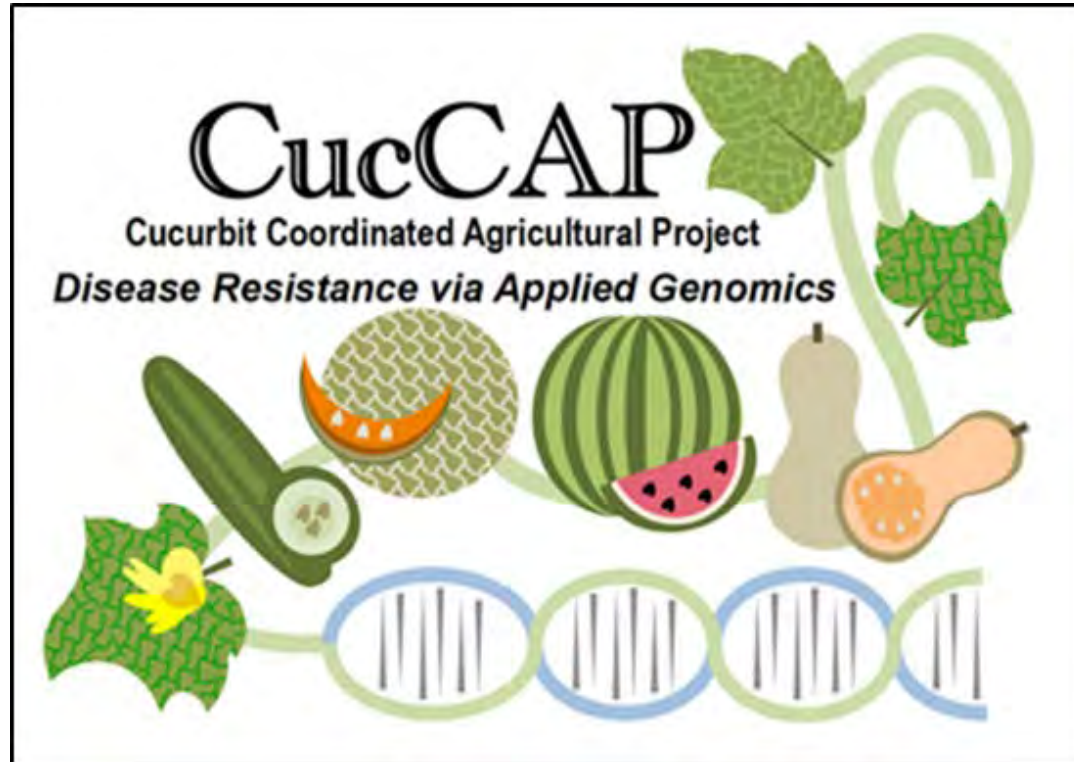


CucCAP: Leveraging applied genomics to increase disease resistance in cucurbit crops



***A collaborative project
among 21 research groups***

Participating institutions:

Boyce Thompson Institute
Cornell University
Michigan State University
North Carolina St University
Texas A&M University
University of Georgia
University of Puerto Rico
USDA-ARS – Salinas CA, Charleston
SC, Madison WI
West Virginia State University

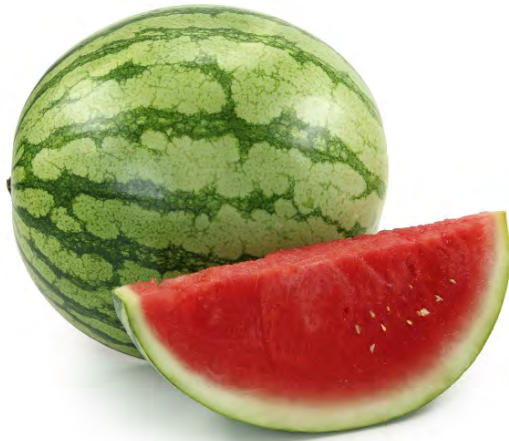
Funded by USDA-SCRI



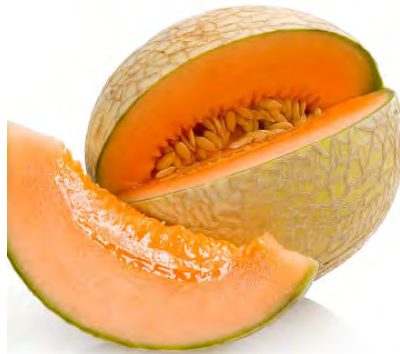
United States Department of Agriculture
National Institute of Food and Agriculture

PD: Rebecca Grumet
Michigan State University

“Advance understanding of the genomics of the Cucurbitaceae family and their application to practical breeding programs.”



watermelon



melon

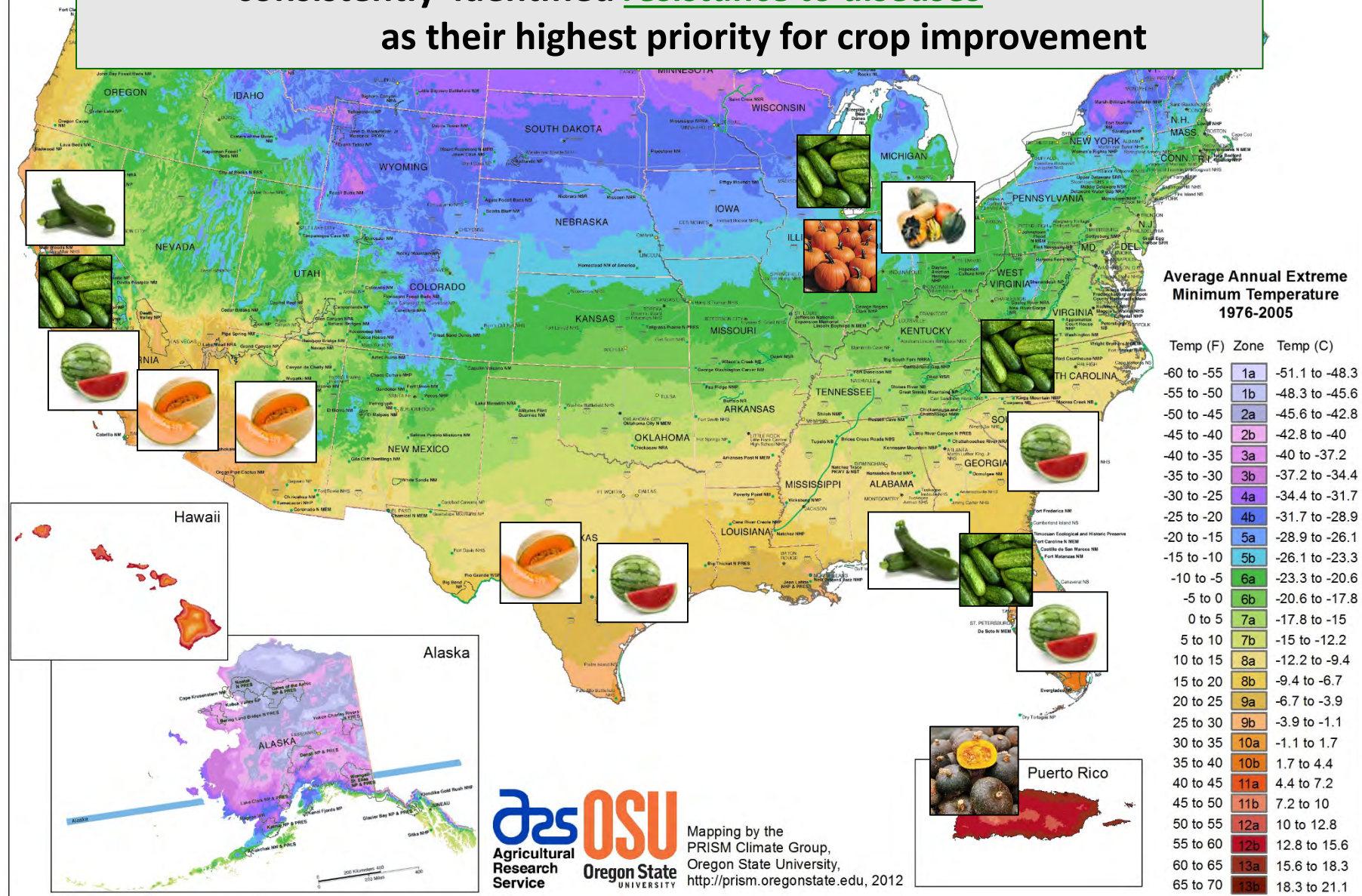


cucumber



squash, pumpkin

Survey of cucurbit growers, shippers, and processors consistently identified *resistance to diseases* as their highest priority for crop improvement



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Objectives

- I. Develop genomic and bioinformatic breeding tool kits for accelerated crop improvement
- II. Use these tools to facilitate efficient introgression of disease resistance into commercially valuable cucurbit cultivars
- III. Perform economic impact analyses of cost of production and disease control, and provide readily accessible information to facilitate disease control.

(I) Develop genomic and bioinformatic tools

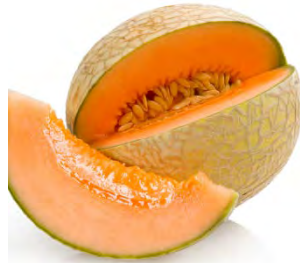
- A. *Develop sequence and phenotype databases and bioinformatics tools and provide access via the Cucurbit Genomics website*
- B. *Genetically characterize diversity of USDA PI collections of the four crops and provide a community resource for genome-wide association studies (GWAS)*

Draft genome sequences are available for the four major cucurbit species:



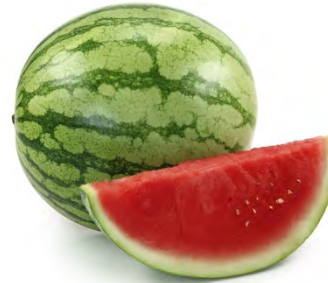
**Huang et al., 2009
Yang et al., 2012**

Cucumis sativus



Garcia-Mas et al., 2012

Cucumis melo



Guo et al., 2013

Citrullus lanatus



**Sun et al., 2017
Montero-Pau et al. 2018**

*Cucurbita maxima,
moschata, pepo*

A. Develop genomic tools and databases and provide access via the cucurbit genomics website: cucurbitgenomics.org

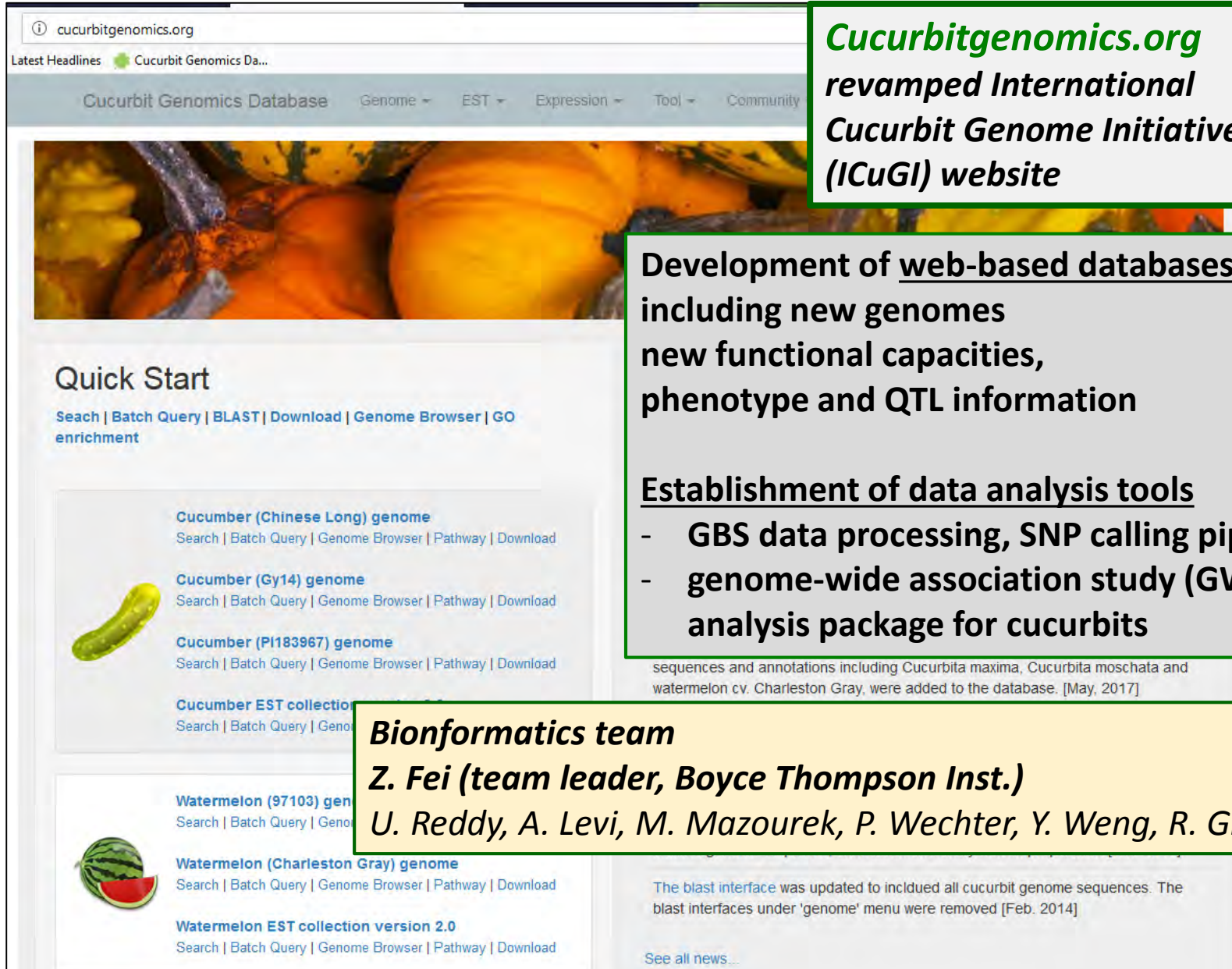
Cucurbitgenomics.org
revamped International
Cucurbit Genome Initiative
(ICuGI) website

Development of web-based databases
including new genomes
new functional capacities,
phenotype and QTL information

Establishment of data analysis tools

- GBS data processing, SNP calling pipeline
- genome-wide association study (GWAS) analysis package for cucurbits

Bionformatics team
Z. Fei (team leader, Boyce Thompson Inst.)
U. Reddy, A. Levi, M. Mazourek, P. Wechter, Y. Weng, R. Grumet



The screenshot shows the cucurbitgenomics.org website. At the top is a navigation bar with links: Cucurbit Genomics Database, Genome, EST, Expression, Tool, and Community. Below the navigation bar is a large image of various cucurbits. Underneath the image is a 'Quick Start' section with links: Search, Batch Query, BLAST, Download, Genome Browser, and GO enrichment. Below this are several featured genomic resources, each with a small image and a list of links: Cucumber (Chinese Long) genome, Cucumber (Gy14) genome, Cucumber (PI183967) genome, Cucumber EST collection, Watermelon (97103) genome, Watermelon (Charleston Gray) genome, and Watermelon EST collection version 2.0. A news section at the bottom right mentions updates to the BLAST interface.

B. Perform Genotyping by sequencing (GBS) analysis of full USDA PI collections of the four species and provide a community resource for genome wide association studies (GWAS)



Examples of fruit type variation in the cucumber PI collection

Photo: M. Colle, Michigan State Univ

Germplasm collections are a critical resource:

- Conserve biological diversity
- Provide source of valuable characteristics for plant breeders (e.g., disease resistances)

Molecular genetic characterization can help us evaluate the extent and nature of variation that exists within the collection

- Manage and preserve diversity
- Facilitate breeding efforts

Genotyping by sequencing (GBS) analysis of full USDA PI collections of the four species to assess diversity

The U.S. National Plant Germplasm System maintains

1,314 cucumber

2,043 melon

1,311 watermelon

1,580 squash (*C. pepo*, *moschata*, *maxima*)



Grow populations – *CucCAP* Crop teams

High throughput DNA preparation – *MSU*

GBS – *Cornell*

Data analysis – Genome alignment, SNP calling and processing – *Bioinformatic (Boyce Thompson Inst)*

Full collections from the U.S. National Plant Germplasm System were genotyped by GBS

	No. Pls	No. reads	Unique tags	No. used for SNP	No. SNPs	No. SNPs (bi-allelic)	Average SNP density
Cucumber (<i>C. sativus</i>)	1234	1.35 billion	31.6 million	441,021	109,058	48,533	10.6 kb
Melon (<i>C. melo</i>)	2077	1.7 billion	31.6 million	441,021	109,058	48,533	14.6 kb
Watermelon (<i>C. lanatus</i>)	1365	0.8 billion	31.6 million	441,021	109,058	48,533	15.7 kb
Squash (<i>C. pepo</i>)	830	0.95 billion	31.6 million	441,021	109,058	48,533	5.4 kb

All GBS data will be publicly available on the Cucurbit Genomics Website –

Cucumber GBS and SNP data are currently available

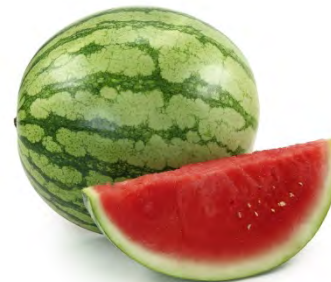
Watermelon and melon data are available upon request (not yet published)

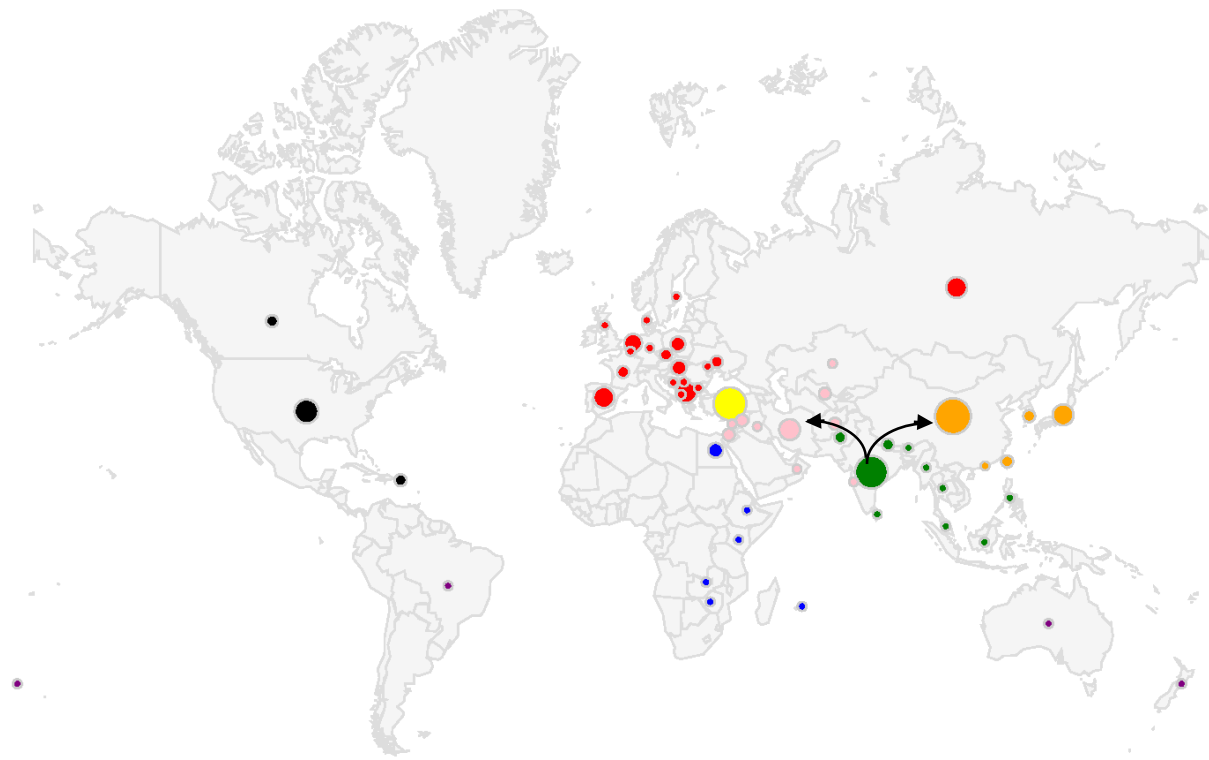
alignment
SNP calling
filtering

~ 3000 markers per chromosome



wiseGEEK





**Geographical distribution
of the 1,234 cucumber
accessions in the U.S.
National Plant
Germplasm System -
NPGS.**

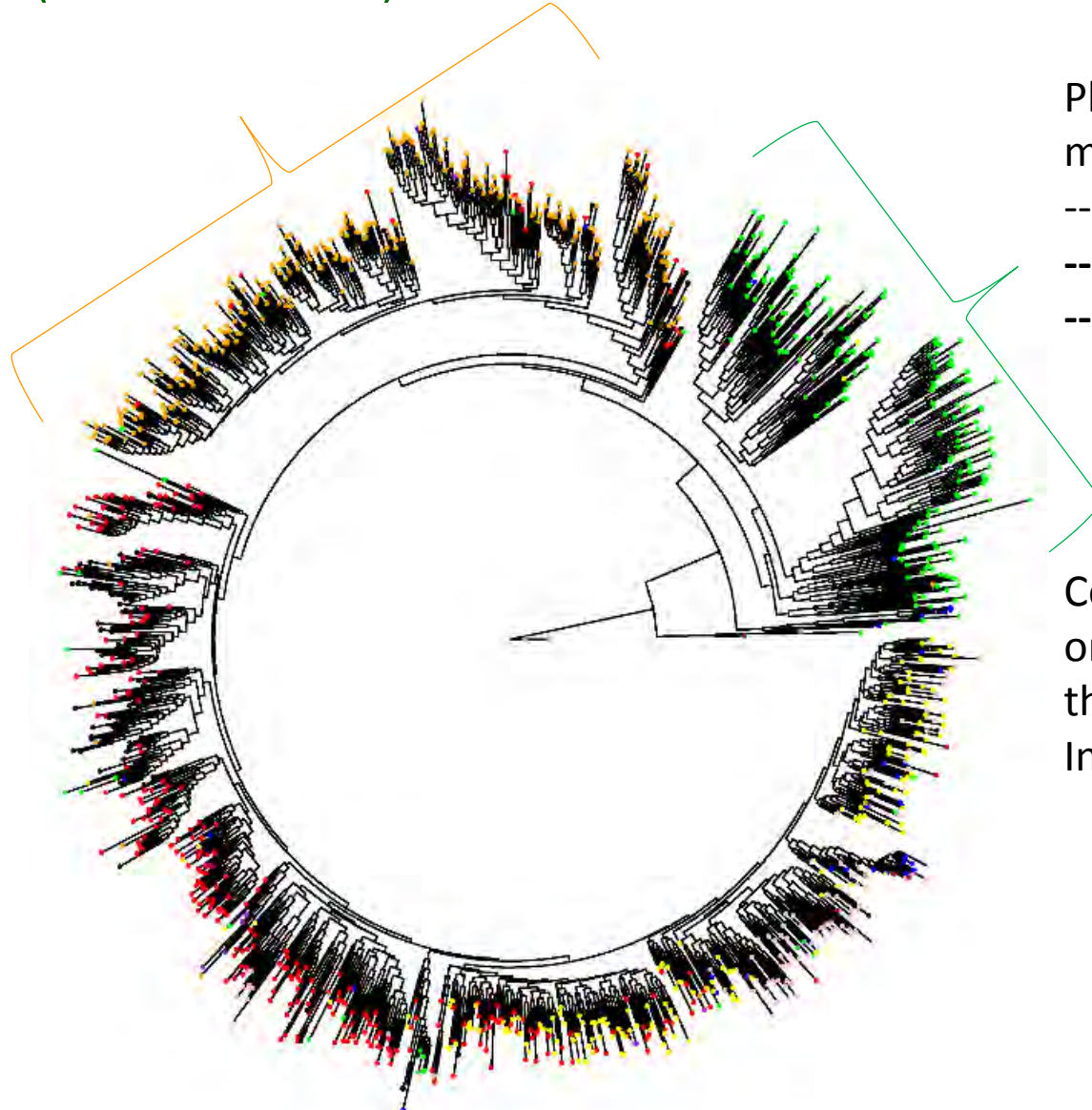
Xin Wang et al., 2018

**Cucumber originated in Asia –
primary and secondary centers of diversity in India and Southeast Asia.**

**Subsequent to domestication in India, cucumber moved both East and West, following
distinct trajectories in each case.**

Phylogenetic relationships of the U.S. cucumber collection

(1234 accessions)

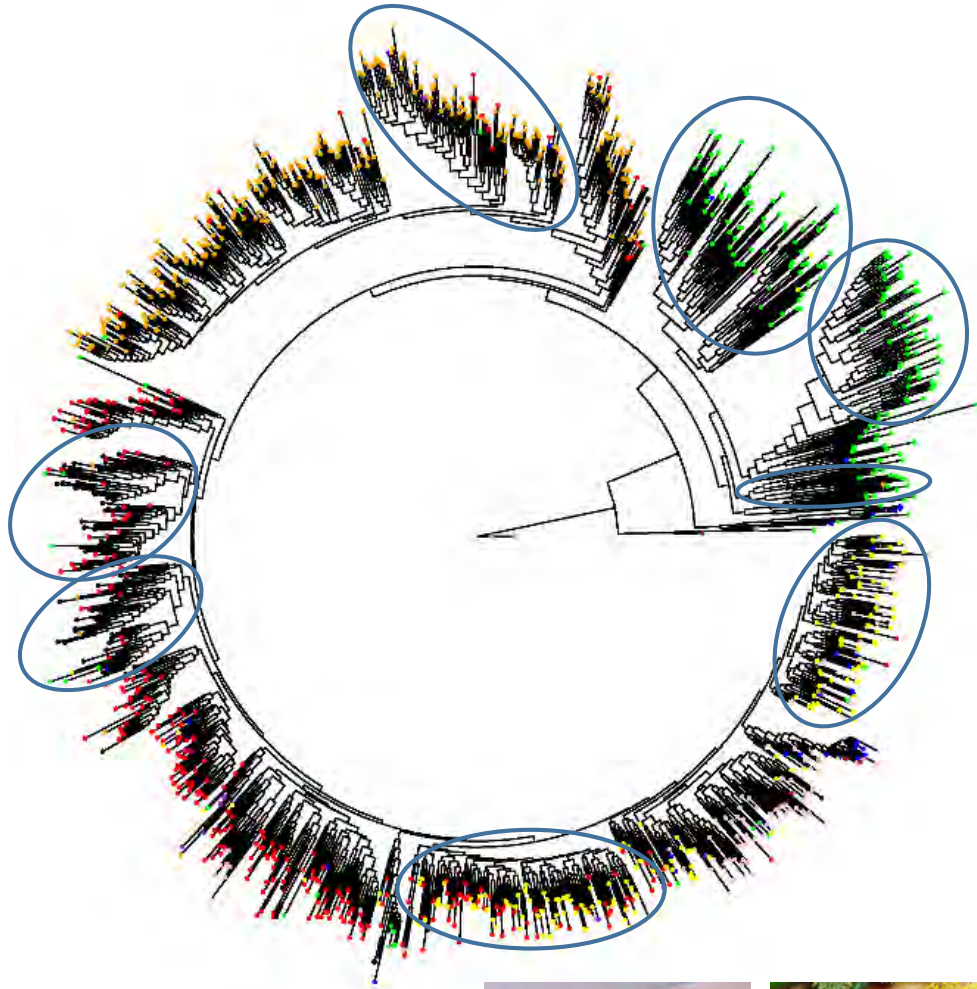


Phylogenetic analysis identified three major clades:

- **India/South Asia**
- **East Asia**
- **Central/West Asia, Turkey, Europe, Africa, North America.**

Consistent with India as the center of origin for cucumber, the clade with the deepest branches was the India/South Asia group.

Wang et al., 2018



Within the major groupings subclades could be observed

- Subclades within India showed separation reflecting geography among Indian states
- Japan and South Korea clustered separately from the rest of the East Asian samples (primarily from China)
- Turkey had two divergent subclades, one clustering with Central/West Asia, the other with Europe
- The North American accessions had two sub-clades, one largely comprised of pickling cultigens and one fresh market



Disease screening data combined with the genetic analysis allows us to ask questions about the sources of resistance and their relationships to each other

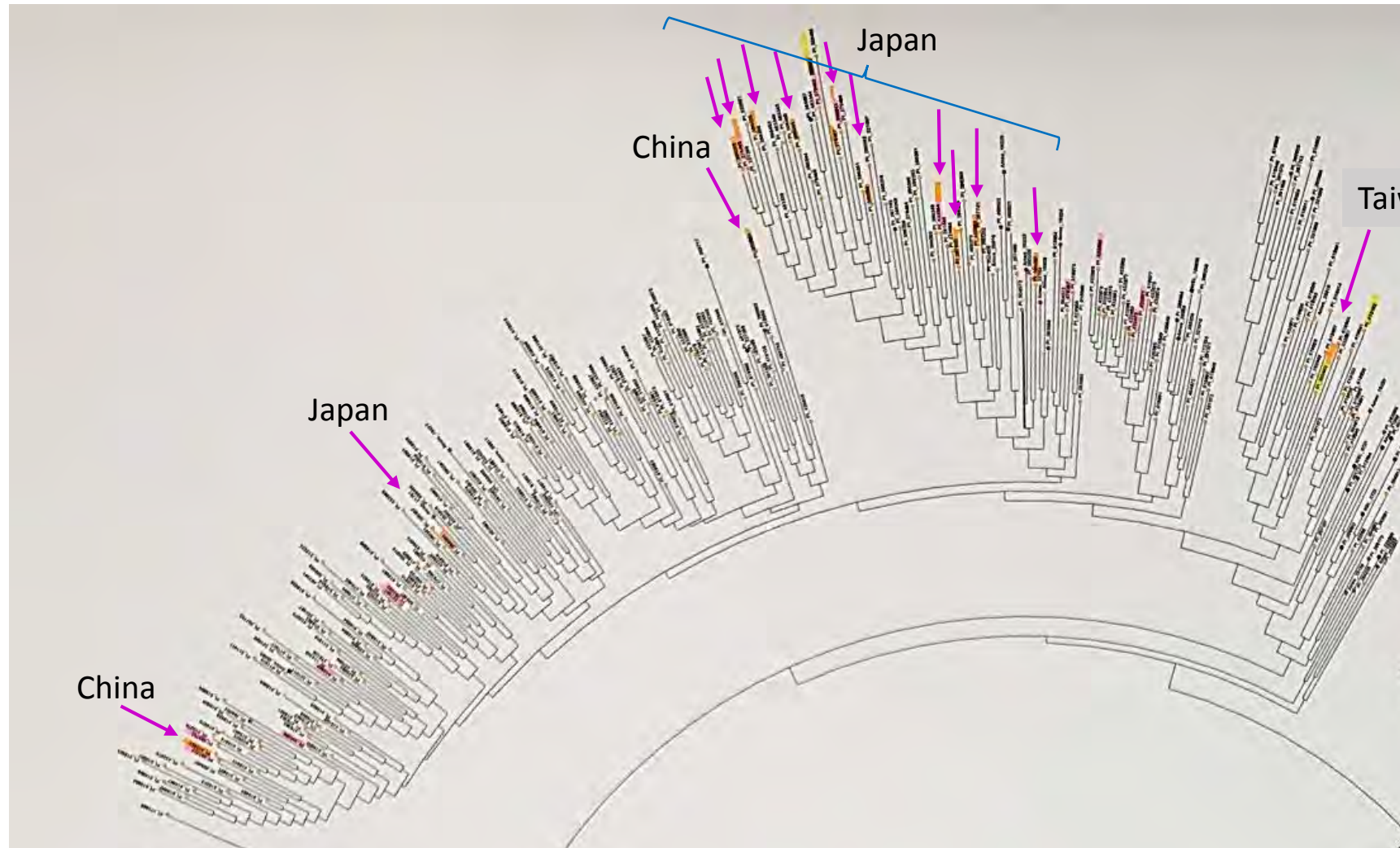
Historical data from replicated field trials produced by the Wehner lab were available for 600-700 accessions for a variety of traits, including three disease resistances:

**Downy mildew
Anthracnose
Gummy stem blight**



Images from Cornell extension and APS net

Gummy stem blight resistance



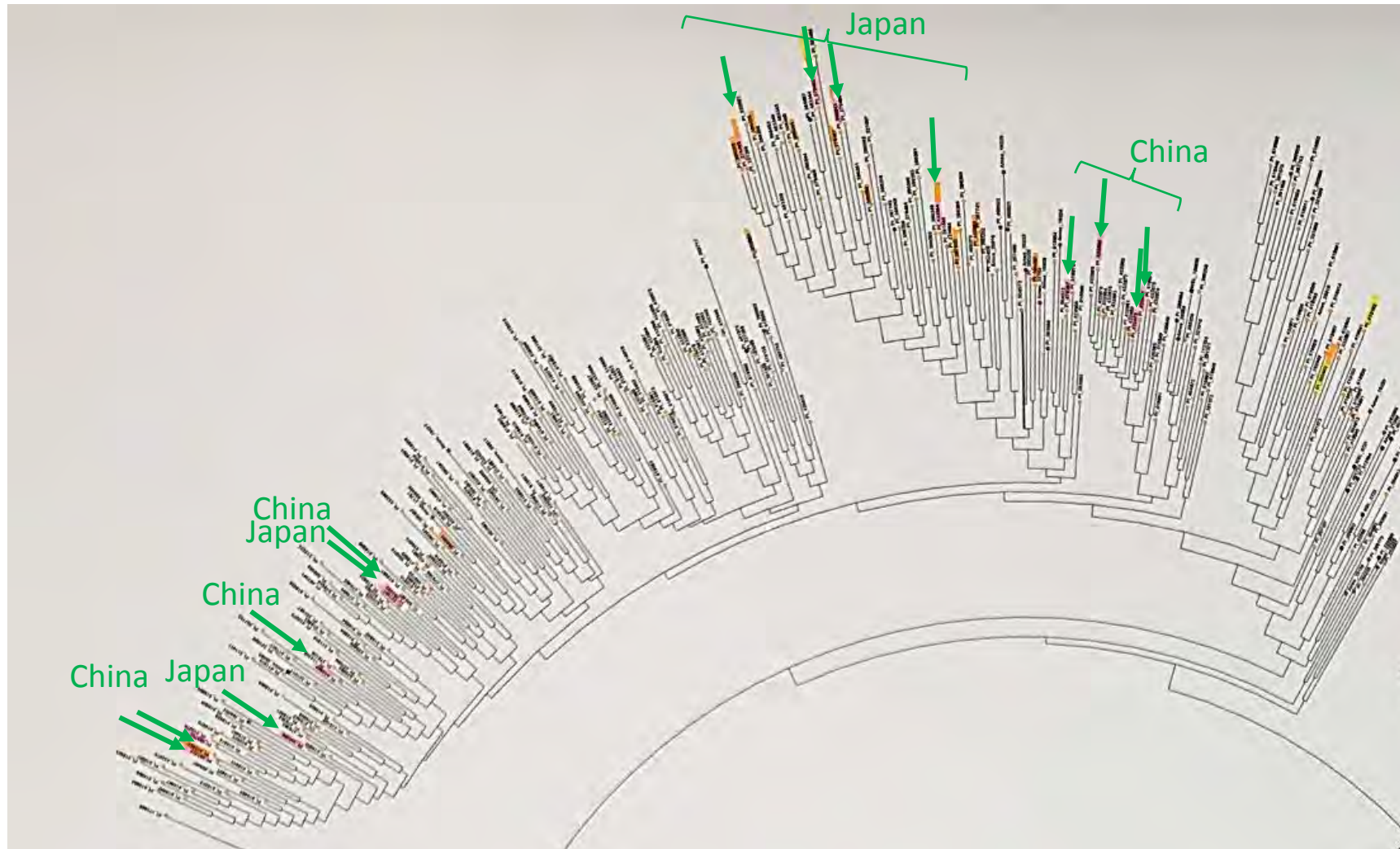
Many tightly clustered accessions

Virtually all from Japan
(reflect disease pressure? active breeding?)

A few from South Asia
(center of origin)
(3 Pakistan; 1 India)

Virtually absent from other clades and other parts of the world

Downy mildew resistance



Virtually all from East Asia found only in specific sub-clades different sub-clades than gummy stem blight

A few from South Asia

Virtually absent from other parts of the world

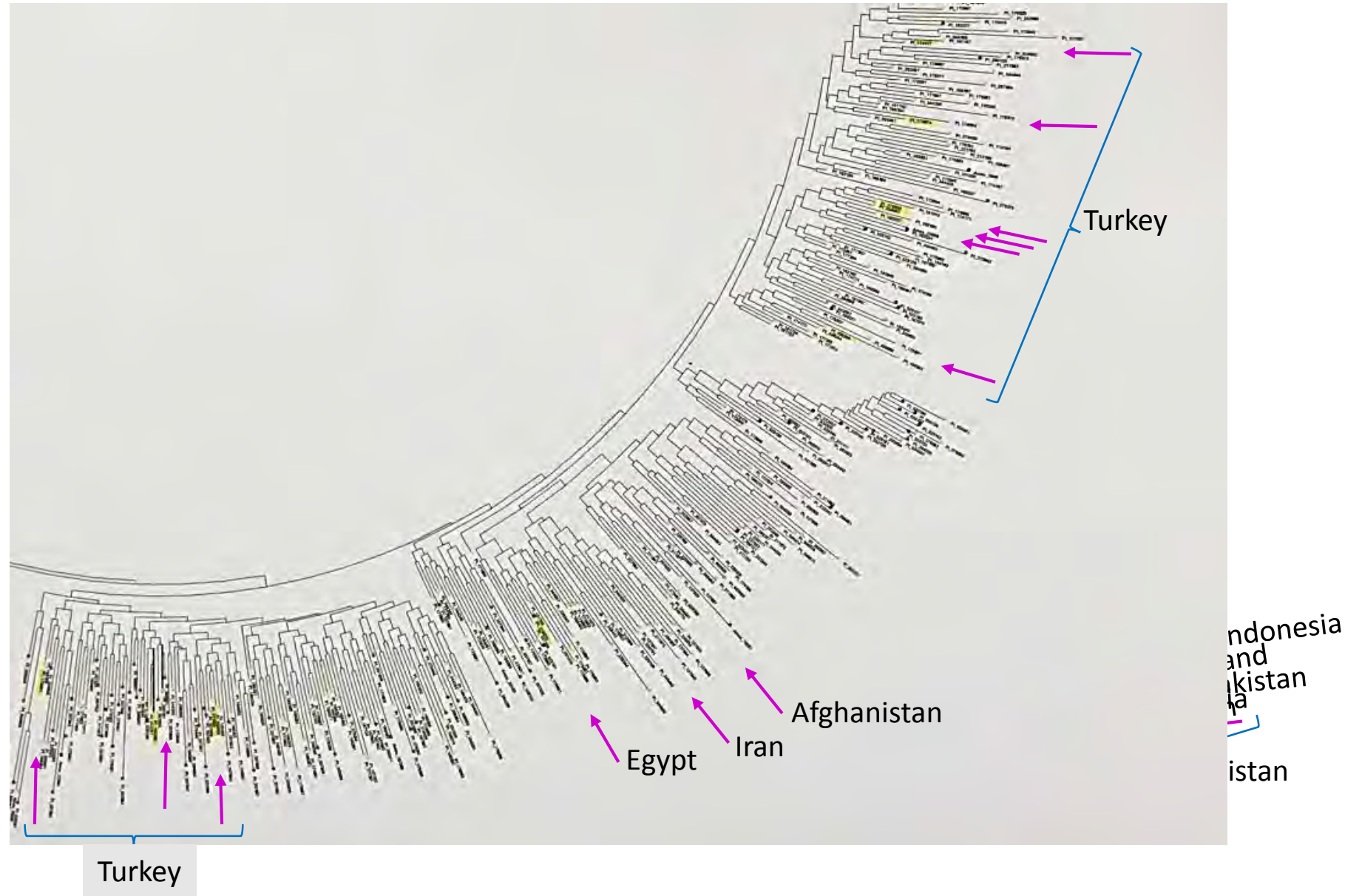
Anthracnose resistance

Numerous
accessions from
South Asia
clustered in
specific subclades

Numerous
additional
scattered
accessions from
West Asia,
Middle East and
Turkey

Virtually absent
from East Asia

Suggests origins
in South Asia and
subsequent
movement
westward



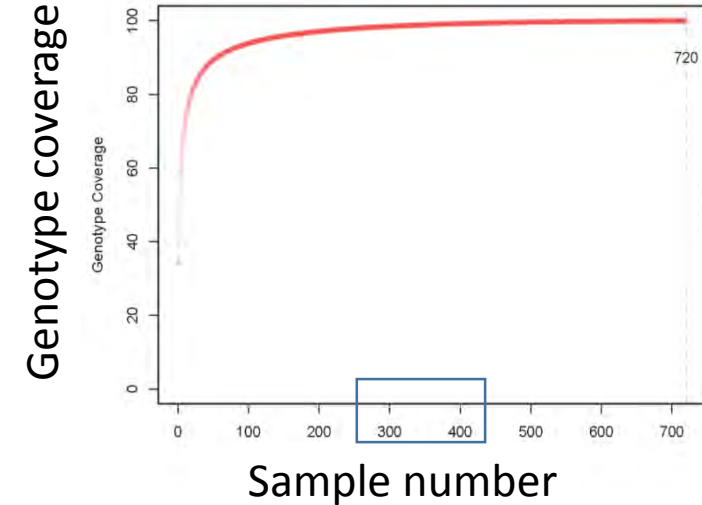
Provide a community resource for genome wide association studies (GWAS)

Use GBS data and crop knowledge to
define a functional panel of 350-400 accessions/species

Genocore analysis is being used to identify a subset of
accessions to capture >95% allelic diversity
Combined with historical varieties providing key
horticultural, disease resistance traits

On-going for all four crops:

- Selection of functional panels
and genomic re-sequencing
(10-20x) of selected accessions for
high resolution mapping
- Creating seed stocks of the
selected accessions



The functional panels will provide:

- a set of diverse lines
- associated sequence data
- SNP datasets
- genetic maps

Lines and data will be publicly accessible for future phenotypic
and GWAS analysis of any traits of interest.

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Which diseases? Cucurbit disease priorities

Table 1. Major disease threats to cucurbit crop production as identified by cucurbit industry stakeholders.		
Disease	Identified as industry priority	Also affects:
Downy mildew	cucumber	melon, watermelon, squash
<i>Fusarium</i> wilt	watermelon	melon, cucumber
Gummy stem blight	watermelon	melon, cucumber, squash
<i>Phytophthora</i> rot	cucumber, watermelon, squash	melon
Powdery mildew	melon, watermelon, squash	cucumber
Viruses (CMV ² ; CYSDV ³ ; PRSV-W ⁴ ; CGMMV ⁵)	melon ^{2,3} , watermelon ^{4,5}	cucumber ^{3,5} , squash ^{2,4}



Fusarium wilt
watermelon



CYSDV
melon



Phytophthora rot
cucumber



Powdery mildew
squash

Breeding challenges to incorporate disease resistance:

- Source of resistance (does it exist/can we find it? What kind of material is it in?)
- Ability to introduce resistance genes into valuable cultivars without carrying negative traits associated with poorly adapted materials.
- Monitor transfer of resistance
Disease screening can be costly, difficult, environmentally variable
can be confounded by the need to effectively pyramid resistances to multiple pathogens

...potential to increase efficiency using genomic-assisted breeding



Photos: T. Wehner, North Carolina State Univ

- **Breeding efforts are underway for each priority crop/disease combination**
 - Identify resistance, determine genetic basis, map resistance, develop and verify marker, introgress and pyramid resistances, develop advanced lines
 - Status varies for each crop – disease combination

Table 2. Current status of resistance breeding for the priority cucurbit diseases.

Crop and disease	Sources of resistance	Elite germplasm for introgression	Field testing locations	Resistant parental line	Phenotypic data for GWAS	Segregating populations	Analysis of inheritance	QTL analysis segregating populations	Marker development	Introgression into cultivated types	Advanced breeding lines for release	Cultivars for release to farmer
Watermelon												
Fusarium race2 (Fus)	PI 482246-USVL246 ^{FR2} ; PI 482252-USVL252 ^{FR2} (55,68 ^a)	Standard: Charleston Gray Icebox: Sugar Baby	SC	X	x	X				x		
Fusarium race 1	Calhoun Gray		SC, NC, GA	x	x	X	77*	77		X		
Gummy stem blight (GSB)	PI 482276-UGA1081 (57,58); PI 526223-UGA157		SC, NC	x	x	X				X		
Phytophthora (Phyt)	PI 494531-USVL531MDR (53,69); PI 560003- USVL003MDR (56)		SC, NC	X		X	70,71b*			X		
Powdery mildew (PM)			GH ^b									
CGMMV	Currently evaluating		SC	X	x	x	140					
PRSV-W	PI 595203 (60)											
Melon												
Powdery (PM)	MR-1 (59)	Cantaloupe: TopMark, Impac Honeydew: Green Flesh Honeydew or PMR Honeydew	CA1,2, AZ	x		X	73*					
Fusarium (Fus)	MR-1 (59)		CA1	x		X	68*			X	X	
CYSDV	PI 313970 (46,50,518); TGR1551 (74)		CA1, AZ	X	x	X	51,74					
CMV	PI 161375 (66); Freeman cucumber (141)		CA1,2, AZ				66,141*					
Cucumber												
Downy mildew (DM)	PI 197088; PI 330628 (54)	Slicer: Poinsett 76	WI, NC	X		X	78	78		X	X	
Phytophthora (Phyt)	PI 109483 (52)	Pickling: NC-25, GY14	MI, NY		9	X						
Squash												
Phytophthora (Phyt)	PI 211996 (64); PI 483347; PI 634693	Butternut: Burpee Butterbush	NY	X			145					
Powdery (PM)	C. martenezii (63)	Tropical pumpkin: Soler,Taina Dorada	PR	x			63	75	x	x	x	x
PRSV-W	Menina, Nigerian Local (61,62)		PR	X			142,146			x	X	X
CMV	Menina, Nigerian Local (61,62)		PR	x			142			x	X	X

^a Reference numbers marked in bold are from members of the CucCAP team. ^b due to need for containment, testing limited to greenhouse * Simply inherited (1-2 genes).

Watermelon

*Identify QTL for resistance,
develop markers, breed for resistance*



A. Levi, P. Wechter



S. Kousik



K-S. Ling

Fusarium wilt

Gummy stem blight

Powdery mildew

***Phytophthora capsici* fruit rot**

**Cucumber green mottle
mosaic virus (CGMMV)**

**Watermelon strain of papaya
ringspot virus (PRSV-W)**

*Team Leader: A. Levi
USDA, Charleston SC*

Identify QTL for resistance, race 1, 2
(Meru and McGregor, 2016; Branham et al., 2016, 2017, 2018)

Characterize inheritance of resistance
(Gusimi et al., 2017)



C. McGregor



Release resistant breeding line
(Levi and Kling, 2017)



A. Levi

Melon

*Identify QTL for resistance,
develop markers, breed for resistance*

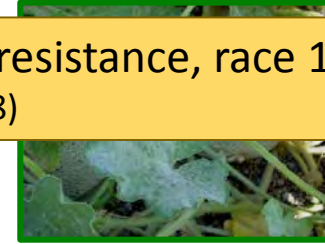


P. Wechter
J. McCreight

Fusarium wilt

Identify QTL for resistance, race 1
(Branham et al., 2018)

Powdery mildew



S. Kanyil, J. McCreight

**Cucurbit yellow stunting
disorder virus (CYSDV)**

Characterize inheritance of
resistance
(McCreight et al., 2017)



W. Wintermantel
J. McCreight

Cucumber mosaic virus (CMV)



W. Wintermantel
M. Mazourek
J. McCreight

Team Leader: J. McCreight
USDA, Salinas CA

Cucumber

*Identify QTL for resistance,
develop markers, breed for resistance*



Y. Weng, T. Wehner

Downy mildew

Identify QTL for resistance
(Wang et al., 2016, 2018)

***Phytophthora capsici* fruit rot**



Release resistant breeding line
(Grumet and Colle, 2017)

R. Grumet

Team Leader: Y. Weng
USDA, University of Wisconsin

Squash

*Identify QTL for resistance,
develop markers, breed for resistance*



Powdery mildew

Identification of a marker for resistance
(Holdsworth et al., 2016)

Phytophthora capsici

**Watermelon strain of papaya
ringspot virus (PRSV-W)**



M. Mazourek

*Team Leader: M. Mazourek
Cornell University*



Development of phenotyping methods
(Seda-Martínez et al.)

Identified QTL



**L. Wessel-Beaver,
A. Linares Ramirez**



**THANK
YOU!**

PD: R. Grumet, *Michigan St. Univ.*

Team Leaders:

Watermelon – Amnon Levi, *ARS, Charleston SC*

Melon – Jim McCreight, *ARS, Salinas CA*

Cucumber – Yiqun Weng, *Univ. Wisconsin*

Squash – Michael Mazourek, *Cornell Univ.*

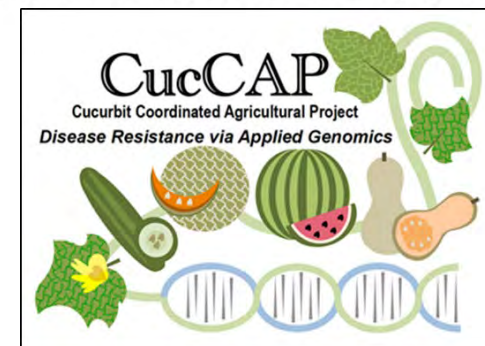
Genomics/Bioinformatics – Zhangjun Fei, *Boyce Thompson Inst.*

Extension – Jonathan Schultheis, *North Carolina State Univ.*

Economics – Marco Palma, *Texas A&M Univ.*



United States Department of Agriculture
National Institute of Food and Agriculture



Other project co-PDs

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Kai-Shu Ling, *ARS, Charleston SC*

Cecilia McGregor, *Univ Georgia*

Lina Quesada, *NC State Univ*

Angela Linares Ramirez, *Univ Puerto Rico*

Umesh Reddy, *West Virginia St Univ*

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