular stem and root staining (VRS)

USDA-ARS and University of California Davis – Cooperative Project - Prepared by Dr. Mauricio Ulloa

Present Summary for FOV4 Resistance

- ✓ **Upland Germplasm Evaluated from the USDA-ARS Collection, College Station, TX**
  - **More than 400 obsolete cultivars and/or SA lines Upland Lines**
  - **Identified Upland SA lines tolerant to FOV4**
- ✓ **Developed Upland Progeny with identified Resistant/Tolerant Sources**
  - **More than 500 crosses combinations**
  - **More than 1,500 progeny developed and so far about 400 FOV4 evaluated**
  - **Identified an Upland RBTN source Now clean of AP and tested-Negative for AP from NMSU**
  - **Identified and Developed new progeny resistant to FOV4 from PS6xUzbekistan germplasm**

Seed Increase for Germplasm Release under Clean or Uninfested FOV4 Field

Continue Breeding Efforts 2012-Present
of Dr. Ulloa, USDA-ARS, Lubbock, TX with the CA group -
Dr. Hutmacher. Univ. of CA.

UPLAND FOV4 TOLERANCE/RESISTANCE UPDATE

Sources of Breeding/Selection made for FOV4 Tolerance/Resistance

DEVELOPING FOV4 TOLERANT BREEDING LINES OR PROGENY

- Upland TM-1 x Pima-S6 and Upland Shorty X Pima-S6 AND Reciprocal-Progeny
- Upland SA-Lines (obsolete cultivars) X US63 (Uzbek x Pima-S6) AND Reciprocal-Progeny
- Upland SA-Lines (obsolete cultivars)
- Upland SA-Lines (obsolete cultivar) X Upland SA-Lines (obsolete cultivars) AND Reciprocal-Progeny
- Upland RBTN-NMSU Line X Upland SA-Lines (obsolete cultivars) AND Reciprocal-Progeny

✓ Cleaning lines of AP (transgenic contamination – USDA-ARS and UC Davis)

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Additional Research of Inheritance, Genetic and QTL Mapping for FOV race 4 Resistance Ongoing

Molecular marker such as Microsatellite or SSR

SSR marker evaluated on susceptible (S) Upland Shorty [No. 1, (*Gossypium hirsutum* L.)]; resistant (R) Pima-S6 [No. 2, (*G. barbadense* L.)], and R F_2 (Shorty x Pima-S6) single plants.

- Previous research identified/developed approximately 150 SSRs published (www.cottongen.com) with Major and Minor association to Disease Resistance which include all FOV races and root-knot nematode (RKN)
- SSR markers have been and could be used for marker assisted selection or MAS.

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Reported Loci/genes associated to FOV Resistance

Different gene-specificity for FOV resistance:

- *Fov1* (race 1) – chromosomes 6,8,11, **12, 16** and 19 (Ulloa et al., 2011; Wang et al. 2017 submitted).
- *Fov3* (race 3) – Chromosomes – Ongoing (Uzbek - USDA Collaboration)
- *Fov4* (race 4) – chromosomes 3,6,8,**14** and **17** (Ulloa et al. 2013; Wang et al. 2014; Ulloa et al. 2016; Wang et al. 2017 submitted).
- *FW*^{R} (race 7) - chromosome 17 (Wang et al., 2009).
- Australian race of *Fov* – chromosomes 6, 22, and 25 (Becerra et al., 2012).

- Explaining 10% - 80% FOV4 resistance variation.
- Approximately – 10 SSRs with major and 55 with minor Association with FOV resistance.

Information about FOV4 Research - California Selected Publications

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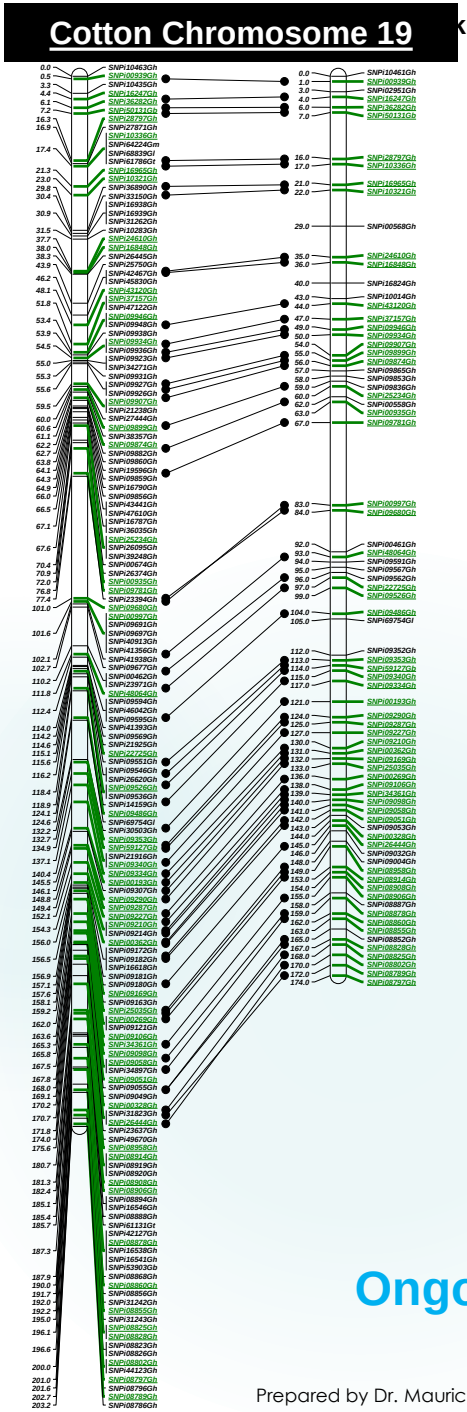

Biomarker - SNP Discovery

Map molecular markers such as SNPs using the 63K Array Cotton Chip

Approach to map more than 25,000 SNPs on the 26 Cotton Chromosomes using around 1,900 lines, progeny, and accessions

Examples of mapping Populations or progeny-lines from different cross-combinations

- Acala Phytogen 72 x Upland Stoneville 474
- Acala Phytogen 72 x NM67
- TM-1 x Pima-S6
- Upland TM-1 x Pima 3-79 (USDA-ARS)
- Pima-S7 x Acala NemX (UC Riverside)
- Cotton Accessions from the Cotton Collection or SA-lines

Ongoing Genetic Mapping and QTL Analyses

Prepared by Dr. Mauricio Ulloa, USDA, ARS

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Agriculture Research Service

FOV4 Gene Expression

Next-Gen Sequencing Illumina MiSeq and HiSeq

Discovery of genes involved in FOV race 4 (FOV4) resistance for molecular breeding

Collection of infected roots with FOV races 1 and 4, and non-infected roots

		Inoculation			Control	Entry
Entry		FOV race 1		FOV race 4	No Inoculation	Total
Pima-S6 (PS6)		5 plants		5 plants	5 plants	15
Pima-S7 (PS7)		5 plants		5 plants	5 plants	15
Pima 3-79		5 plants		5 plants	5 plants	15
Upland TM-1		5 plants		5 plants	5 plants	15
Upland Shorty		5 plants		5 plants	5 plants	15
Acala FBCX-2b		5 plants		5 plants	5 plants	15

- PS7 and 3-79 are susceptible to FOV4 and are tolerant to FOV race 1 (FOV1), but 3-79 is more susceptible to race 1 than PS7.
- Upland Shorty and TM-1 are very susceptible to FOV1. Shorty also is susceptible to FOV4.
- FBCX-2 (tolerant to race 1) and TM-1 are moderately tolerant to FOV4. TM-1 is less tolerant than FBCX-2 for race 4.

Prepared by Dr. Mauricio Ulloa, USDA, ARS

Next Generation Sequencing

Illumina MiSeq & HiSeq Sequencers

Infected Root and Non-infected Root Genes (Transcriptome) – Many TB of data

from FOV4 Resistant and Susceptible genotypes from Upland and Pima Cottons

Exploring gene diversity and discovering molecular markes [SNP-Biomarkers] for assisting breeding

SNP-Biomarkers target important genes of different plant functional processes

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SUMMARY

- **Investigate the Inheritance of FOV4.**
  - Different disease response between Upland vs. Pima cottons
  - Improving assay & selection criteria of tolerant/resistant Upland/Acala cottons during the breeding process
  - Pathogenicity evaluation of races and new FOV biotypes
- **Discover candidate genes involved in FOV resistance.**
  - Validate Identified SNPs/Alleles/Genes from the SNP63k array and Genome Reference(s) on diverse Genotypes
  - Use of Next-Gen Sequencing (NGS) for gene discovery
- **Develop Upland germplasm resistant to FOV.**
  - Continue searching for new sources of FOV resistance

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