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CALIFORNIA **Riverside**



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UNIVERSITY

➤ 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



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



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 F₁ and F₂. (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, 2013.
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



2007/2008



- PUBLIC PIMA GERMPLASM LINES JOINTLY 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 JOINTLY 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. Vincent)

➤ Projected New Pima Releases

- **2018 Pedigree: Pima-S6 x Pima-S7**

Field Site with Fusarium wilt - FOV4



Susceptible

Resistant

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

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)



Susceptible Check
Pima-S7 (PS7)

Resistant Check
Pima-S6 (PS6)



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

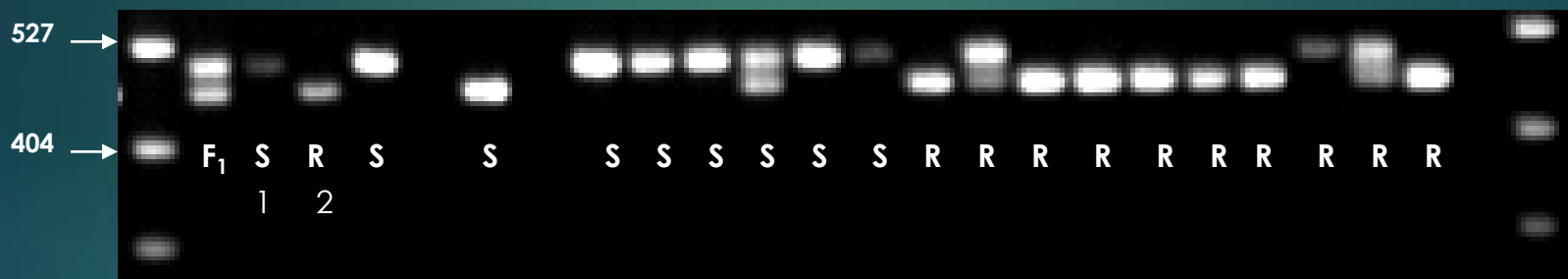
✓ **Cleaning lines of AP (transgenic contamination – USDA-ARS and UC Davis)**

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



bp

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 F₂ (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.

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

2005	Kim, Y. ¹ , R.B. Hutmacher ^d , R.M. Davis ^d . 2005. Characterization of California Isolates of <i>Fusarium oxysporum</i> f. sp. <i>Vasinfectorum</i> . Plant Disease 89:366-372.	2009	Ulloa, M., R. Percy, R.B. Hutmacher, J. Zhang, R.M. Davis, S.D. Wright. 2009. Registration of Four Pima Cotton Germplasm lines (SJ-07P-FR01 – FR04) Possessing Good Levels of Resistance to Fusarium wilt race 4 With Moderate Yields, and Good Fibers. J. Plant Registration. 3(2):198-202.
2005	Hutmacher, R.B. ^d , R.M. Davis ^d , S.D. Wright ^g , M. Ulloa ^c , D.S. Munk ^g , B.H. Marsh ^g , B.A. Roberts ^g , R.N. Vargas ^g , M.P. Keeley ^b . 2005. New Fusarium Issues for California Cotton. Cotton Industry – National Cotton Council and J. Cotton Sci.	2011	Bennett, D.W. Spurgeon, W.R. DeTar, J.S. Gerik, R.B. Hutmacher, B.D. Hanson. 2011. Efficacy of four soil treatments against <i>Fusarium oxysporum</i> f. sp. <i>Vasinfectorum</i> Race 4 on cotton. J. Plant Disease. 95:967-976..
2006	Ulloa, M. ^d , R.B. Hutmacher ^d , R. M. Davis ^g , S.D. Wright ^g , R. Percy ^c , B. Marsh ^g . 2006. Breeding for Fusarium Wilt Race 4 Resistance in Cotton Under Field and Greenhouse Conditions. J. Cotton Sci. 10:114-127.	2011.	Ulloa, M., C. Wang, R.B. Hutmacher, S.D. Wright, R. Michael Davis, S.A. Saski, P.A. Roberts. 20___. Mapping Fusarium Wilt Race 1 Resistance Genes in Cotton by Inheritance, QTL, and Sequencing Composition. Molec. Genetic Genomics. 286(1): 21-36.
2006	Hutmacher, R.B. ^a , R.N. Vargas ^g , S.D. Wright ^g . 2006. Methods to Enable the Coexistence of Diverse Cotton Production Systems. Agricultural Biology In California Series, Publication # 8191. Univ. of CA Div. Agric. Nat. Resources, http://anrcatalog.ucdavis.edu	2011	Bennett, R.S., W. O'Neill, L. Smith, R.B. Hutmacher. 2011. Commercial detergents effective against conidian and chlamydospores of <i>Fusarium oxysporum</i> f. sp. <i>Vasinfectorum</i> . J. Cotton Science. 15:162-169
2006	Ulloa, M. ^a , R. Percy ^c , R.B. Hutmacher ^g , R.G. Cantrell ^h . 2006. Registration of SJ-U86 Cotton Germplasm Line with High Yield and Excellent Fiber Quality. Crop Sci. 46:2336-2338.	2013	Ulloa, M., Hutmacher, R.B., Roberts, P.A., Wright, S.D., Nichols, R.L. and Davis, R.M. 2013. Inheritance and QTL mapping of Fusarium wilt race 4 resistance in cotton. Journal of Theoretical and Applied Genetics. 126:1405-1418.
2007	Ulloa, M. ^a , R.G. Percy ^c , R.B. Hutmacher ^g , R.G. Cantrell ^h . 2007. Notice of release of one Upland cotton germplasm line (SJ-U86) for California possessing superior lint yield, fiber traits and heat tolerance. Germplasm Release. Crop Sci.: 47:106-107.	2013	Hutmacher, R.B., Ulloa, M., Wright, S.D., Campbell, B.T., Percy, R.G., Wallace, T., Myers, G., Bourland, F., Weaver, D., Chee, P., Thaxton, P., Zhang, J., Smith, W., Dever, J., Kuraparthi, V., D. Bowman, D. Jones, J.Burke. Elite Upland Cotton Germplasm-Pool Assessment of Fusarium wilt resistance in California. Agronomy Journal 105:1635-1644. 2013.
2007	Ulloa, M. ^a , Percy, R. ^c , R.B. Hutmacher ^g , J.F. Zhang ^e . 2007. The Future of cotton breeding in the Western United States. Paper #1355. Proc. World Cotton Research Conference IV, September 11-14, 2007, Lubbock, TX, Cotton Council International, 15 pp. electronic: http://www.icac.org/meetings/wcrc/wcrc4/presentations/data/html	2015	Cianchetta, A., T.W. Allen, R.B. Hutmacher, R.C. Kemerait, T.L. Kirkpatrick, G.W. Lawrence, K.S. Lawrence, J.D. Mueller, R.L. Nichols, M. Olsen, C. Overstreet, J.E. Woodward, R.M. Davis. 2015. Survey of <i>Fusarium oxysporum</i> f. spp. <i>Vas infectorum</i> in the United States. J. Cotton Science. 19: 328-336.
2007	Bennett, R.S. ^a , R.M. Davis ^g , R.B. Hutmacher ^g . 2007. Fusarium oxysporum f, Sp, vasinfectorum Race 4 in California: A Reemerging Threat to Cotton Production. Paper #1383. Proc. World Cotton Research Conference IV, September 11-14, 2007, Lubbock, TX, Cotton Council International, 9 pp. electronic: http://www.icac.org/meetings/wcrc/wcrc4/presentations/data/html	2015	Ulloa, M., Hutmacher, R., Percy, R.G., Wright, S., Burke, J.J. Release of Pima SJ-FR05, Pima SJ-FR06, Pima SJ-FR07, Pima SJ-FR08, and Pima SJ-FR09 Pima cotton with improved FOV4 resistance, and good lint yield and fiber quality. Germplasm Release. p.1-2. 2015.
2008	Bennett, Rebecca S., R.B. Hutmacher, R. Michael Davis. 2008. Seed Transmission of <i>Fusarium oxysporum</i> f. sp. <i>vasinfectorum</i> Race 4 in California. J. Cotton Science. 12: 160-164.	2016	Ulloa, M., Hutmacher, R.B., Percy, R.G., Wright, S.D., Burke, J.J. Registration of five pima cotton germplasm lines (SJ-FR05 - FR09) with improved resistance to fusarium wilt race 4 and good lint yield and fiber quality. Journal of Plant Registrations. 10:154-158. 2016.
2008	Ulloa, M., Percy, R.G., Hutmacher, R.B., Wright, S. and Davis, M. 2008. Release of SJ-07P-FR01, SJ-07P-FR02, SJ-07P-FR03, and SJ-07P-FR04 'Cotton'. USDA Germplasm Release. Pp. 79-81.	2016	Ulloa, M., Wang, C., Saha, S., Hutmacher, R.B., Stelly, D.M., Jenkins, J.N., Roberts, P. Analysis of root-knot nematode and fusarium wilt disease resistance in cotton (<i>Gossypium</i> spp.) using chromosome substitution lines from two alien species. Genetica. 144(2):167-179. 2016
2008	Gilbert, C.A., N. Zhang, R.B. Hutmacher, R. M. Davis, C.D. Smart. 2008. Development of a DNA-based Macroarray for the Detection and Identification of <i>Fusarium oxysporum</i> f. sp. <i>vasinfectorum</i> in cotton tissue. J. Cotton Science 12:165-170.		

Cotton Chromosome 19

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



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.



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



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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