

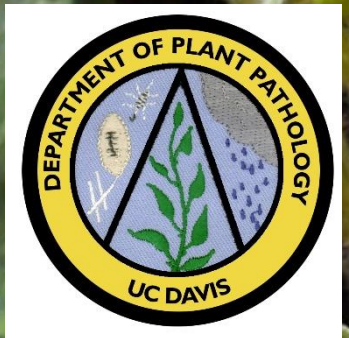
Cucumber Green Mottle Mosaic Virus: A cucurbit infecting virus new to North America

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Staff Research Associate

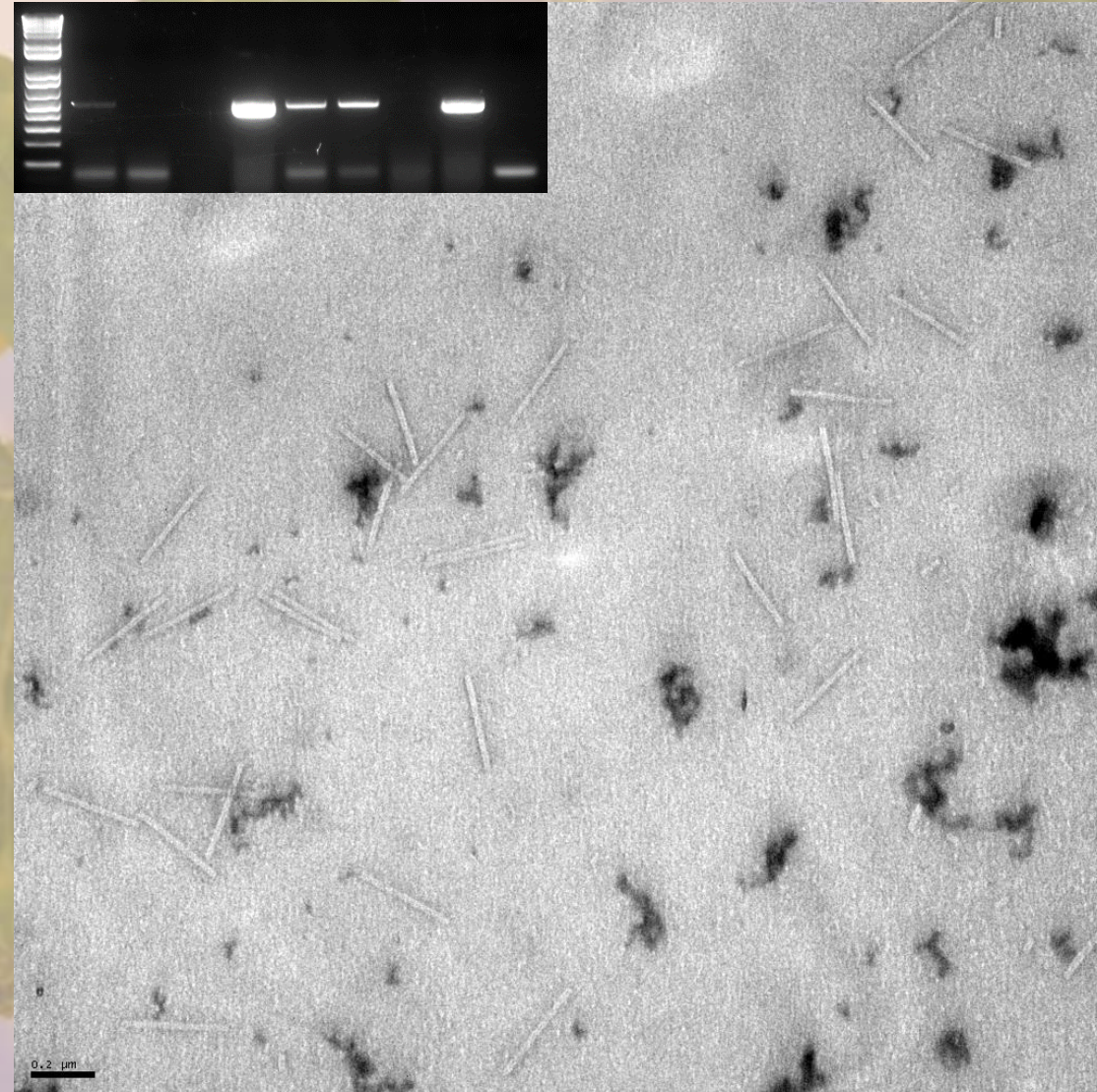
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Yolo County 2013

- Commercial cucurbit seed fields with unusual symptoms in Yolo County
- CDFA virologist Dr. Tongyan Tian was called to examine the field
- TEM imaging suggested direction, RT-PCR confirmed the virus
- Dr. Bryce Falk was contacted to do a transmission study in the CRF



TEM and cucumber images by Dr. Tongyan Tian, CDFA

plant disease

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Disease Notes

First Report of *Cucumber green mottle mosaic virus* on Melon in the United States

T. Tian and K. Posis, Plant Pest Diagnostics Center, California Department of Food and Agriculture (CDFA), Sacramento 95832; C. J. Maroon-Lango, V. Mavrodieva and S. Haynes, USDA APHIS (PPQ), BARC-East, Beltsville, MD 20705; and T. L. Pittman and B. W. Falk, Department of Plant Pathology, University of California, Davis 95616

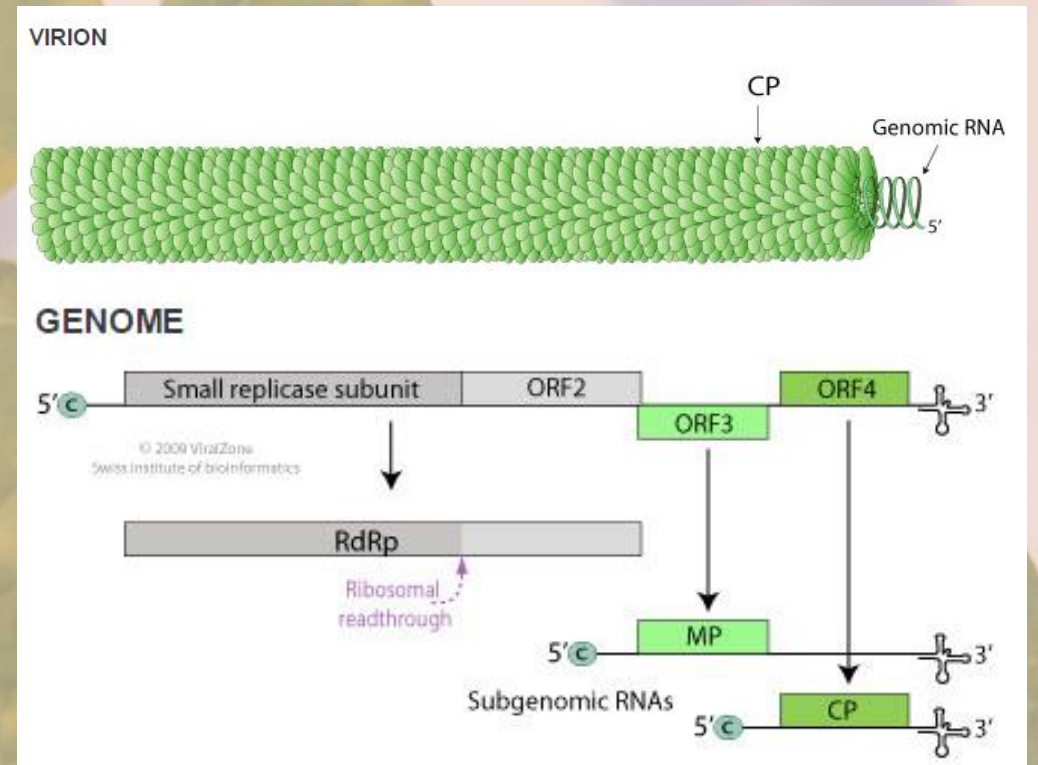
2014

- Dr. Brenna Aegerter examined seedless watermelon fields in San Joaquin County
- Samples arrived in August, tested positive for CGMMV
- More samples arrived other counties, positive samples found from Fresno and Kern counties



Cucumber Green Mottle Mosaic Virus

- 6422 nucleotide genome
- Group IV +ssRNA virus
- Family *Virgaviridae*
 - Genus *Tobamovirus*



Top image from viralzone.expasy.org, produced by the Swiss Institute of Bioinformatics.

Bottom image from NCBI.NIH.gov accession NC_001801.1, National Center for Biotechnology Information.

Disease Cycle



Infected fruit



Contaminated seeds



1%-5% infected seedlings

Secondary spread by mechanical transmission, infection rates up to 100%



Symptoms

- Light mottling and mosaic
- Uneven fruit ripening, necrotic flesh
- Lesions on peduncle





Cucumber



Melon



Watermelon

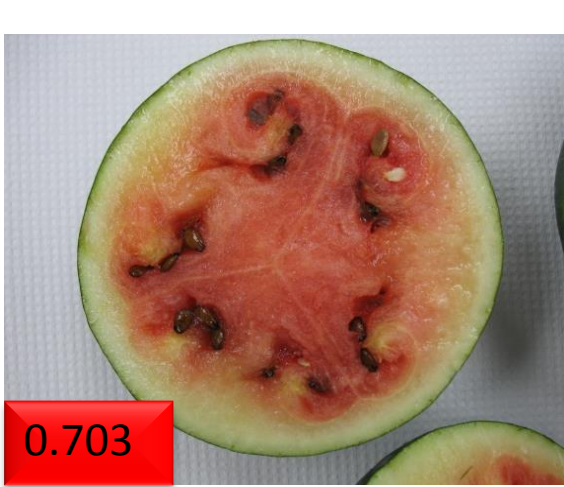




0.088



0.094



0.703



0.146



0.147



0.147



1.418



1.688

Watermelons (Sugar Baby) from Yolo, 2013 and CGMMV ELISA O.D. Data and images by Dr. Tongyan Tian, CDFA



Field infection management

- Infected fields are immediately destroyed (herbicide, tillage)
- Host species (melon, cucumber, squash, etc) can't be grown at site for a period of 2-3 years
- Non-host crops allowed
- Equipment used in the field has to be decontaminated on site with 10% bleach solution

Research Objectives

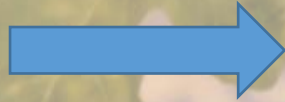
- Validate testing methods (ELISA, RT-PCR, immunostrips)
- Validate seed sampling and testing to assess and quantify virus load
- Confirm host range bioassays
- Phylogenetic analysis of CA introductions-detection of all genotypes
- Soil detection of virus
- Produce new antibodies to CGMMV for detection assays

Contained Research Facility

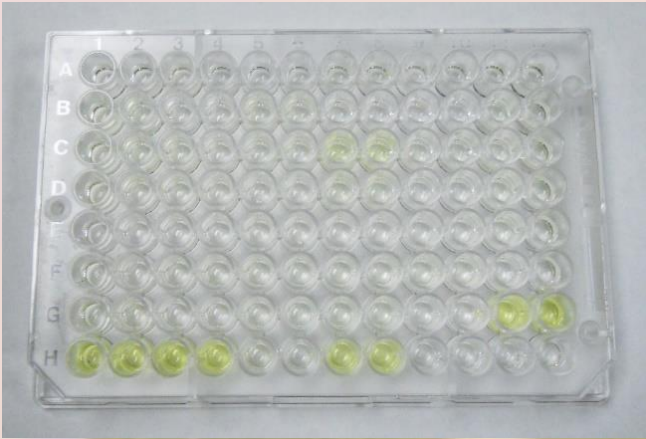
- USDA listed quarantined pathogen
- All work inside biosecurity 3 facility for plant pests and diseases
- Negative air flow, air filtration
- All water sterilized before entering municipal system
- No organisms or infectious material leave
- Oversight by USDA, CDFA, Yolo County ag commissioner



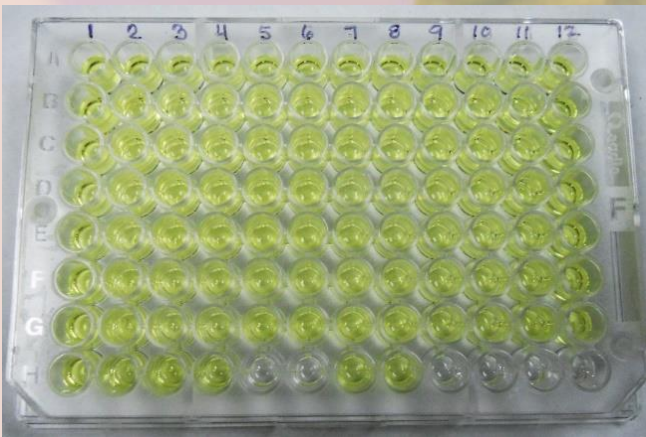
Grow out experiments



Seedling ELISA results



Seed ELISA results



Individual Seedlings

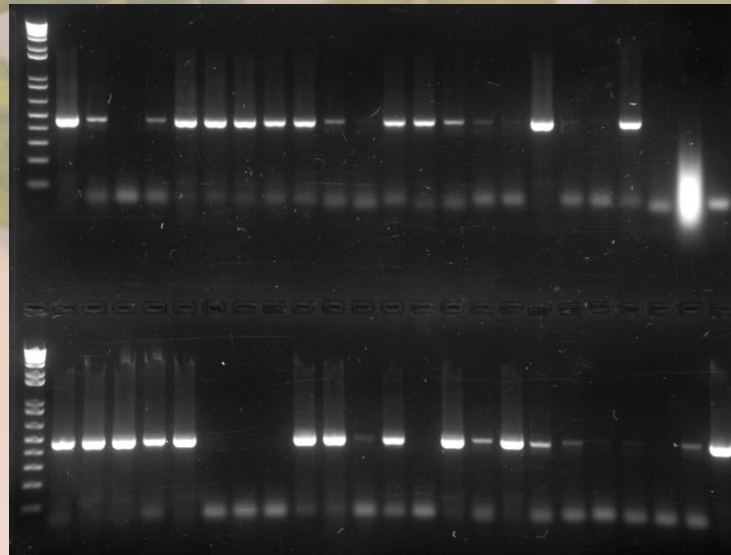
Individual Seeds

Homogenize each sample in buffer

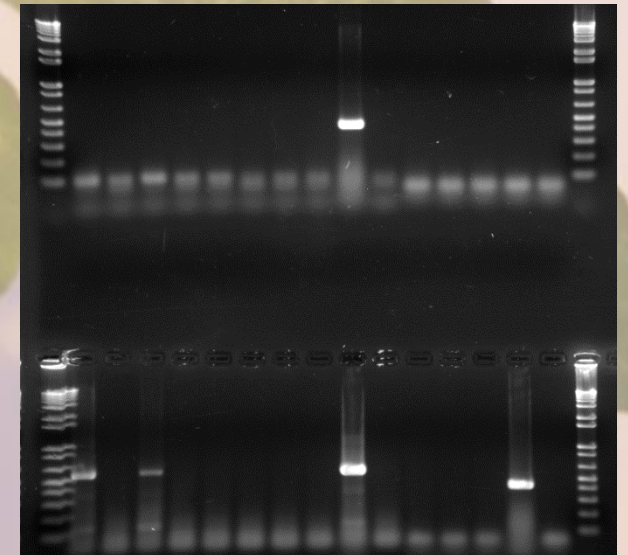
ELISA testing

Total RNA extraction, RT-PCR

Seed RT-PCR results



Seedling RT-PCR results



Experiment	Date Planted	Variety	Type	Number Tested	ELISA positive	RT_PCR positive
1	9/25/2014	Laredo	seedlings	55	1 (1.8%)	1 (1.8%)
1	9/25/2014	Laredo	seeds	25	25 (100%)	6 (24%)
2	10/14/2014	watermelon	seedlings	86	4 (4.65%)	4 (4.6%)
2	10/14/2014	watermelon	seeds	96	96 (100%)	39 (40.6%)
3	10/17/2014	Laredo	seedlings	72	1 (1.4%)	1 (1.4%)
3	10/17/2014	Laredo	seeds	116	116 (100%)	38 (32.8%)
4	1/26/2015	Suski melon	seedlings	85	0	0
4	1/26/2015	Suski melon	seeds	91	100%	52 (57%)

Host Index Testing

Testing herbaceous species for a suitable candidate for host index testing. Also testing breeder lines for resistance to CGMMV

Nicotiana tabacum 'Glurk'
N. tabacum 'V20'
N. tabacum
N. debneyi
N. rustica
N. glutinosa
N. edwardsonii
Datura metel
D. stramina
Spinacia oleracea
Chenopodium amaranticolor
C. quinoa
C. capitatum
Solanum physalifolium



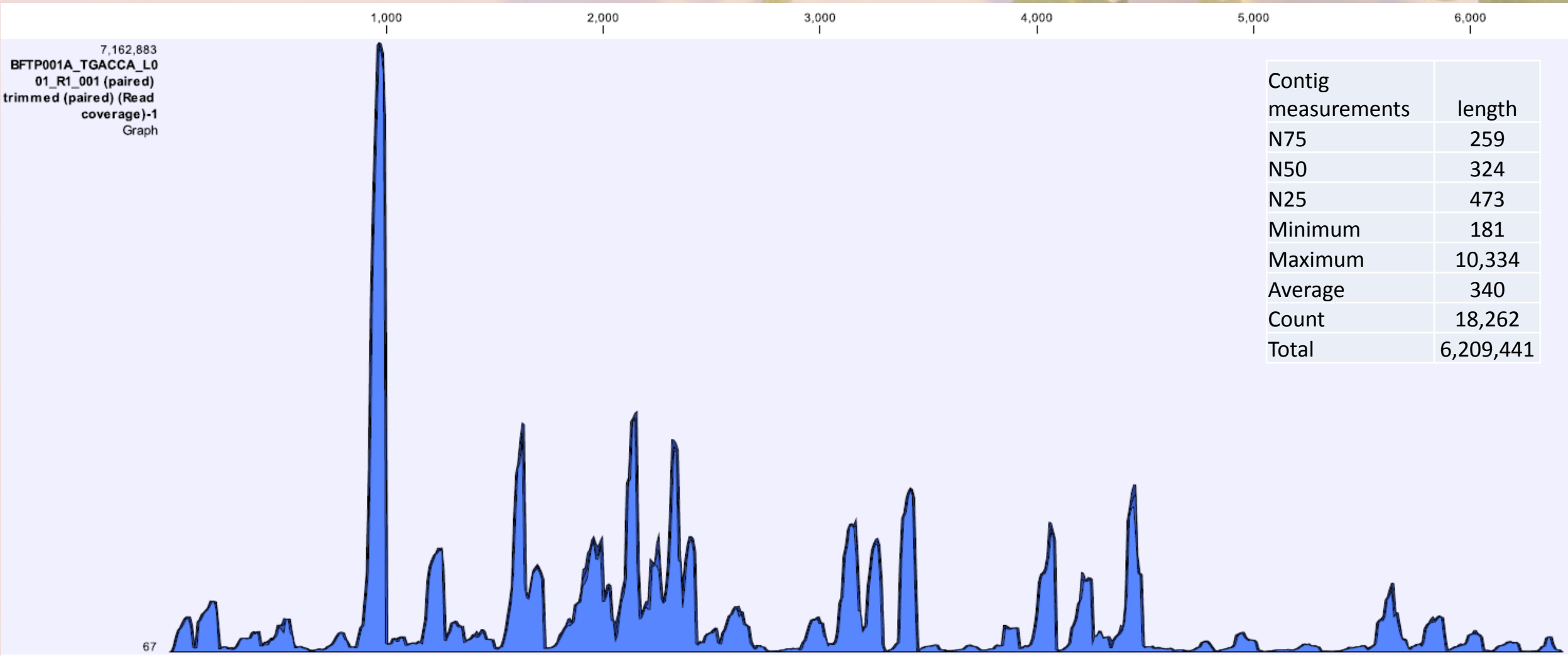
Variety resistance testing

- Screening varieties for resistance to virus infection
- Rub inoculate seedlings with virus, test for infection at 7 days





Sanger sequencing using primer walking to generate the Yolo 2013 sequence covering nucleotides 43-6343; ongoing to ensure appropriate coverage of all regions.

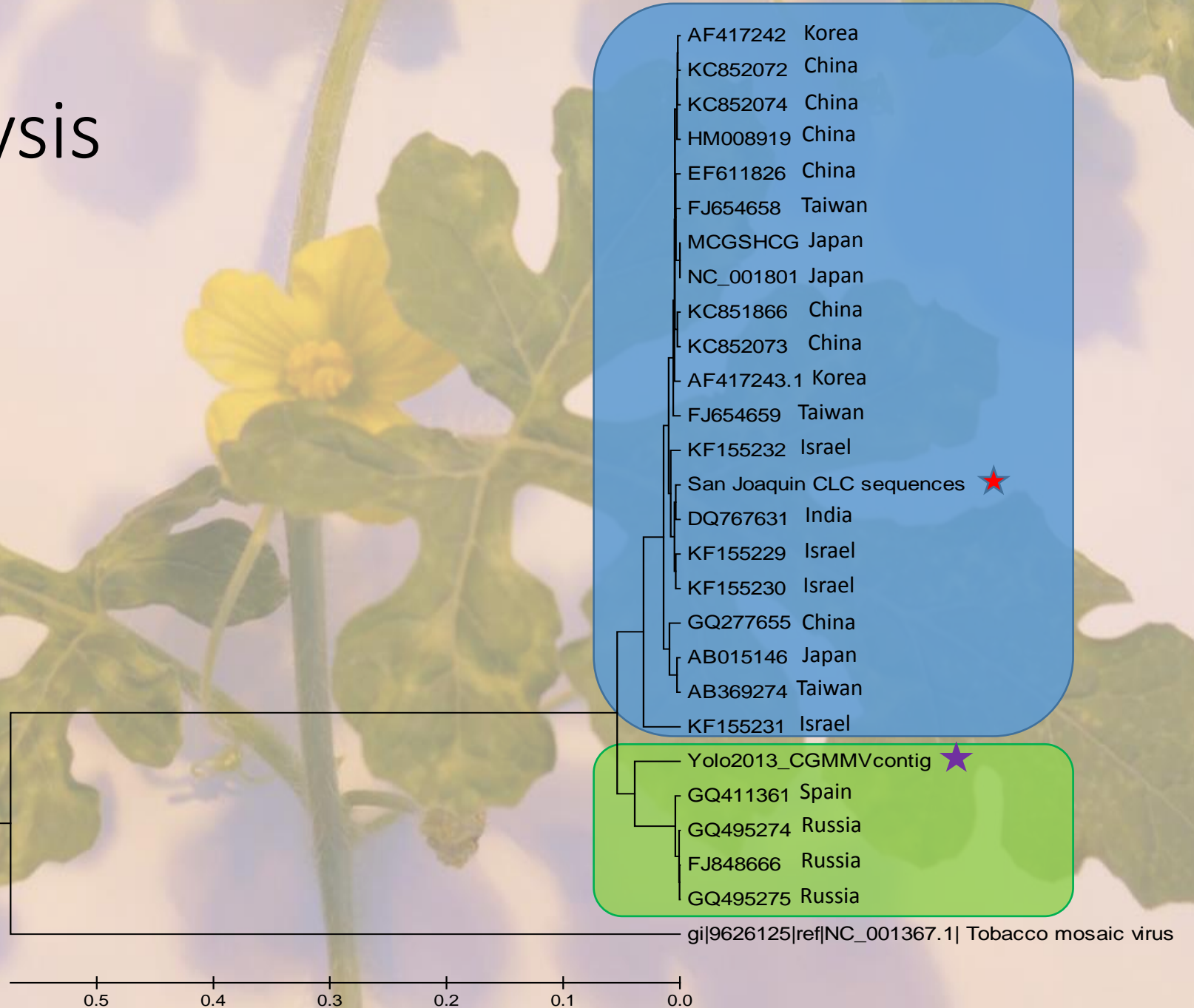


Deep sequencing reads mapped to CGMMV genome from San Joaquin 2014 isolate, using Illumina HiSeq platform. Sequence analysis is ongoing.

Sequence Analysis

Trimmed sequences covered nucleotides 43-6343

Yolo and San Joaquin isolates share 89% sequence similarity



Ongoing and Future research

- Permit acquired for sampling soil from infected fields to examine long term viability of virus in soils
- Greenhouse experiments with soil and plant debris for soil infectivity assay
- Continue grow-out assays for robustness of data, optimize diagnostic tools
- Develop CGMMV antibodies to our isolates
- Sequencing of isolates from Kern and Fresno Counties



Thank you!



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- Materials provided by Dr. Tongyan Tian, CDFA and Dr. Brenna Aegerter, UCCE San Joaquin County
- Research help and support by Falk lab

