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

…the beginning of a new project to develop genomic resources for the cucurbit community

USDA NIFA

United States Department of Agriculture
National Institute of Food and Agriculture

Over the past ~10 years, the USDA has prioritized different crops and crop groups for genomic projects
Cucurbits had not been one of the targeted crop groups

During the past year we had the opportunity to develop a project for cucurbits meeting the objective to:
"Advance understanding of the genomics of the Cucurbitaceae family and their application to practical breeding programs."

Opportunity to bring together cucurbit breeders/geneticists/genomicists

PI: 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*.
Socioeconomics – Marco Palma, *Texas A&M Univ*.

21 co-PIs
11 institutions

Other project co-PIs

Mary Hausbeck, *Michigan St Univ*
Shaker Kousik, *ARS, Charleston SC*
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*
Louis Ribera, *Texas A&M*
Christine Smart, *Cornell Univ*
Pat Wechter, ARS, *Charleston SC*
Todd Wehner, *NC State Univ*
Linda Wessel-Beaver, *Univ Puerto Rico*
Bill Wintermantel, *ARS, Salinas CA*

Cucurbit Industries

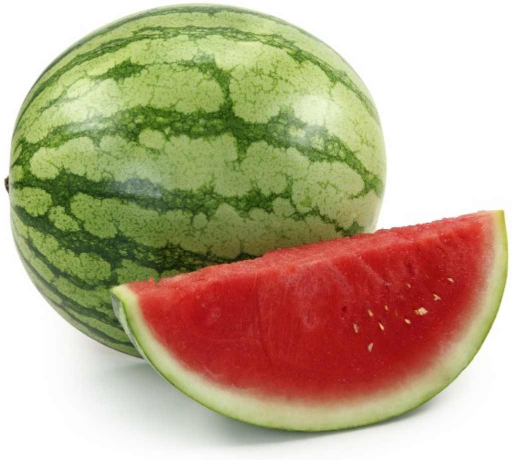
Farm gate value of cucurbit crops in the U.S. ~1.65 billion/year

watermelon

melon

cucumber

squash, pumpkin



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

Consultation with industry (growers, shippers, processors) identified *resistance to diseases* as the highest priority for crop improvement

Diseases cause

- **severe reductions in fruit yield and quality,**
- **increased labor and expenses for disease control,**
- **environmental impacts from application of pesticides**
- **potential outright loss of the crop in the field or at point of sale.**

Disease-resistant varieties are the most cost-effective and environmentally desirable solution

Are many diseases impacting cucurbit crops which ones to work on?

Primary diseases impacting cucurbit crops

Table 1. Major disease threats to cucurbit crop production as identified by cucurbit industry stakeholders.

Disease	Identified as industry funding priority[1]	Also affects:
Downy mildew	cucumber	melon, watermelon, squash/pumpkin
***Fusarium* wilt**	watermelon	melon, cucumber
Gummy stem blight	watermelon	melon, cucumber, squash/pumpkin
***Phytophthora* rot**	cucumber, watermelon, squash/pumpkin	melon
Powdery mildew	melon, watermelon, squash/pumpkin	cucumber
Viruses (CMV[2]; CYSDV[3]; PRSV-W[4]; CGMMV[5])	melon[2,3], watermelon[4,5]	cucumber[3,5], squash/pumpkin[2,4]

***Fusarium* wilt**
watermelon

CYSDV
melon

***Phytophthora* rot**
cucumber

Powdery mildew
squash

Breeding challenges:

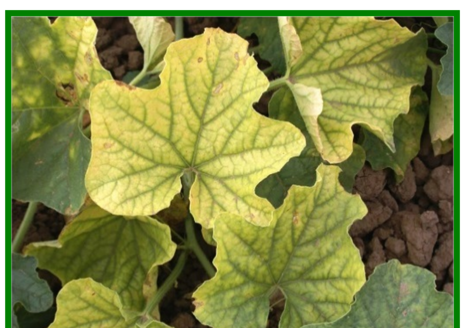
Source of resistance (does it exist? What kind of material is it in?)

Ability to move desired genes without carrying negative traits associated with poorly adapted materials.

Performance of the disease screening to monitor transfer of resistance can be costly and difficult

> **Can be confounded by the need to effectively pyramid resistances to multiple pathogens**

…potential to increase efficiency using genomic-assisted breeding



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

Objectives

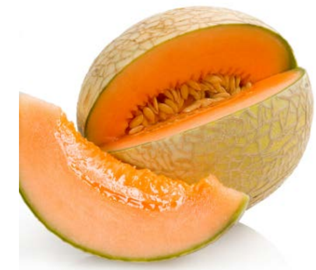
(a) Develop genomic and bioinformatic breeding tool kits for accelerated crop improvement across the Cucurbitaceae

(b) Use these tools to facilitate efficient introgression of disease resistance into commercially valuable cucurbit cultivars

(c) Perform economic impact analyses of cost of production and disease control and provide readily accessible information to facilitate disease control.

Advantages to now

***Draft genome sequences for the four major cucurbit species*:**



Cucumber (*Cucumis sativus*) (2009)

Melon (*Cucumis melo*) (2012)

Watermelon (*Citrullus lanatus*) (2013)

Squash (*Cucurbita pepo*) (2016)

Constantly improving genomic technologies,
reduced cost of sequencing

Among possible approaches considered, team has chosen to invest in GBS

(a) Develop genomic and bioinformatic breeding tool kits

Z. Fei, U. Reddy, A. Levi, M. Mazourek, P. Wechter, Y. Weng

- ***i. Develop communal sequence and phenotype databases and bioinformatics tools for watermelon, melon, cucumber and squash***

- ***ii. Perform GBS analysis of PI collections of the four species to provide a community resource for genome wide association studies (GWAS)***

- ***iii. Provide access to cucurbit genomics tools and databases via the International Cucurbit Genome Initiative (ICuGI) website***

(a) Develop genomic and bioinformatic breeding tool kits

- ***i. Develop communal sequence and phenotype databases and bioinformatics tools for watermelon, melon, cucumber and squash including*:**

1. **Establishment of a GBS data processing and SNP calling pipeline, as well as a genome-wide association study (GWAS) analysis package for cucurbits.**

2. **Development of breeder-friendly web-based databases for cucurbit phenotype, genotype and QTL information**

3. **Establishment of community-standardized gene/trait descriptors and nomenclature for cucurbits**

(a) Develop genomic and bioinformatic breeding tool kits

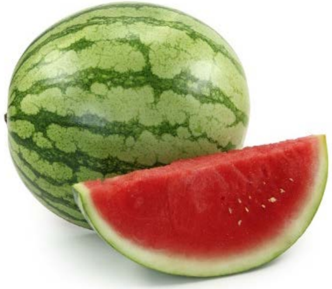
- ***ii. Perform GBS analysis of PI collections of the four species to provide a community resource for genome wide association studies (GWAS)***

The U.S. National Plant Germplasm System maintains

1,314 cucumber
2,043 melon
1,311 watermelon
1,580 squash (*Cucurbita pepo, C. moshcata* and *C. maxima*) PIs

→ Diversity in the collection will be genotyped by GBS for 1000-1500 accessions/crop.

High throughput DNA preparation – MSU
GBS - Cornell



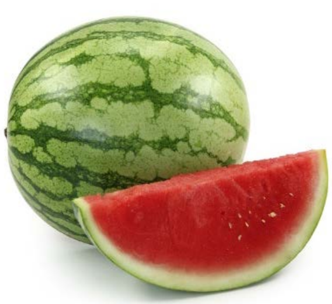
(a) Develop genomic and bioinformatic breeding tool kits

- ***ii. Perform GBS analysis of PI collections of the four species to provide a community resource for genome wide association studies (GWAS)***

GBS data will be used to <u>define a genome-informed core population</u> of 384 PIs for each species that best represents diversity present in the crop.

Individual plants from the core collections will be self-pollinated and re-sequenced by GBS

-- the genome-informed core collections will provide a set of diverse lines
-- their associated sequence data, SNP datasets, and genetic maps
will be available for future phenotypic and GWAS analysis
of any traits of interest.

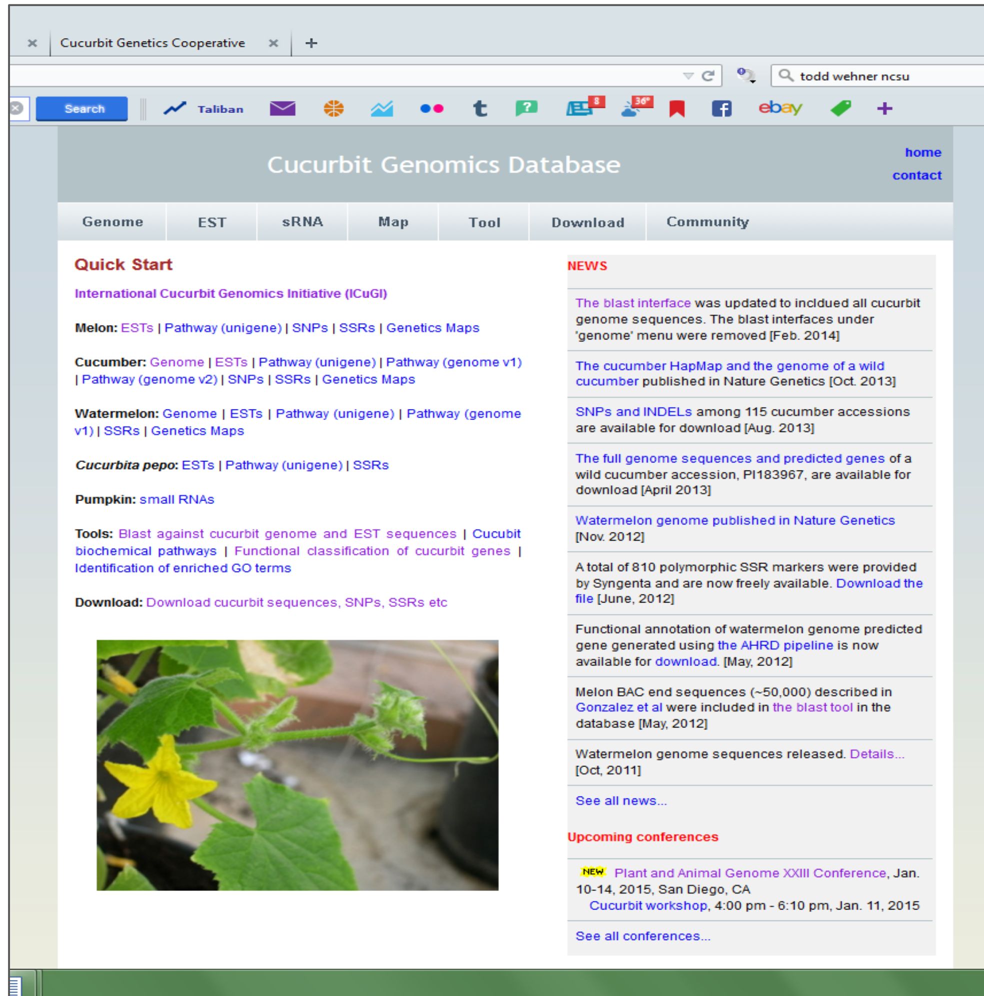


(a) Develop genomic and bioinformatic breeding tool kits

- *iii. Provide access to cucurbit genomics tools and databases via the International Cucurbit Genome Initiative (ICuGI) website*, genomics and bioinformatics workshops open to all members of the cucurbit scientific and breeding communities

The International Cucurbit Genomics Initiative (ICuGI) website, which hosts the Cucurbit Genomics Database is currently established and managed by Z. Fei through Cornell University

We will be able to build on this website to add additional features and capacity



(b) Perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars.

- Identify sources and determine the genetic basis for resistance to key cucurbit diseases

- Utilize genomic approaches to identify and map resistances to key diseases

QTL mapping of resistances will use a combination of:
GBS of segregating progeny from biparental mapping populations
GWAS analysis of PI accessions

Initial QTL regions will be subsequently refined by fine mapping

- Develop and verify molecular markers for efficient trait selection and gene pyramiding

- Introgress resistances into advanced breeding lines

- Introgress resistances into advanced breeding lines

- Breeding efforts are underway for each priority crop/disease combination
  - Status varies
    - Identified resistance → advanced lines nearing release

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	x	x	X	77*	77		X		
Gummy stem blight (GSB)	PI 482276-UGA1081 (57,58); PI 526223-UGA157		NC, GA	x	x	X				X		
Phytophthora (Phyt)	PI 494531-USVL531MDR (53,69); PI 560003- USVL003MDR (56)		SC, NC	x	x	X				X		
Powdery mildew (PM)			SC, NC	X		X	70,71b*			X		
CGMMV	Currently evaluating		GH[b]									
PRSV-W	PI 595203 (60)		SC	X	x	x	140					
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 Pickling: NC-25, GY14	WI, NC	X		X	78	78		X	X	
Phytophthora (Phyt)	PI 109483 (52)		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

Fusarium wilt race 1

Gummy stem blight

Powdery mildew races 1 & 2

***Phytophthora capsici* fruit rot**

C. McGregor

S. Kousik

Cucumber green mottle mosaic virus (CGMMV)

S. Kousik

Watermelon strain of papaya ringspot virus (PRSV-W)

K-S. Ling

A. Levi

Team Leader: A. Levi

Melon

Identify QTL for resistance, develop markers, breed for resistance

P. Wechter
J. McCreight

Fusarium wilt race 1 & 2

Powdery mildew

S. Kousik, J. McCreight

Cucurbit yellow stunting disorder virus (CYSDV)

Cucumber mosaic virus (CMV)

W. Wintermantel
J. McCreight

W. Wintermantel
M. Mazourek
J. McCreight

Team Leader: J. McCreight

Cucumber

Identify QTL for resistance,
develop markers, breed for resistance

Y. Weng, T. Wehner

Downy mildew

Phytophthora capsici **fruit rot**

R. Grumet

Team Leader: Y. Weng

Squash

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

M. Mazourek

Phytophthora capsici

Watermelon strain of papaya ringspot virus (PRSV-W)

L. Wessel-Beaver

Team Leader: M. Mazourek

CucCAP Stakeholder Advisory Board

Organization	Representative
National Watermelon Promotion Board	Mark Arney
National Watermelon Association	Robert Morrissey
California Melon Research Board	Milas Russel
California Melon Research Board	Steve Smith
Pickle Packers International	Brian Bursiek
Swanson Pickles and PPI	John Swanson
Stony Brook Oil (squash processor)	Greg Woodworth
Martin Farms (squash grower)	Mitch Meyler
Bayer Crop Science	Jovan Djordjevic
HM Clause	Alyson Thornton
Hollar Seeds	Bruce Carle
Johnny's Selected Seeds	Rob Johnston
Magnum Seeds, Inc.	Ken Owens
Monsanto	Nischit Shetty
Sakata Seed	Jeff Zischke
Syngenta	Jim Brusca
United Genetics Seeds Co.	Xuemei Zhang

External Evaluators: Nurit Katzir, Phil McClean, Allen Van Deynze

Thank you!