CucCAP:

Leveraging applied genomics to improve disease resistance in cucurbit crops

Second Annual CucCAP Team Meeting
March 27-28, 2017
Charleston SC
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AGENDA

and

PARTICIPANTS
AGENDA

Second annual CucCAP team meeting – March 27-28, 2017

Monday, March 27
8:00-8:15  Arrival, welcome
8:15-8:30  Introductions
   (team members, industry advisory board, external reviewers, germplasm curators, guests)
8:30-8:45  Overview of project progress, plans for meeting (Grumet)
8:45-9:15  Bioinformatics team report, questions, discussion (Fei et al)
9:15-9:45  Watermelon team report, questions, discussion (Levi et al)
9:45-10:00  Break
10:00-10:30  Melon team report, questions, discussion (McCreight et al)
10:30-11:00  Cucumber team report, questions, discussion (Weng et al)
11:00-11:30  Squash team report, questions, discussion (LaPlant et al)
11:30-12:00  Discussion: Cucurbit gene nomenclature (Weng lead)
12:00-1:00  Lunch
1:00-1:30  Extension team report, questions, discussion (Schultheis et al)
1:30-2:00  Socioeconomics team report, questions, discussion (Palma)
2:00-2:30  CucCAP website, overview, questions, discussion (Lorscheider)
2:30-2:45  Break
2:45-3:15  Views from the cucurbit industries – needs and concerns
3:15-3:45  Views from the seed industry
3:45-5:00  Breakout Session I - Websites
   A.  CucCAP website (Schulthies, Lorscheider leads)
   B.  ICuGI website (Fei lead)
   Barbecue Dinner

Tuesday, March 28
8:00-8:15  Arrive
8:15-9:30  Breakout Session II
   A.  Core collections – selection criteria, obtaining germplasm
       Sequencing strategy, Maintenance (Weng, Fei leads)
       (curators, seed company reps, members of crop teams)
   B.  Cucurbit disease resources (Smart lead)
       (commodity reps, extension, crop team members)
9:30-10:30  Breakout Sessions III
   A.  Cucurbit Crop Germplasm Committee, Cucurbit Vulnerability Statement
       (McCreight lead)
       (curators, seed company reps, members of crop teams)
   B.  Extension plans and industry needs (Schulthies lead)
       (commodity reps, extension, socioeconomic, crop team members)
10:30-10:45  Break
10:45-12:00  Wrap up discussions, feedback from advisory board and external reviewers
CucCAP Team

Project Director

*Rebecca Grumet*, Professor, Department of Horticulture
1066 Bogue Street, Michigan St Univ., East Lansing MI 48824 ([grumet@msu.edu](mailto:grumet@msu.edu))

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**Watermelon (Citrullus lanatus)**
*Amnon Levi*, Research Geneticist, Vegetable Research Laboratory
2700 Savannah Highway, USDA-ARS, Charleston SC 29414 (Amnon.levi@ars.usda.gov)

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*Jim McCreight*, Research Leader, US Agricultural Research Station
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lbeaver@upr.edu  
(squash team)

Bill Wintermantel  
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USDA-ARS, US Agricultural Res. Station  
1636 E Alisal St  
Salinas CA 93905  
Bill.Wintermantel@ars.usda.gov  
(melon team)
### Stakeholder Advisory Board

<table>
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<tr>
<th>Organization</th>
<th>Representative</th>
<th>Position</th>
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<tr>
<td><strong>Commodity Groups - Growers, Shippers, Processors, Marketing</strong></td>
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<tr>
<td>National Watermelon Promotion Board</td>
<td>Mark Arney</td>
<td>Executive Director, National Watermelon Promotion Board</td>
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<tr>
<td>National Watermelon Association</td>
<td>Robert Morrissey</td>
<td>Executive Director, National Watermelon Association</td>
</tr>
<tr>
<td>California Melon Research Board</td>
<td>Milas Russell</td>
<td>Chair Elect, California Melon Research Board President, Sandstone Melon Company</td>
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<tr>
<td>California Melon Research Board</td>
<td>Steve Smith</td>
<td>Chair, California Melon Research Board Co-Owner Turlock Fruit Company</td>
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<tr>
<td>Pickle Packers International</td>
<td>Brian Bursiek</td>
<td>Executive Vice President, Pickle Packers International</td>
</tr>
<tr>
<td>Swanson Pickles and Pickle Packers International</td>
<td>John Swanson</td>
<td>President Swanson Pickle Company; Research Board, Pickle Packers International</td>
</tr>
<tr>
<td>Martin Farms (squash grower, shipper)</td>
<td>Mitch Beyler</td>
<td>Partner, John B. Martin and Sons Farms, Inc.</td>
</tr>
<tr>
<td>Stony Brook Wholehearted Foods (squash processor)</td>
<td>Greg Woodworth</td>
<td>Founder, Stony Brook Wholehearted Foods</td>
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<td><strong>Seed Industry</strong></td>
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<tr>
<td>Bayer Crop Science</td>
<td>Jovan Djordjevic/ Suren Baliji</td>
<td>Global R&amp;D Lead, Melons and Watermelons, Bayer Crop Science</td>
</tr>
<tr>
<td>HM Clause</td>
<td>Kishor Bhattarai</td>
<td>Phytopathology Project Manager, HM Clause, Vegetable Seeds Division, Limagrain</td>
</tr>
<tr>
<td>Hollar Seed Company</td>
<td>Bruce Carle</td>
<td>Plant Breeder, Hollar Seed Company</td>
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<tr>
<td>Johnny’s Selected Seeds</td>
<td>Rob Johnston/ Lindsay Wyatt</td>
<td>Chairman, Johnny’s Selected Seeds</td>
</tr>
<tr>
<td>Monsanto</td>
<td>Nischit Shetty</td>
<td>NAFTA Cucurbit Lead for Monsanto Vegetable Seeds</td>
</tr>
<tr>
<td>Sakata Seeds</td>
<td>Jeff Zischke/ Benito Juarez</td>
<td>Director of Research, Vegetables, Sakata Seed</td>
</tr>
<tr>
<td>United Genetics Seeds Co.</td>
<td>Xuemei Zhang</td>
<td>Melon Breeder, United Genetic Seeds</td>
</tr>
</tbody>
</table>

**Industry Participants:**
Isabel Armas, Daniel Ludeking, Rijk Zwaan
Cucurbit Crop Curators

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CucCAP PROJECT OBJECTIVES,

TIMELINES and METRICS
CucCAP PROJECT OBJECTIVES

Each objective includes integrated research and extension/outreach [E/O] approaches:

**Obj. 1. Develop genomic approaches and tools for cucurbit species.**
- Develop genomic and bioinformatic platforms for: genotyping by sequencing (GBS); sequence data processing and analysis; and genotype, phenotype and QTL databases
- Perform GBS analysis of PI collections and core populations of the four species to provide a community resource for genome wide association studies (GWAS) for current and future traits of interest
- [E/O] Provide access to cucurbit genomics tools and databases via the International Cucurbit Genome Initiative (ICuGI) website, and by genomics and bioinformatics workshops open to all members of the cucurbit scientific and breeding communities

**Obj. 2. Perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars.**
- Utilize genomic approaches to map resistance loci for key cucurbit diseases
- Develop and verify molecular markers for efficient trait selection and gene pyramiding
- Introgress resistances into advanced breeding lines
- [E/O] Provide web-based and face to face information via field trials, extension venues, and scientific meetings regarding breeding materials, markers, and breeding progress

**Obj. 3 Perform economic impact analyses of cost of production and disease control and provide readily accessible information to facilitate disease control.**
- Define, parameterize, simulate, and validate production variables based on cucurbit production crop budgets
- Use a risk-based simulation model to analyze economic potential of the disease resistant cucurbit cultivars
- [E/O] Develop a centralized cucurbit disease website, including content in English and Spanish, providing information about diagnostics and links to disease clinics; disease control recommendations; disease alerts and links to forecasting tools

**Disease priorities identified by the cucurbit industries:**

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<th>Disease</th>
<th>Identified as commodity funding priority</th>
<th>Also affects:</th>
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<tr>
<td>Downy mildew</td>
<td>cucumber</td>
<td>melon, watermelon, squash/pumpkin</td>
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<td>Fusarium wilt</td>
<td>watermelon</td>
<td>melon, cucumber</td>
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<td>Gummy stem blight</td>
<td>watermelon</td>
<td>melon, cucumber, squash/pumpkin</td>
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<tr>
<td>Phytophthora rot</td>
<td>cucumber, watermelon, squash/pumpkin</td>
<td>melon</td>
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<tr>
<td>Powdery mildew</td>
<td>melon, watermelon, squash/pumpkin</td>
<td>cucumber</td>
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<tr>
<td>Viruses (CMV(^b), CYSDV(^c), PRSV-W(^d), CGMMV(^e))</td>
<td>melon(^b,c), watermelon(^c,d)</td>
<td>cucumber(^c,d), squash/pumpkin(^b,d)</td>
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</table>
### Project Structure – Team Organization

#### CucCAP Teams

<table>
<thead>
<tr>
<th>Team</th>
<th>PD, Co-PDs and Co-PIs</th>
<th>Institution*</th>
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</thead>
<tbody>
<tr>
<td><strong>Watermelon</strong></td>
<td><strong>PD: Rebecca Grumet</strong> (RG)</td>
<td>MSU</td>
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<tr>
<td></td>
<td><strong>Team Leader: Amnon Levi</strong> (AL)</td>
<td>ARS-SC</td>
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<td></td>
<td>Shaker Kousik (SK)</td>
<td>ARS-SC</td>
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<td></td>
<td>Kai-Shu Ling (KSL)</td>
<td>ARS-SC</td>
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<td></td>
<td>Cecilia McGregor (CM)</td>
<td>UGA</td>
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<td></td>
<td>Lina Quesada (LQ)</td>
<td>NCSU</td>
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<td></td>
<td>Pat Wechter (PW)</td>
<td>ARS-SC</td>
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<td></td>
<td>Todd Wehner (TW)</td>
<td>NCSU</td>
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<tr>
<td><strong>Melon</strong></td>
<td><strong>Team Leader: Jim McCreight</strong> (JM)</td>
<td>ARS-CA</td>
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<td>Bill Wintermantel (BW)</td>
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<td><strong>Cucumber</strong></td>
<td><strong>Co-PD, Team Leader: Yiqun Weng</strong> (YW)</td>
<td>ARS-WI</td>
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<td></td>
<td>Rebecca Grumet (RG)</td>
<td>MSU</td>
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<td></td>
<td>Mary Hausbeck (MH)</td>
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<td>Todd Wehner (TW)</td>
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<td><strong>Squash</strong></td>
<td><strong>Team Leader: Michael Mazourek</strong> (MM)</td>
<td>CU</td>
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<td>Christine Smart (CS)</td>
<td>CU</td>
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<td></td>
<td>Linda Wessel-Beaver (LWB)</td>
<td>UPR</td>
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<tr>
<td><strong>Genomics/bioinformatics</strong></td>
<td><strong>Team Leader: Zhangjun Fei</strong> (ZF)</td>
<td>BTI</td>
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<td>Umesh Reddy (UR)</td>
<td>WVSU</td>
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<td></td>
<td>Amnon Levi (watermelon) (AL)</td>
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<td>Yiqun Weng (cucumber) (YW)</td>
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<td><strong>Socioeconomics</strong></td>
<td><strong>Team Leader: Marco Palma</strong> (MP)</td>
<td>TAMU</td>
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<td>Louis Ribera (LR)</td>
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<td><strong>Extension/Outreach</strong></td>
<td><strong>Team Leader: Jonathan Schultheis</strong> (JS)</td>
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<td>Lina Quesada (watermelon) (LQ)</td>
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<td>Jim McCreight (melon) (JM)</td>
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<td>Angela Linares Ramírez (ALR)</td>
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<td></td>
<td>Zhangjun Fei (bioinformatics) (ZF)</td>
<td>CU</td>
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</tbody>
</table>

*Institution abbreviations: ARS-CA (Salinas), SC (Charleston), WI (Madison); BTI-Boyce Thompson Inst; CU-Cornell Univ; MSU-Michigan St Univ; NCSU-North Carolina St Univ; TAMU- Texas A&M Univ; UGA-Univ Georgia; UNH-Univ New Hampshire; UPR-Univ Puerto Rico; WVSU-West Virginia St Univ
## TIMELINE CHART

### Objective

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#### 1.1. Develop genomic and bioinformatics platforms

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1.1.1. Genotyping by sequencing

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1.1.2. Sequence data processing/analysis

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1.1.3. ICuGl database development

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1.1.4 Community standardized nomenclature

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<tbody>
<tr>
<td>YW (ARS-WI), AL (ARS-SC)</td>
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<tr>
<td>JM (ARS-CA), MM (CU)</td>
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1.1.5. Genomic, bioinformatics workshops

<table>
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<th>Personnel/Institution</th>
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<tr>
<td>ZF (BTI), UR (WVSU), members of crop teams</td>
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#### 1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)

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<th>Year</th>
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1.2.1. GBS of cucurbit species, establish molecular-informed core populations

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<th>Year</th>
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1.2.2. Population genetics and GWAS analyses

<table>
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<th>Year</th>
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<td>UR (WVSU), ZF (BTI)</td>
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<td>YW (ARS-WI), RG (MSU)</td>
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</tbody>
</table>

#### (b) Obj. 2. Genomic assisted breeding for disease resistance

**2.1 QTL map resistances:**

- Watermelon
  - CGMMV
  - Fusarium race 1
  - Fusarium race 2
  - gummy stem blight
  - Phytophthora
  - powdery mildew
  - PRSV-W

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<tr>
<td>KSL (ARS-SC), AL (ARS-SC)</td>
<td>Sc</td>
</tr>
<tr>
<td>AL (ARS-SC), PW (ARS-SC)</td>
<td>Sc,P</td>
</tr>
<tr>
<td>PW (ARS-SC), AL (ARS-SC)</td>
<td>P</td>
</tr>
<tr>
<td>CM (UGA), TW (NCSU)</td>
<td>PFSQ</td>
</tr>
<tr>
<td>SK (ARS-SC)</td>
<td>PFS</td>
</tr>
<tr>
<td>SK (ARS-SC)</td>
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</tr>
<tr>
<td>AL (ARS-SC), KSL (ARS-SC)</td>
<td>FSQ</td>
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</tbody>
</table>

- Melon
  - powdery mildew
  - Fusarium
  - CYSDV
  - CMV

<table>
<thead>
<tr>
<th>Personnel/Institution</th>
<th>Year</th>
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</thead>
<tbody>
<tr>
<td>SK,PW (ARS-SC), JM (ARS-CA)</td>
<td>PFSQ</td>
</tr>
<tr>
<td>PW (ARS-SC)</td>
<td>F</td>
</tr>
<tr>
<td>JM (ARS-CA), WW (ARS-CA)</td>
<td>F</td>
</tr>
<tr>
<td>JM (ARS-CA), MM (CU)</td>
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</tr>
</tbody>
</table>

- Cucumber
  - downy mildew
  - Phytophthora

<table>
<thead>
<tr>
<th>Personnel/Institution</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>YW (ARS-WI), TW (NCSU)</td>
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</tr>
<tr>
<td>RG (MSU)</td>
<td>SQ</td>
</tr>
</tbody>
</table>

- Squash
  - Phytophthora
  - PRSV-W
  - CMV

<table>
<thead>
<tr>
<th>Personnel/Institution</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM (CU), CS (CU)</td>
<td>PF</td>
</tr>
<tr>
<td>MM</td>
<td>PFQ</td>
</tr>
<tr>
<td>MM</td>
<td>PFQ</td>
</tr>
</tbody>
</table>

**2.2 Marker development and verification:**

- Watermelon
  - Fusarium race 1
  - Fusarium race 2
  - gummy stem blight
  - Phytophthora
  - powdery mildew
  - PRSV-W

<table>
<thead>
<tr>
<th>Personnel/Institution</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL (ARS-SC), PW (ARS-SC)</td>
<td>R</td>
</tr>
<tr>
<td>PW (ARS-SC), AL (ARS-SC)</td>
<td>R</td>
</tr>
<tr>
<td>CM (UGA), TW (NCSU)</td>
<td>R</td>
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<tr>
<td>SK (ARS-SC)</td>
<td>R</td>
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<tr>
<td>SK (ARS-SC)</td>
<td>R</td>
</tr>
<tr>
<td>AL (ARS-SC), KSL (ARS-SC)</td>
<td>R</td>
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</tbody>
</table>

- Melon
  - powdery mildew
  - Fusarium
  - CYSDV
  - CMV

<table>
<thead>
<tr>
<th>Personnel/Institution</th>
<th>Year</th>
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</thead>
<tbody>
<tr>
<td>SK (ARS-SC)</td>
<td>M</td>
</tr>
<tr>
<td>PW (ARS-SC)</td>
<td>RM</td>
</tr>
<tr>
<td>WW (ARS-CA), JM (ARS-CA)</td>
<td>RM</td>
</tr>
<tr>
<td>JM (ARS-CA), MM (CU)</td>
<td>RM</td>
</tr>
<tr>
<td>2.2.3. Cucumber</td>
<td>YW (ARS-WI), TW (NCSU)</td>
</tr>
<tr>
<td>2.2.4 Squash</td>
<td>MM(CU), LWB(UPR)</td>
</tr>
<tr>
<td>- powdery mildew</td>
<td>MM(CU), LWB(UPR)</td>
</tr>
<tr>
<td>- Phytophthora</td>
<td>MM(CU), LWB(UPR)</td>
</tr>
</tbody>
</table>

| 2.3. Introgress resistance into advanced breeding lines | Develop breeding lines (B), introgress into cultivated (I), advanced lines (A), release to breeders (R) |
| 2.3.1. Watermelon | AL (ARS-SC), PW (ARS-SC) | B | I | IA | AR |
| - Fusarium race 1 | PW (ARS-SC), AL (ARS-SC) | B | B | I | 1 |
| race 2 | CM (UGA), TW (NCSU) | B | B | I | 1 |
| - gummy stem blight | SK (ARS-SC) | B | I | I | A |
| - Phytophthora | SK (ARS-SC) | B | I | I | A |
| - powdery mildew | AL (ARS-SC), KSL (ARS-SC) | B | I | I | 1 |
| - PRSV-W | AL (ARS-SC), KSL (ARS-SC) | B | I | I | 1 |
| 2.3.2. Melon | SK (ARS-SC), JM (ARS-CA) | B | I | I | IA |
| - powdery mildew | PW (ARS-SC) | B | B | I | IA |
| - Fusarium | JM (ARS-CA), WW (ARS-CA) | I | I | IA | IAR |
| - CYSDV | JM (ARS-CA) | I | I | IA | IAR |
| - CMV | AL (ARS-SC), PW (ARS-SC) | B | B | I | 1 |
| 2.3.3. Cucumber | YW (ARS-WI), TW (NCSU) | B | I | I | R |
| - downy mildew | RG (MSU) | B | I | I | 1 |
| - Phytophthora | Already exists | MM(CU), CS(CU) | I | I | AR | AR |
| 2.3.4 Squash | Already exists | MM(CU), CS(CU) | I | I | AR | AR |

(b) Obj. 3. Economic impact analyses, disease control information

| 3.1 Perform economic analysis, cost of production/disease control |
| 3.1.1. Define, parameterize, simulate, validate production variables | LR (TAMU), MP (TAMU) | X | X |
| 3.1.2. Estimate the potential economic impacts to the cucurbit industry | LR (TAMU), MP (TAMU) | X | X |

<p>| 3.2 Provide readily accessible information to facilitate disease control |
| 3.2.1. Develop a centralized cucurbit disease website | LQ (NCSU), JS (NCSU) | X | X |
| 3.2.2. Develop and post diagnostic resources and disease control information in English and Spanish; prepare diagnostic poster | LQ (NCSU), MH (MSU), CS (CU), ALR (UPR) | X | X | X | X |
| 3.2.3 Provide disease alerts and forecasting tools | X | X | X | X | X |
| 3.2.4. Field days and demonstration plots | Crop and extension teams | X | X | X | X |</p>
<table>
<thead>
<tr>
<th>Crop and disease</th>
<th>Sources of resistance</th>
<th>Elite germplasm for introgression</th>
<th>Field testing locations</th>
<th>Resistant parental line</th>
<th>Phenotypic data for GWAS</th>
<th>Segregating populations</th>
<th>Analysis of inheritance</th>
<th>QTL analysis segregating populations</th>
<th>Marker development</th>
<th>Introgression into cultivated types</th>
<th>Advanced breeding lines for release</th>
<th>Cultivars for release to farmer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watermelon</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Fusarium race 2</td>
<td>PI 482246-USVL246F; PI 482252-USVL252F</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Fusarium race 1</td>
<td>Calhoun Gray</td>
<td>SC</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<tr>
<td>Gummy stem blight (GB)</td>
<td>PI 482276-UGA1081; PI 526223-UGA157</td>
<td>NC, GA</td>
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<td>x</td>
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<td>x</td>
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<tr>
<td>Phytophthora (Phyt)</td>
<td>PI 494531-USVL531MDR; PI 560003-USVL003MDR</td>
<td>SC, NC</td>
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<td>x</td>
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<tr>
<td>Powdery mildew (PM)</td>
<td>PI 313970; TGR1551</td>
<td>SC</td>
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<td>x</td>
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<td>x</td>
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<td>CGMMV</td>
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<tr>
<td>PRSV-W</td>
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<td>x</td>
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<td>x</td>
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<tr>
<td>Melon</td>
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<td>Powdery (PM)</td>
<td>MR-1</td>
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<td>CYSDV</td>
<td>PI 197088; PI 330628</td>
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<td>CMV</td>
<td>PI 161375; Freeman cucumber</td>
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<tr>
<td>Cucumber</td>
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<td>Downy mildew (DM)</td>
<td>PI 109483</td>
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<tr>
<td>Phytophthora (Phyt)</td>
<td>PI 211996; PI 483347; PI 634693</td>
<td>Butternut: Burpee Butterbush</td>
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<tr>
<td>Powdery (PM)</td>
<td>C. martenezii</td>
<td>Tropical pumpkin: Soler,Taina Dorada</td>
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<tr>
<td>PRSV-W</td>
<td>Menina, Nigerian Local</td>
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<tr>
<td>CMV</td>
<td>Menina, Nigerian Local</td>
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</table>
### PROJECT METRICS

#### Metrics to be used in CucCAP project evaluation

<table>
<thead>
<tr>
<th>Short term metrics (1-2 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. State of the art, genotyping by sequencing (GBS) and data analysis platforms are developed for cucurbit species.</td>
</tr>
<tr>
<td>2. GBS sequence data are obtained for 1000-1600 PIs for each of the four cucurbit crops.</td>
</tr>
<tr>
<td>3. Community-standardized cucurbit gene/trait descriptors and nomenclature are established.</td>
</tr>
<tr>
<td>4. Germplasm lines with resistance to <em>Fusarium</em> r.1,2, <em>Phytophthora</em>, powdery mildew, and PRSV are established for watermelon; for CYSDV in melon, and <em>Phytophthora</em> in cucumber.</td>
</tr>
<tr>
<td>5. Markers developed for KASP-based assay for downy mildew in cucumber and powdery mildew and ZYMV in squash.</td>
</tr>
<tr>
<td>6. Field trials and field days are held to test and demonstrate disease resistant materials (average 1/yr/crop).</td>
</tr>
<tr>
<td>7. Representative farms are developed for economic analyses for three locations for each of the four commodities.</td>
</tr>
<tr>
<td>8. The CucCAP Cucurbit Disease Extension Website is established.</td>
</tr>
<tr>
<td>9. Participation in outreach to 15-20 stakeholder groups per year via industry events and field days.</td>
</tr>
<tr>
<td>10. A Cucurbit Genomics and Bioinformatics workshop is delivered at PAG 2017 attended by members from at least 20 cucurbit research laboratories.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medium term metrics (3-4 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Population structure analysis is performed and molecular-directed core populations are established for the four cucurbit crops.</td>
</tr>
<tr>
<td>2. Breeder-friendly databases to store and distribute genomic, phenotypic, and genotypic information and development of associated data analysis tools are implemented (<a href="http://www.icugi.org">www.icugi.org</a>). Based on current traffic, at least 1000 unique visitors are expected per week.</td>
</tr>
<tr>
<td>3. GWAS analyses are performed for CGMMV, <em>Fusarium</em>, gummy stem blight, <em>Phytophthora</em> and PRSV-W in watermelon; CYSDV in melon and <em>Phytophthora</em> in cucumber.</td>
</tr>
<tr>
<td>4. QTL associated with CGMMV, <em>Fusarium</em> r.2, gummy stem blight, <em>Phytophthora</em>, powdery mildew, and PRSV in watermelon; CMV, CYSDV, <em>Fusarium</em> and powdery mildew in melon; downy mildew, <em>Phytophthora</em> in cucumber; and CMV, PRSV and powdery mildew in squash have been identified.</td>
</tr>
<tr>
<td>5. Molecular markers have been developed for <em>Fusarium</em> r.1 in watermelon; CMV, CYSDV, <em>Fusarium</em> and powdery mildew in melon; CMV, PRSV and powdery mildew in squash.</td>
</tr>
<tr>
<td>6. Breeding lines with resistance to <em>Fusarium</em> r.1,2 and PRSV in watermelon; CMV, CYSDV, <em>Fusarium</em> and powdery mildew in melon; downy mildew in cucumber; and <em>Phytophthora</em> in butternut squash are available to researchers and seed companies.</td>
</tr>
<tr>
<td>7. Cucumber lines carrying multiple disease resistances (downy mildew/powdery mildew/ZYMV) developed by marker assisted selection.</td>
</tr>
<tr>
<td>8. Field trials and field days are held to test and demonstrate disease resistant materials (average 2/yr/crop).</td>
</tr>
<tr>
<td>9. Cucurbit disease informational materials in English and Spanish are developed and posted on the CucCAP disease website for each of the priority diseases.</td>
</tr>
<tr>
<td>10. Stakeholders use website and social media tools to obtain information about disease outbreaks, diagnosis and control. Based on prior experience with cucurbit disease tracking and informational websites, 1000-2000 hits per week are expected during peak growing season.</td>
</tr>
<tr>
<td>11. Participation in outreach to 15-20 stakeholder groups each year via industry events and field days.</td>
</tr>
<tr>
<td>12. Representative farms are compared to information available through USDA agencies and Extension service budgets for accuracy, and inputs are used to develop stochastic economic feasibility model by region.</td>
</tr>
<tr>
<td>13. Scenarios developed using project findings are run through economic feasibility models.</td>
</tr>
<tr>
<td>14. The Cucurbitaceae 2018 conference is hosted, expected attendance of 200-250 international cucurbit scientists from public and private sector.</td>
</tr>
<tr>
<td>15. Cucurbit genomics workshops are delivered at PAG 2018, 2019 and Cucurbitaceae 2018; expected attendance at Cucurbitaceae 2018, 100-200 people.</td>
</tr>
<tr>
<td>16. 15 graduate students and 3 post-docs are trained in cucurbit genetics, genomics, disease and economic analysis.</td>
</tr>
<tr>
<td>17. 4-5 refereed articles are published by each crop group</td>
</tr>
<tr>
<td>Long term metrics</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>1. Sustainable data management, storage, and statistical analysis systems for cucurbit sequence, QTL, marker and phenotype data are available for the cucurbit research and breeding community.</td>
</tr>
<tr>
<td>2. Sustainable community resources for cucurbit GWAS analysis are available for the four crops.</td>
</tr>
<tr>
<td>3. Advanced breeding lines with resistance to Fusarium race 1,2, gummy stem blight, Phytophthora, powdery mildew, PRSV and GCMMV in watermelon; CMV, CYSDV, Fusarium and powdery mildew in melon; and combined downy mildew, powdery mildew, and ZYMV in cucumber.</td>
</tr>
<tr>
<td>4. Breeding lines with resistance to critical cucurbit diseases are used in breeding programs to improving/pyramiding resistance into commercial cucurbit cultivars.</td>
</tr>
<tr>
<td>5. Markers developed from major QTL are used in breeding programs to improve disease resistance in commercial cucurbit cultivars.</td>
</tr>
<tr>
<td>6. Markers are adopted by at least one fee for service genotyping lab serving the US breeding community.</td>
</tr>
<tr>
<td>7. A sustainable web-based resource is available for information about cucurbit disease diagnosis and control.</td>
</tr>
<tr>
<td>8. The cost and time frame for development of cucurbit cultivars with comprehensive disease resistance packages is reduced.</td>
</tr>
<tr>
<td>9. Cucurbit producers experience reduced losses, improved crop quality and reduced input costs and labor due to increased disease resistance.</td>
</tr>
<tr>
<td>10. There is reduced pesticide used to control cucurbit diseases.</td>
</tr>
</tbody>
</table>
TEAM PROGRESS REPORTS

and

PLANS FOR THE COMING YEAR
Genomics and Bioinformatics Team

Team members:
Zhangjun Fei (Boyce Thompson Institute)  Michael Mazourek (Cornell University)
Umesh Reddy (West Virginia St. Univ.)  Pat Wechter (USDA, ARS)
Amnon Levi (USDA, ARS)  Rebecca Grumet (Michigan State University)
Yiqun Weng (USDA, ARS)  

Objectives Year 1
Develop common genomic approaches and tools for cucurbits

<table>
<thead>
<tr>
<th>(a) Obj. 1. Develop common genomic approaches and tools for cucurbits</th>
<th>Personnel/Institution</th>
<th>Yr 1</th>
<th>Yr 2</th>
<th>Yr 3</th>
<th>Yr 4</th>
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</thead>
<tbody>
<tr>
<td>1.1. Develop genomic and bioinformatics platforms</td>
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</tr>
<tr>
<td>1.1.1. Genotyping by sequencing</td>
<td>ZF (BTI)</td>
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<td>X</td>
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<tr>
<td>1.1.2. Sequence data processing/analysis</td>
<td>ZF (BTI)</td>
<td>X</td>
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<tr>
<td>1.1.3. ICuGi database development</td>
<td>ZF (BTI)</td>
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<tr>
<td>1.1.4 Community standardized nomenclature</td>
<td>YW (ARS-WI), AL (ARS-SC)</td>
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<td>X</td>
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<tr>
<td>1.1.5. Genomic, bioinformatics workshops</td>
<td>ZF (BTI), UR (WVSU), members of crop teams</td>
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<td>X</td>
<td>X</td>
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<tr>
<td>1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)</td>
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<tr>
<td>1.2.1. GBS of cucurbit species, establish molecular-informed core populations</td>
<td>ZF (BTI), RG (MSU)</td>
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<tr>
<td>- watermelon</td>
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<td>- cucumber</td>
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<td>- squash</td>
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<tr>
<td>1.2.2. Population genetics and GWAS analyses</td>
<td>UR (WVSU), ZF (BTI)</td>
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<td>AL (ARS-SC)</td>
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<td>JM (ARS-CA)</td>
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<td>X</td>
<td>X</td>
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<td>- squash</td>
<td>MM (CU)</td>
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Work in progress and plans
1.1. Develop genomic and bioinformatic platforms for cucurbit crops
1.1.1. Genotyping by sequencing
In closely working with Cornell Genomic Diversity Facility, we have set up the genotyping-by-sequencing (GBS) platform for the four cucurbit species: watermelon, melon, cucumber and squash.

1.1.2. Sequence data processing/analysis
We have evaluated and compared the performance of TASSEL-GBS (http://www.maizegenetics.net/tassel) and GATK (https://www.broadinstitute.org/gatk) in SNP calling using GBS data. Based on the results from this analysis we have established a GBS data analysis pipeline.
1.1.3. ICuGI database development
We are in the process of re-implementing the ICuGI database using the GMOD Tripal system (http://gmod.org/wiki/Tripal) and the Chado database schema (http://gmod.org/wiki/Chado). The implementation of the database is close to done. Genome sequences of melon, watermelon Charleston Gray and wild cucumber have been processed and included in the database. Genome syntenies between watermelon, melon and cucumber have been identified and a genome synteny browser have been implemented in the database. The database is currently run on an old development server (http://tripal.feilab.net/). We recently purchased a new high-end web server and the system is currently under configuration. The database will be moved to the new server once the configuration is done, and the link will be distributed to the CucCAP teams for suggestions and final tweaks.

1.1.4 Community standardized nomenclature.
This has not begun.

1.1.5. Genomic, bioinformatics workshops
A workshop on the ICuGI database has been scheduled at the Solcuc2017 meeting in Sept., 2017 at Valencia, Spain.

1.2. Perform GBS analysis of PI collections, establish core populations of the four species, and provide community resource for genome wide association studies (GWAS)

1.2.1. GBS of cucurbit species, establish molecular-informed core populations
Each crop team has grown PI seedlings and sampled leaf tissue for processing in the Grumet lab using high throughput DNA extraction protocols established at the outset of the CucCAP project. At this time, DNA has been prepared from the full PI collection for cucumber, along with additional lines representing different market classes and historical cultivars (1615 samples). The majority of the PI collection for watermelon (1344/1384 samples) also has been completed (as of 3/1/17); the remaining samples will be received and processed shortly. After quality checks, all samples were sent in 96-well format to Cornell for GBS, where GBS is performed in 384-plex format. At this time GBS data has been received for 26 plates (14 for cucumber; 12 for watermelon) and quality of this data has been evaluated. DNA preparations are currently underway for melon; 1900 samples have been received, 1520 (13 plates) have been processed and shipped. An additional 855 are in process and should be completed by late March. The squash PI collection spans three commercially important species. 165 accessions of *C. maxima* have been genotyped with a PstI digest. Tissue for 319 accessions of the *C. moschata* collection has been collected and is awaiting transfer to the Grumet lab for DNA preps. The entire available *C. pepo* collection is currently being planted and we expect to begin DNA preps in April.

<table>
<thead>
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<th>Table 1 Status of cucurbit GBS</th>
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<tr>
<td>Batch</td>
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<td>2</td>
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<td>3</td>
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<td>4</td>
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</tbody>
</table>
### 1.2.2. Population genetics and GWAS analyses
We are waiting to get the GBS data for all samples of each species. All GBS data from each species will be processed together and SNPs called. The resulting SNPs will be used for population genomics analyses and for the analysis to identify core collections.

### 1.2.3 Genomic resequencing of core collections
During last year’s annual meeting, we agreed to change our plan to genotype the core collection using whole genome resequencing instead of GBS. We were trying to identify cheap services for library construction and sequencing to accommodate our budget. For library construction, we identified Cornell Genomic Diversity Facility as our service provider, who charges $33 for each library ([http://www.biotech.cornell.edu/brc/genomic-diversity-facility/price-list](http://www.biotech.cornell.edu/brc/genomic-diversity-facility/price-list)). For sequencing, we will use Novogene HiSeq X Ten system, which cost $18 per 100 Gb raw data.

Recently, we submitted 45 DNA samples to Cornell Genomic Diversity Facility for library construction. We expect to get the constructed libraries and sequences in about two months.
Watermelon Team

Team members:
Amnon Levi (USDA, ARS)  Cecilia McGregor (Univ. Georgia)
Shaker Kousik (USDA, ARS)  Pat Wechter (USDA, ARS)
Kai-shu Ling (USDA, ARS)  Todd Wehner (North Carolina St. Univ.)

Overall objectives: Identifying quantitative trait loci (QTL) associated with resistance to major and emerging diseases, developing useful molecular markers and utilizing the genomic tools to incorporate resistance into watermelon cultivars.

Major diseases: Gummy stem blight, Fusarium wilt, Powdery mildew, Phytophthora fruit rot, Papaya ringspot virus (PRSV) and Cucumber green motile mosaic virus (CGMMV).

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<tr>
<th>Objective</th>
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<th>Year</th>
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<tr>
<td>(a) Obj. 1. Develop common genomic approaches and tools for cucurbits</td>
<td>(initials as in Table 3)</td>
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<tr>
<td>1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)</td>
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<td>1.2.1. GBS of cucurbit species, establish molecular-informed core populations</td>
<td>ZF (BTI), RG (MSU) X X</td>
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<tr>
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<td>AL (ARS-SC), TW (NCSU) X X</td>
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<td>1.2.2 Population genetics and GWAS analysis</td>
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<td>AL (ARS-SC)</td>
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<td>(b) Obj. 2. Genomic assisted breeding for disease resistance</td>
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<td>2.1 QTL map resistances:</td>
<td>Screen for resistance (Sc), develop populations (P), phenotype (F), sequence (S), QTL map (Q)</td>
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<td>KSL (ARS-SC), AL (ARS-SC) Se</td>
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<td>SK (ARS-SC) SQ</td>
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<tr>
<td>- Phytophthora</td>
<td>SK (ARS-SC) SQ</td>
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<tr>
<td>- powdery mildew</td>
<td>AL (ARS-SC), KSL (ARS-SC) SF</td>
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<td>- PRSV-W</td>
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<td>2.2 Marker development and verification:</td>
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<td>2.2.1. Watermelon</td>
<td>AL (ARS-SC), PW (ARS-SC) R</td>
<td>RV</td>
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<td>- Fusarium race 1</td>
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<td>V</td>
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<td>2.3. Introgress resistance into advanced breeding lines:</td>
<td>Develop breeding lines (B), introgress into cultivated (I), advanced lines (A), release to breeders (R)</td>
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<td>2.3.1. Watermelon</td>
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<td>I IA</td>
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<td>PW (ARS-SC), AL (ARS-SC) B</td>
<td>I</td>
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<tr>
<td>race 2</td>
<td>CM (UGA), TW (NCSU) B</td>
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</tr>
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<td>- gummy stem blight</td>
<td>SK (ARS-SC) B</td>
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<tr>
<td>- Phytophthora</td>
<td>SK (ARS-SC) B</td>
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<td>- powdery mildew</td>
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<td>- PRSV-W</td>
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Work in progress and plans
1.2. Perform GBS analysis of PI collections, establish core populations of the four species, and provide community resource for genome wide association studies (GWAS) (Takshay Patel and Todd C. Wehner)

1.2.1. GBS of cucurbit species, establish molecular-informed core populations

i. **Objective:** Develop molecular markers for high resistance to gummy stem blight (GSB) using genome-wide association studies (GWAS) in the USDA watermelon germplasm collection, and introgress GSB resistance into watermelon cultivars.

We are collecting and increasing *Citrullus* PI accessions, heirloom cultivars, and gene mutant type-lines. Seed increase of the 2000 PI accessions is being accomplished by seed companies, USDA scientists, and university researchers. Each is increasing 1 to 10 accessions per year using controlled pollination in greenhouse or field.

A list of the PI accessions and gene type-lines is being developed to use for the DNA sampling. The sampling protocol is from Michigan State University, and will involve 1000 PI accessions sampled at the seedling stage, with one plant per accession.

**Genotyping:** A group of 384 watermelon accessions are being selected to develop a core watermelon populations. The core watermelon population will be used to develop sequence-based molecular markers (SNPs) using the genotyping by sequencing (GBS) method at Cornell University. We expect to get several thousand of SNPs for the association analysis.

**Association analysis:** Collected phenotypic and genotypic data will be analyzed using R packages: SNPassoc, snpMatrix, GenABEL and pbatR. The result of the analysis will allow us to locate and identify SNP markers associated with GSB resistance.

**Gene type lines**
Sixty-two available accessions (red text indicates difficult to find) are index

Collection and seed increase of the watermelon gene type-lines will include all cultivars, breeding lines, and PI accessions in the gene mutant list at Cucurbit Genetics Cooperative. Examples include: PI 189225 (*db, Ar-2-I*), NC-517 (*C*), PI 482261 (*Ctr*), Bush Charleston Gray (*dw-1*), PI 595203 (*zym-CH, zym-FL*). Below is the list of 42 type lines.

Watermelon gummy stem blight resistance (Luis Rivera and Todd C. Wehner)

Objective: Develop molecular markers for high resistance to gummy stem blight (GSB) using genome-wide association studies (GWAS) in the USDA watermelon germplasm collection, and introgress GSB resistance into watermelon cultivars.

Phenotyping: The WmGsb population was developed by intercrossing the most resistant accessions of *Citrullus* four times (I4), followed by crossing with elite cultivars of watermelon (I4F1), followed by intercrossing without selection, while maintaining wild and elite types in the populations (I4F1I4), followed by self-pollinations of plants at random (I4F1I4S1). The 296 lines will be screened in the MAF greenhouse and the field at Clinton NC. Resistance will be rated several times on each plot, in an experiment having 2 years, 4 replications, and 2 locations (greenhouse and field).
2.1.1.1 CGMMV
Evaluating the watermelon PI collection for resistance to *Cucumber green mottle mosaic virus* (CGMMV) and conducting genome-wide association mapping to identify SNPs associated with CGMMV resistance (KS Ling, A Levi)

*Cucumber green mottle mosaic virus* (CGMMV) is an emerging disease on watermelon and other cucurbit crops in North America, including Canada and the United States (Ling et al., 2014; Tian et al., 2014). CGMMV is a tobamovirus, similar to *Tobacco mosaic virus* (TMV). This virus was first discovered in Europe and has caused serious epidemics in several Asian countries, like China, Japan, and Korea, and more recently in Australia and the Middle East (Jordan and Israel). It is a seed-borne virus. It is highly contagious and poses a serious threat to the entire cucurbit industries in the U.S. Currently, there is no known resistance sources available for watermelon and other cucurbits. Our primary objective has been to evaluate the entire USDA watermelon germplasm collection with the prospective of identifying and developing potential sources of resistance to CGMMV.

Due to its seed-borne nature and global seed trade CGMMV geographic distribution has been expanding rapidly and it became a major threat to all major cucurbit crops and the entire cucurbit industries in the U.S. and around world. The CGGMV has been identified as an emerging virus on cucumber, melon, watermelon and other cucurbit crops in Canada and the United States (Ling et al., 2014; Tian et al., 2014) and in Australia (http://www.nt.gov.au/d/cgmmv/).

- We have concluded the preliminary screening in a containment greenhouse of the entire USDA watermelon germplasm (~1,600 accessions) through mechanical inoculation, symptom observation and appropriate lab testing.
- Plants from seven accessions (including 3 *Citrulus lanatus* and 4 *C. colocynthis*) with potential resistance (tolerance) to CGMMV were selected based on symptom expression and lower virus titer in tests.
- Various number of seeds were collected from these selected plants.
- Secondary screening for resistance to CGMMV using seedlings generated from the S1 seeds (various number of seeds were collected from these selected plants) are underway and the results will be analyzed and presented at the project meeting.
- Resistant plants will be saved for selfing to produce S2 seeds. Cross pollination will be conducted to generate segregation populations for study genetic inheritance of the resistance.
In addition to watermelon, to determine whether any of the USDA cucumber germplasm has resistance to CGMMV, we screened 174 core collection of cucumber germplasm. Unfortunately, none of the test materials was resistant to CGMMV. However, more cucumber germplasm collections are available for screening if needed.

Furthermore, we also screened 18 rootstocks. Although *lagenaria siceraria* and *C. lanatus* genotypes were susceptible, several Cucurbita hybrids (*Cucurbita maxima* x *Cu. Moshata*) were resistant to CGMMV. These CGMMV-resistant rootstocks may be useful to protect grafted watermelon from CGMMV infection through root contacts in contaminated soil.

Finally, seeds for approximately 200 accessions of *Cu. Maxima* and *Cu. Moshata* have been requested from the USDA-ARS germplasm resource centers and will be used to screen for resistance to CGMMV.

References:

2.1.1.2- Fusarium race 1,2
Genetic mapping of QTL associated with resistance to Fusarium oxysporum races 1 and 2 in *Citrullus lanatus* var. *citroides*. (P. Wechter, S. Branham, and A. Levi)

_Fusarium oxysporum* f. sp. _niveum_ which causes fusarium wilt of watermelon, is considered one of the most important diseases of watermelon production in the United States. There are currently no economical or even viable chemical control strategies or methods that can control this soil-borne pathogen. To date, only a few watermelon lines have been identified and reported as resistant or tolerant to this pathogen. Unfortunately, although some of these lines were reported more than twenty-five years ago, no commercial cultivar is available with resistance to the most serious of the races of this fungus, race 2.

_Development of Germplasm lines and Genetic Populations_

- Seeds of both *Citrullus lanatus* var. _citroides_ USVL246-FR2 and USVL252-FR2, both developed in our work, have been requested and disseminated to eight seed companies and numerous researchers for use in breeding programs and fusarium studies. More than 4000 seeds were distributed in FY2016.
- F2:3 population (\(N = 173\)) derived from crossing the Fusarium wilt races 1 and 2 resistant *Citrullus lanatus* var. *citroides* line with the susceptible *Citrullus lanatus* var. *citroides* USVL114 line.
- Two hundred and twenty-five single seed descent lines have been taken to the F6 stage from a cross of _Fusarium oxysporum_ f. sp. _niveum* (*Fon*) race 1 and 2 resistant *Citrullus lanatus* var. _citroides_ USVL246 by a susceptible *Citrullus lanatus* var. _citroides_ P1582114. These will be carried to the recombinant inbred line (RIL) population (F\(_{++}\)).
- Two reciprocal F2:3 genetic populations USVL252-FR2 x PI 244019 (S3; resistant to papaya ringspot virus PRSV) have been developed. The first population includes 178 F2:3 families, while the population derived from the reciprocal cross includes 195 F2:3 families. These populations have been developed with the generous support of Sakata Seeds (Dr. Nihat Guner).
Evaluating genetic populations and genetic analyses to identify QTL associated with resistance to Fusarium wilt races 1 and 2

- Two rounds of Fon race 2 greenhouse inoculation studies were performed.
- DNA was isolated from 180 of the F2 plants from this cross, and F3 seed generated from each. All 180 of these DNAs have been sequenced using genotyping by sequencing (GBS) procedure.
- Genotype and phenotype data have been analyzed and we have identified one major and four minor QTL for race 2 resistance (Branham et al. 2016).
- Genetic mapping with this population resulted in a saturated map with 2495 SNP markers (Branham et al. 2016).
- F2:3 lines have been assayed for resistance to Fon race 1.
- QTL associated with race 1 resistance have been identified and mapped to the genome.
- The two reciprocal F2:3 genetic populations USVL252-FR2 x PI 244019 will be evaluated for resistance for. Leaf samples were collected from all F2 plants for GBS-SNP analysis following their evaluation for resistance to Fusarium wilt races 1 and 2.

Breeding resistance in to watermelon cultivars

- USVL252-FR2 and USVL246-FR2 have been crossed into Sugar Baby, Charleston Grey and Calhoun Grey.
- Backcrossing (BC2F2) of the above into the recurrent parent have been performed.

References:

2.2.1.1. Converting a Fusarium wilt race 1-resistance QTL to a DNA marker (S. Branham, P. Wechter, and A. Levi)

Year 2 progress: 2.1.1; 2.2.1; 2.3.1

Fusarium wilt (FW) race 1 is a major disease of watermelon throughout the United States. In a recent study (Lambel et al. 2014), we identified a major quantitative trait locus (QTL) on chromosome 1 of watermelon associated with resistance to FW race 1. There is a need to develop a SNP marker(s) for marker assisted selection (MAS) to precisely predict the presence of resistance in large genetic populations.

We have been developing genetic populations F1, F2, BC1R, BCS [Calhoun Gray (R) x Sugar Baby (S); or Calhoun Gray (R) x Black Diamond (S)] segregating for resistance to FW race 1. To identify a tightly linked DNA marker, we performed QTL-seq analysis. We bulked the DNA of most resistant versus most susceptible F2 plants. The parent genomes and the resistant and susceptible bulked DNAs (Lambel et al. 2014) were re-sequenced. SNPs were called from genomic alignments and a delta-
SNP index was calculated as the frequency of the resistant allele in the susceptible bulk subtracted from the frequency in the resistant bulk.

QTL-seq analysis increased the resolution of the QTL on chromosome 1 from 6.5 Mb (Lambel et al. 2014) to 1.5 Mb. The resequencing data have been used for the construction of 20 KASP primers in this region. Of the 20 KASP primers, 10 are located in the area of highest association with resistance (0-500 kb) while the other 10 KASP primers are spread across regular intervals of the remainder of the major QTL (500 kb -1.5 Mb). The KASP primers will be used in a genetic analysis to identify the SNP which is most tightly linked to resistance to FW race 1 in watermelon.

Reference:
Overview of objectives for project duration

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<th>Objectives</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
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1. Progress for Year 2

1.1 Population Development:

WPop GSB 1: PI 482276 x Crimson Sweet population of 220 F2:3 lines. **Complete**

WPop GSB 2: PI 526233 x Sugar Baby population for 96 F2:3 lines. **Complete**

WPop GSB 3: Backup population of resistant *C. amarus* (PI 482276) x susceptible *C. amarus*. **In progress**

1.2 Phenotyping

Phenotyping of gummy stem blight is very challenging, particularly when phenotyping a large number of plants. We tested several protocols spray and agar drop inoculation protocols in greenhouses/growth rooms/growth chambers. As expected, growth chambers gave the most constant results. The spray and drop methods gave comparable results, but the spray method takes less time when having to inoculate many plants. We secured space at the UGA Envirotрон facility (Griffin, GA) which will enable us to carry out all screenings in growth chambers with complete temperature and humidity control. Population screening will start at this facility in April 2017. The parental lines and a subset of F2:3 families will also be phenotyped in the field in Attapulgus (GA) in summer 2017. The purpose of this is to compare field level resistance to what we observe in the growth chamber screens. A subset is used due to space limitations at the field site.

1.3 Sequencing

Initially it was proposed that Genotyping-by-sequencing would be used for genotyping of the populations. However, at the 2016 meeting in East Lansing it was proposed that QTL-Seq should instead be used. In order to do QTL-Seq, phenotyping needs to be completed in order to select the lines that will make up the
resistant and susceptible bulks. All the leaf samples have been collected and are in the -80°C, ready for genotyping once phenotyping is complete.

1.4 QTL Mapping
See above

2. Conclusion and Future Work
Our schedule has changed somewhat due to the need to complete phenotyping before genotyping can start. However we are still confident that we can complete our project goals.

2.1.1.4. Phytophthora fruit rot of watermelon (S. Kousik)

Project metrics (time line) for research on Phytophthora fruit rot and powdery mildew of watermelon

- Develop germplasm lines with resistance to Phytophthora fruit rot and powdery mildew for watermelon: Completed.
- Develop populations for phenotyping resistance to Phytophthora fruit rot and powdery mildew of watermelon: Completed
- Sequence and map Phytophthora fruit rot and powdery mildew QTL in watermelon: In progress.
- Introgress Phytophthora and powdery mildew resistance into cultivated type watermelon: In progress
- Participation in outreach to stakeholder groups per year via industry events and field days: Completed

Phytophthora fruit rot of watermelon has been a major problem in watermelon growing areas in the Southeastern U.S. (FL, GA, SC, NC and VA). In recent years it has also become a problem in watermelon growing areas in Maryland (MD), Delaware (DE) and Indiana (IN) (Kousik et al., 2016). At the U.S. Vegetable Laboratory (USDA, ARS) in Charleston we have developed several germplasm lines with high levels of resistance to Phytophthora fruit rot. In these studies we used the germplasm line USVL531-MDR which was resistant to 20 different \textit{P. capsici} isolates from across the U.S.A. Studies to determine inheritance of resistance to Phytophthora fruit rot using the same population described for powdery mildew (USVL531-MDR X USVL677-PMs) were conducted as USVL531 is resistant to both these diseases. Fruit from parents, F1, F2 and back cross populations were harvested when mature and placed on wire shelves in a walk-in-humid chamber. Each fruit was inoculated with a 7-mm agar plug from an actively growing colony of \textit{Phytophthora capsici} as described before (Kousik et al., 2014a,b). Data on fruit rot was recorded five days after inoculation. Initial observations of the data indicated that inheritance to Phytophthora fruit rot is more complex than powdery mildew. We are currently compiling and analyzing the data from this study. As mentioned above, we have extracted DNA from parents and F2 plants for GBS. Of the F2 plants we self-pollinated 186 plants kept in a net house to generate F2:3 populations for further evaluation. We will evaluate F3 families in 2017. Similarly we will phenotype the populations from USVL003-MDR x USVL677-PMs for resistance to Phytophthora fruit rot in 2017.

Powdery mildew of watermelon (Shaker Kousik)

Powdery mildew of watermelon (\textit{Citrullus lanatus}) continues to be a constant problem throughout the southeast. Our recent survey of watermelon researchers also indicated that powdery mildew was considered an important priority for research across the U.S. (Kousik et al., 2016). We have developed USVL531-MDR which is resistant to powdery mildew and Phytophthora fruit rot and have provided the
seeds of this germplasm line to many seed companies through and MTA. USVL531-MDR is an egusi type watermelon with white flesh and low brix (<2% TSS) and was derived from PI 494531 after five cycles of screening and selections. USVL677-PMS was derived from PI 269677 after five cycles of screening and selection for high levels of susceptibility to powdery mildew and Phytophthora fruit rot for use in genetic studies. A simple inheritance of resistance study on powdery mildew of watermelon caused by *Podosphaera xanthii* was conducted on the segregating population derived from the cross of USVL531-MDR x USVL677-PMS. A total of 713 plants were evaluated. Of these 66 plants were of the resistant parent (USVL531-MDR) and 81 plants of susceptible parent (USVL677-PMS). Of the segregating population, 112 were F1, 311 F2, 64 BCF1S and 80 BCF1R. All the plants were inoculated using a suspension (10^5 conidia/ml) of powdery mildew conidia in sterile water plus 0.02% tween 20 as described before (Kousik et al., 2011). The powdery mildew isolate prevalent in the Charleston, SC area was used. This isolate behaves a melon Race 1 based on its reaction on melon (*Cucumis melo*) differentials. The isolate also is capable of causing powdery mildew on various watermelon cultivars including Mickey Lee, Dixie Lee and Crimson Sweet. Powdery mildew ratings on a 0-10 scale of increasing disease severity was recorded for hypocotyl, cotyledons and true leaves. Resistance to powdery mildew in cotyledons and true leaves appears to be a dominant trait in USVL531-MDR. We have completed extracting DNA from parents, and 180 F2 plants for GBS analysis. Of the F2 plants we self-pollinated 186 plants kept in a net house to generate F2:3 populations for further evaluation. We will evaluate F3 families in 2017. Fruit from F2 plants with powdery mildew resistance, uniform red flesh and decent brix (>7) were collected and have been advanced till F4 and further advancement to F5 is in progress. We conducted a progeny test on 23 red fleshed F4 lines using 16 plants per line and identified several lines that are homozygous for resistance (Figure 1). Currently we are growing these in the greenhouse to get self-pollinated fruit to determine fruit quality and for advancing the line further to develop powdery mildew resistant inbred lines for release.

Segregating populations (F1, F2, BCF1R, BCF1S) from cross of USVL003-MDR and USVL677-PMS were developed in 2016. USVL003-MDR is resistant to powdery mildew and Phytophthora fruit rot whereas USVL677 is susceptible to both these diseases. USVL003 is an egusi type watermelon with white flesh and low brix and was derived from PI 560003 after five cycles of screening and selections. Studies on inheritance of resistance to powdery mildew and Phytophthora fruit rot will be conducted in 2017 spring and summer.

We have recently hired a graduate student (Ph.D.) through Clemson University who will be conducting her Ph.D. research project at USVL, USDA, ARS in Charleston, SC on breeding for resistance to powdery mildew of watermelon.

Figure 1. Progeny test of advanced watermelon progenies with red flesh and resistance to powdery mildew. Line # 8 (plants on left) in the figure is a homozygous highly susceptible line compared to line # 29 (on right) that is homozygous for resistance to powdery mildew.
References


2.1.1.6 PRSV-W (A Levi, K-S Ling)

Identification of QTL associated with papaya ringspot virus (PRSV) in watermelon

Genetic populations F2:F3 have been generated using PRSV-susceptible Clc parent USVL252-FR2 crossed with the PRSV-resistant Clc parents PI 244017 (Pop 52-17) or PI 244019 (Pop 52-19) mentioned above. The genetic populations have been prepared with the generous help of Dr. Nihat Guner, Sakata Seeds). The populations are being prepared for evaluation for PRSV-resistance (as described by Ling et al. 2009). Leaf samples have been collected and following evaluation for PRSV resistance, DNA will be isolated and used in GBS analysis for identification of SNPs and QTL associated with PRSV-resistance.

Reference

Melon Team

Team members:
Jim McCreight (USDA, ARS) Pat Wechter (USDA, ARS)
Shaker Kousik (USDA, ARS) Bill Wintermantel (USDA, ARS)
Michael Mazourek (Cornell Univ)

Table 4. TIMELINE CHART

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<th>Objective</th>
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<td>(a) Obj. 1. Develop common genomic approaches and tools for cucurbits</td>
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<tr>
<td>1.2. Perform GBS analysis of PI collections, establish core populations,</td>
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<td>provide community resource for genome wide association studies (GWAS)</td>
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1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)

1.2.1. GBS of cucurbit species, establish molecular-informed core populations

Melon

Melon accessions (n = 2,038) from the National Plant Germplasm System (NPGS) and additional heirloom melons were planted to collect leaf tissue for DNA isolation. A total of 22 plates will be send to Michigan State University (MSU) for extraction. To date, 21 plates have been sent; the last plate will be sent shortly. Four plates of high quality DNA have been sent thus far from MSU to Cornell for GBS analysis.

1.2.2. Melon

Powdery Mildew (Podosphaera xanthii) resistance in MR1xAY RIL

Charleston, South Carolina Race 1

- First round of phenotyping of MR1xAY RIL against Race 1 was completed in June 2016.
- Second round phenotyping of MR1xAY RIL against Race 1 was completed in March 2017.
- Total of 210 RIL lines were phenotyped
- Melon powdery mildew race differential lines confirmed the isolate was race 1 (Figure 1.)
- Three replications of four plants were evaluated in both rounds.
- First round was inoculated on May 16, 2016, and rated on May 27th and June 7th.
- Second round was inoculated January 17, 2017, and rated on February, and March 1, 2017.
  - Conidial suspension (10^5 conidia/mL in distilled water with 0.02% tween 20)
• Uniformly sprayed on the plants.
• First observation powdery mildew was recorded on hypocotyl, cotyledons and true leaves in first and second rounds. Mildew was also recorded for stems in the second round.
• Second observation recorded powdery mildew on upper true leaves.
• *MR1xAY RIL* populations will be phenotyped for resistance to powdery mildew race 2 during the fall of 2017.

![Image](image_url)

**Figure 1.** Powdery mildew reactions on melon powdery mildew race differentials included in the assays of the *MR1xAY RIL* population. Top row from left to right: Iran H, ‘PMR 45’, ‘PMR 5’, and ‘Edisto 47’. Bottom row from left to right: WMR 29, PI 414723, MR-1, and ‘Védrantais’. Severe powdery mildew was only observed on leaves of Iran H and ‘Védrantais’, indicating that the isolate was race 1. (Charleston)

**California and Arizona Field tests**
Three replicated field tests planted; 138 RILs plus race differentials; subjected to natural infection.

• Imperial Valley, CA (University of California, Desert Research and Extension Center, Holtville)
  - watered March 4, 2016
  - Disease reaction evaluated mid-June
  - Variable plant stands (due reportedly to specific site conditions)
  - Virtually no powdery mildew due to abnormally high temperatures.
  - Plant and fruit data collected
• Yuma, AZ (Yuma Agricultural Research Center),
  - watered March 31
  - Disease reaction evaluated mid- to late June
  - Stands fairly uniform
  - CMV present in late April
  - Virtually no powdery mildew due to abnormally high temperatures.
  - Plant and fruit data collected
• Westside (Westside Research and Extension Center, Five Points)
  - Planting scheduled for early to mid-June
Evaluation in early to mid-September

- Plant and fruit data collected.

- ADDITIONAL
  - Leafminers were uniformly distributed throughout the test. RILs and race differentials were qualitatively (susceptible or resistant) evaluated for leafminer infestation. MR-1 and PI 124111 were susceptible, but several powdery mildew race differentials exhibited resistance: PI 414724, PI 313970, PI 482420 (TGR 1551) and PI 482431 (TGR 1937). PI 313970 was previously reported resistant to leafminer, and PI 371795 was variable for resistance (Kennedy et al. 1978. J. Amer. Soc. Hort. Sci. 103:571–574). PI 414723 was derived form PI 371795 through selection for uniform reaction to melon aphid, *Aphis gossypii* (McCreight et al. 1992. Cucurbit Genet. Coop. Rpt. 15:51–52).

**Fusarium Wilt resistance in MR1xAY RIL**

- 205 RIL have now been generated from a cross of MR-1 x Ananas Yokneam.
- GBS has been completed on 90 RIL lines and we are currently waiting for data from an additional plate that will be provided in April 2017.
- *Fusarium oxysporum* f. sp. *melonis* (FOM) race 2 assays for resistance of the RILs. Two individual tests of 190 RIL were evaluated.
- QTL analysis will be performed as soon as data from the second GBS plate are available.
- New isolates of FOM race 1 are being tested for pathogenicity due to loss of virulent isolates at USVL.
- 150+ RILs have been grown in California and in South Carolina and evaluated for phenotype for future selection for introgression to U.S. western shipper-type cantaloupe.
- ADDITIONAL: 90 RIL lines were assayed for Alternaria leaf spot resistance, QTL were identified for resistance. (Daley et al. 2017. Phytopathology. DOI 10.1094/PHYTO-06-16-0246-R)
- ADDITIONAL: RILs will be tested for carotenoids in summer 2017 in collaboration with Dr. Li Li, ARS, Ithaca, NY.

**CYSDV**

- Planted PI 313970 and ‘Top Mark’ in a greenhouse for crossing and production of the F2 for evaluation in fall 2017 in the field. DNA samples and cuttings will be collected from every plant in mid-September for QTL mapping and producing F2:F3 in a greenhouse at Salinas.
- ADDITIONAL related research on resistance in melon to sweetpotato whitefly
  - Third year of a field evaluation of resistance is planned for spring 2017 (late-April planting date).
  - Data from greenhouse studies of whitefly resistance in no-choice tests were analyzed.
  - Choice tests of whitefly resistance are planned to assess differences in antixenosis (non-preference) among sweetpotato whitefly-resistant sources.

**CMV**

- Increase advanced CMV-resistant lines (western U.S. shipping type cantaloupe, and honeydew) developed by M. Kyle-Jahn and H.M. Munger for assessment of CMV resistance in controlled-inoculation greenhouse tests and adaptation and fruit quality in field tests at three locations in Arizona and California.
Additional Related Virus Information and Activity

- The Wintermantel Lab maintains live and frozen stocks of *Cucumber mosaic virus* (CMV), *Cucurbit yellow stunting disorder virus* (CYSDV), and other viruses common in the southwestern US production region. In addition, the lab maintains stocks of the whitefly, *Bemisia tabaci* MEAM1, and both green peach aphid (*Myzus persicae*) and melon aphid (*Aphis gossypii*).

- The lab maintains live and frozen stocks of *Cucumber mosaic virus* (CMV), *Cucurbit yellow stunting disorder virus* (CYSDV), and other viruses common in the southwestern US production region. In addition, the lab maintains stocks of the whitefly, *Bemisia tabaci* MEAM1, and both green peach aphid (*Myzus persicae*) and melon aphid (*Aphis gossypii*).

- Testing of selected material from experimental trials by RT-PCR to determine prevalence of CYSDV and other common cucurbit viruses.

Primers previously developed and confirmed effective in the Wintermantel Lab were used to test plant melon plants from summer CYSDV resistance evaluations for the presence of not only CYSDV, but also other viruses that are periodically associated with melons. This allowed determination of what other viruses may have contributed to severity in melon fields. Results demonstrated an abundance of CYSDV in late spring/early summer 2016 research trials from the desert, as well as additional prevalence of CMV and Squash vein yellowing virus in these melons. *Squash vein yellowing virus* (SqVYV) is an important pathogen of watermelon that emerged in California two years ago, but evidence to date suggests little if any impact on melon production. CMV and CYSDV, however, both have potential for significant impact on melon production and were both prevalent in trials, although the predominant symptoms were yellowing, caused by CYSDV.

- CMV – 5/7 were positive
- CYSDV – 7/10 were positive
- SqVYV – 3/11 were positive
- Potyviruses – 0/7 were positive (Checked with a general potyvirus primer set and also specifically for PRSV, ZYMV, and WMV.)

- Development and testing of TaqMan probes for quantification of CYSDV in melon.

TaqMan probes were developed for detection and quantification of CYSDV. Probes were designed to an 81 nt region within the RNA dependent RNA polymerase (RdRp) gene of CYSDV RNA1. Evaluation demonstrated reliable detection of CYSDV and a linear relationship with the amount of CYSDV present in starting material (Figure 2), but await more robust evaluation from new field and greenhouse tests.

![Figure 2](image)

*Figure 2*. The assay was performed with SuperScript III Platinum One-Step Quantitative RT-PCR System (Life Technologies) for use with TaqMan probes. The standard curve range is from $10^2$ to $10^7$ copies. Samples graphed with standards were randomly selected from experiments to evaluate linearity.
2.2 Marker development and verification: Refine map (R) develop marker (M), verify (V)

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**Powdery Mildew**
- No planned research for this period

**Fusarium wilt**
- See 2.1.2.

**CYSDV**
- No planned research for this period

**CMV**
- No planned research for this period

2.3. Introgress resistance into advanced breeding lines: Develop breeding lines (B), introgress into cultivated (I), advance lines (A), release to breeders (R)

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**Powdery Mildew**
- Planted three replicated field tests of 138 MR-1 x AY RILs at three locations in California (Imperial Valley and San Joaquin Valley) and Yuma, Arizona. There was scant powdery mildew infection in Imperial Valley and Yuma in June 2016. Powdery mildew was present in the San Joaquin Valley (Five Points) in September that appeared to be a variant of race 5, based on the reactions of 12 commonly used cucurbit powdery mildew race differentials.
- Forty-four North America-originated *C. melo var texanus* accessions (Decker-Walters et al. 2002. DOI 10.1007/s00606-002-0191-3) were evaluated for resistance to powdery mildew in a greenhouse to an unverified race (but where either race S or SD has mostly been present year-round for many years). All were susceptible with one exception that showed resistant phenotype with resistant blisters (McCreight. 2001. Cucurbit Genet. Coop. Rpt. 24:22.). We will self and cross the accessions with ‘Top Mark’ to further characterize the genetic basis of resistance. The *texanus* endornaviruses will also be characterized and compared with previous results form this Salinas lab (see Sabanadzovic et al. 2016).
- ADDITIONAL
  We have consulted with M. Pitrat about the correct/current horticultural nomenclature for the *texanus* accessions (Decker-Walters et al. 2002. DOI 10.1007/s00606-002-0191-3), as they were not considered in the recent revision of melon horticultural classification (Pitrat. 2016. DOI 10.1007/7397_2016_10).

**Fusarium wilt**
- See 2.1.2.
**CYSDV**
- Backcrossed resistant field selections from Fall 2015 for selfing and backcrossing in the greenhouse.
- Evaluated S\(_1\) from several different backcross families for resistance to natural infection in Imperial Valley in Fall 2016.
- CYSDV-resistant single plant selections from S\(_1\) of several backcross populations taken as vegetative cuttings for backcrossing in a greenhouse at Salinas and subsequent selection in fall 2017.

**CMV**
- Increase of advanced CMV-resistant lines for testing in Arizona and California is underway.

1.2. **Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)**

1.2.1. **GBS of cucurbit species, establish molecular-informed core populations**

Melon accessions held by North Central Plant Germplasm Resources Center, Ames, Iowa will be grown in a greenhouse at Salinas and sampled for GBS beginning late in year 1 and continuing through year 2.

Completing production of 200 RILs of MR-1 x Ananas Yokneam (MR1xAY). Seed of each RIL will be produced for remaining studies, including GBS on ~190 RIL for identification of high-quality SNPs for use in QTL analysis and mapping. Finished generation of one hundred and seventy-five F\(_7\) or greater MR1xAY RIL. Will have completed 200 F\(_7\) or greater lines by the end of 2016. Seed has been distributed for powdery mildew screens and fruit quality assessment. Seed being increased for 2017 screens.

Performed GBS of 89 MR1xAY RILs and have identified 2200, high-quality SNPs that have been used to identify 2 QTLs linked to Alternaria leaf blight resistance and to generate a high-density map of MR-1 x AY.

Will perform GBS with another 95 RILs and identify high-quality SNPs from the sequencing data. Will begin the Fusarium assays for Race 1 and 2. Will repeat powdery mildew tests and begin QTL analysis and mapping of Powdery mildew resistance.
Cucumber Team

Team members:
Yiqun Weng (USDA, ARS)
Rebecca Grumet (Michigan St. Univ.)
Todd Wehner (North Carolina St. Univ.)

Objectives

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2. Genomic assisted breeding

Objective 1.2 GBS of PI collection, establish GWAS core
Personnel: Weng (Wang Y, Tan J, Madera AR, Haider KR), Grumet (Grumet R, Hammar S.) and Wehner (Wehner T., Silverman EJ) Labs

2016-2017 progress
1. GBS of PI lines
DNA samples of 1,520 plant introduction (PI) lines and historical cultivars or landraces of cultivated (Cucumis sativus var. sativus) and wild (C. sativus var. hardwickii) cucumber lines have been sent to the Genomics team at Boyce Thompson Institute, Cornell University for genotyping by sequencing. Among them sequencing of 1,420 samples has been done. Data analysis is underway.

2. Phenotyping of morphological traits and DM resistance in cucumber natural populations
Three hundred cucumber lines were grown in the University of Wisconsin Hancock Agricultural Research Station (HARS) for collection of morphological data. One hundred cucumber lines (2 reps, 8 plants per rep) were planted in North Carolina State University experimental field in summer 2016. Data for responses to DM natural infestation were collected.
2017-2018 work plan
1. A core collection (384) will be selected pending GBS data analysis from Bioinformatics team.
2. Seed increase of the 384 PI lines
3. The 384 lines will be planted in 2017 field season at HARS for phenotypic data collection.
   The same set of materials will be grown in North Carolina State University fields for collecting data for responses to natural DM infestation.

Objectives 2.1 and 2.2: QTL mapping, marker development for DM and PFR resistances

A. Fine mapping of DM resistance in cucumber (Weng and Wehner Labs)

2016-2017 progress
We aim to conduct QTL mapping of DM resistance from two resistant sources: PI 330628 (WI7120) and PI 197088. Previous QTL mapping studies have identified 4 QTL, dm2.1, dm4.1, dm5.2 and dm6.1 for DM resistance from WI7120 which together could explain 62-76% phenotypic variations. Among them, dm4.1 and dm5.2 were major-effect QTL (Wang et al. 2016). Using the PI 197088 × Coolgreen RIL population, 11 QTL for DM resistance were identified including dm1.1, dm2.1, dm2.2, dm3.1, dm3.2, dm4.1, dm5.1, dm5.2, dm5.3, dm6.1 and dm6.2. Among them, dm5.2 and dm5.3 are major-effect QTL. The four DMR QTL detected in WI7120 seem to be consistent with the corresponding ones detected in PI 197088 but their contributions to the total observed phenotypic variations are different. The dm5.3 QTL in PI 197088 is also closely linked with, but not the same as pm5.1 (syn. CsMLO1 or CsMLO8, pm-h), which is a major-effect QTL for PM resistance in cucumber.

We focused on three major-effect DMR QTL, dm4.1, dm5.2 from WI7210 and dm5.3 from PI 197088 for fine mapping. F2 and RIL plants carrying respective QTL regions were selected to backcross with the susceptible cucumber line 9930. Backcross derivatives (BC1, BC2) carrying target regions were selected with molecular markers.

2017-2018 work plan
1. Narrow down the QTL region (1.5 LOD interval) of target QTL regions through fine genetic mapping and GWAS; identify candidate genes for dm4.1 and dm5.2.
2. Growth chamber and field evaluation of DM resistance of the NILs (Wisconsin and North Carolina).
B. QTL mapping of *Phytophthora capsici* resistance in cucumber (R Grumet lab - B Mansfeld, Y-C Lin; in collaboration with C. Smart)

B.1 Young fruit resistance to *P. capsici*

2016-2017 progress:

1. **Testing promising PI 104983-derived families with multiple *P. capsici* isolates.** S₆ families were grown in the field in summer 2016. Young fruit were harvested and tested for resistance to four isolates of *P. capsici* obtained from various crops (cucumber, pumpkin, pepper and bean) from locations in Michigan and New York: OP97; 10193; SP98; Bartley’s1 and NY 0664-1. The isolates were originally provided by Mary Hausbeck (MSU) or Chris Smart (Cornell). Prior tests had been done with isolate OP97, originally isolated from cucumber in Michigan. Despite differences in severity among the different isolates, the relative disease rankings of the different lines were consistent, suggesting that the resistance is not isolate-specific. The rate of disease development was markedly reduced in PI 104983-53B with symptoms largely limited to the region of inoculation.

2. **Germplasm release.** An S₆ progeny line with young fruit resistance to *P. capsici*, PI 104983-53B, exhibited as reduced or delayed infection with little or no mycelial growth and sporulation at 5 days post inoculation (mean score 3-4/9 vs. 7-8/9 for susceptible controls), was prepared for release for breeding purposes (Colle and Grumet, 2017). Seed has been requested by, and provided to, three companies.

3. **Initiate introgression and genetic analysis – population development.** Crosses were made in the greenhouse in spring 2016 between resistant S₅ generation, PI 109483-53B (B) plant plants and the susceptible, sequenced pickling cucumber breeding line, Gy14 (G). In summer 2016, F₁ (G x B and B x G) plants were self-pollinated to produce segregating F₂ progeny and were backcrossed to Gy14. Reciprocal F₁ progeny were planted in the field in summer of 2016 and compared to parental genotypes. The F₁ progeny had intermediate phenotypes for infection by *P. capsici* relative to the parents, suggesting quantitative inheritance. There were not differences between the reciprocal crosses. Backcross progeny were tested in the greenhouse in Fall 2016 and BC₁ individuals showing resistance were backcrossed again to Gy14. Doubled haploid families derived from four PI 109483-53 lines were produced by Rijk Zwaan for future analysis.

4. **Field trial in *P. capsici* infested field.** Field trials in a heavily *P. capsici*-infested site maintained in Geneva, New York are being performed by Dr. Chris Smart (Cornell University). The primary purpose of the trial in 2016 was to establish effective inoculation conditions. Two methods were tested: spray at fruiting time with zoospore suspensions, and incorporation of infested vermiculite into the soil between the rows. Inoculations were applied twice during the season. The soil incorporation method was more effective than spraying with zoospores in establishing cucumber fruit infection.
2017-2018 work plan:
1. Screen F₂ progeny from PI-derived resistant lines x Gy14, phenotyping for response to *P. capsici* to initiate inheritance and QTL analyses.
2. Intercross BC progeny to increase resistance levels in a background with better fruit type, more uniform germination, and earlier female flower production.
4. Initiate GWAS analysis for resistance to *P. capsici* using GBS data for cucumber PI accessions and data from prior *P. capsici* screening of the cucumber PI collection.
5. Field trial in *P. capsici* infested field. Further work is needed to establish effective screening conditions in the field. Tests this year will be performed with planting densities used by commercial pickling cucumber producers for machine harvest.

B2. Age-related resistance (ARR) to *P. capsici*

2016-2017 progress:
1. QTL seq analysis. Fruit from F₂ progeny (n=355) of lines that do and do not express ARR were phenotyped for response to *P. capsici*. Pools of resistant and susceptible individuals were sequenced using Illumina Hi-Seq paired-end sequencing. QTL seq analysis is currently in progress.
2. Transcriptomic and metabolomic analysis of peels from ARR+ and ARR- cultivars. A manuscript describing the transcriptomic and metabolomic work has been submitted for publication (Mansfeld et al.). Genes uniquely upregulated in resistant age ARR+ fruit were associated with defense and/or specialized metabolism. Untargeted metabolomic analysis identified ions uniquely abundant in resistant ‘Vlaspik’ 16 dpp peel extracts; the most abundant of which had relative mass defects consistent with terpenoid glycosides.

2017-2018 work plan:
Continue QTL seq analysis for ARR from F₂ populations of Gy14 × Poinsett and Gy14 × Vlaspik. Screen additional F₂ progeny to increase population size. Compare results of transcriptome analysis with QTL seq analysis to help identify genomic regions of greater interest.

Objective 2.3 Advanced line development for downy mildew resistance

2016-2017 Progress:
1. Marker-assisted QTL pyramiding (Weng and Wehner Labs)
Our objective is to develop a new version of the elite pickle cucumber inbred line Gy14 with improved DM resistance to the post-2014 DM strain. We focused on marker-assisted pyramiding of the two major-effect QTL (*dm4.1* and *dm5.2*) of DM resistance from WI7120 into Gy14.
genetic background. Crosses were made between Gy14 and plants carrying \( dm2.1 \), \( dm4.1 \) and \( dm5.1 \) QTL from WI7120. In 2016-2017 period, we have advanced the backcrosses to BC3F1 using marker-assisted selection. In 2016 summer trial, these plants were grown in the University of Wisconsin Hancock Agricultural Research Station for preliminary observations.

2. Breeding line development for DM resistance

2.1. RIL development and evaluation of DM resistance (Wehner lab: T Wehner, EJ Silverman)

The RILs population was developed in 2007 by a cross PI 197088 (HR) × Coolgreen (S). A total of 200 F2 lines were generated and self pollinated in the greenhouse in 2009. The RILs have been tested in 7 years of field evaluations under high disease intensity. The 2017 population contains 146 lines; 71 at S12 generation, 35 at S11 generation, 32 at S10 generation, and 8 at the S9 generation. Several lines are being recovered and advanced for use in genetic studies.

In 2016, we evaluated for high resistance to the new downy mildew in the field in North Carolina. The design was a randomized complete block with 3 replications and 4 disease ratings. Lines were also rated for fruit traits such as skin type (smooth, cracked, netted) and spine color (black, white).

2.2 Inbreds with resistance and quality (Wehner lab: TC Wehner and EJ Silverman)

The population PI 197088 (HR) × Poinsett 76 (MR) contains 72 lines. The plants have been self pollinated in the greenhouse 8 generations and tested in the field for evaluation of yield, quality and resistance. We recovered 9 lines of the 72 that did not advance to S8 in the past greenhouse cycle. We were not able to recover 3 lines last greenhouse cycle and these lines are in the S7 generation. Lines in S6 and S7 are being tested in the field for yield, earliness and quality for release to the industry.

We selected and self-pollinated sub-lines from 41 lines that are at the S8 to S9 generation in the greenhouse in 2016. The lines were evaluated for high resistance to the new downy mildew, as well as fruit quality, in the field in North Carolina. The most resistant lines were crossed in the greenhouse using parents that had intermediate fruit quality, with the objective of improving fruit quality among the highly resistant lines.

2017-2018 work plan (Weng and Wehner Labs)

1. Continue marker-assisted backcrossing in Gy14 genetic background for pyramiding of \( dm4.1 \) and \( dm5.2 \) QTL from WI7120. Conduct field and greenhouse screening tests to evaluate DM resistance and performance of horticulture traits.

2. Develop inbred cucumber populations. Three populations (PI 197088 × Gy14, NC-25, or Poinsett 76) are being developed for inbred development of pickling and slicing type. Eight to 10 lines each have been selected with yield, earliness, quality and resistance. They will be released to industry for use cultivar development. In 2016, we advanced the most resistant families that also had acceptable fruit quality by self pollination in the greenhouse. There were 3 populations of 8, 9 or 10 families each (S1 to S4 generation) to make 1 or 2 sublines each. The resulting 50 families were tested for high resistance to the new downy mildew in
the field in North Carolina. The design was a randomized complete block with 3 replications and 4 disease ratings. Lines were also evaluated for fruit quality. Lines were evaluated for fruit quality on a 1 to 9 scale (1=poor, 9=excellent). A total of 3 lines were selected based on field data collected in 2016. The selected lines were self pollinated and also cross pollinated in pairs in fall 2016 to develop more highly resistant cucumber populations with better fruit quality.

3. Identify new sources of resistance. A new population derived from PI 605996 (HR) × 'Poinsett 76' is being developed to provide new sources of high resistance to downy mildew. The F2 progeny will be self-pollinated and the S1 lines tested in the field for high resistance to natural disease incidence of downy mildew at the Clinton, NC research station. In addition to resistance, lines will be selected for yield, earliness and quality.

4. Field screening of downy mildew resistance for the 384-line GWAS panel.
Squash Team

Team members:
Michael Mazourek (Cornell Univ.)
Linda Beaver (Univ. Puerto Rico)  
Angel Linares (Univ. Puerto Rico)
Chris Smart (Cornell Univ.)

<table>
<thead>
<tr>
<th>Objective</th>
<th>Personnel/Institution</th>
<th>Year</th>
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<tbody>
<tr>
<td>(a) Obj. 1. Develop common genomic approaches and tools for cucurbits</td>
<td>(initials as in Table 3)</td>
<td>1 2 3 4</td>
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<tr>
<td>1.2. Perform GBS analysis of PI collections, establish core populations, provide community resource for genome wide association studies (GWAS)</td>
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<tr>
<td>1.2.1. GBS of cucurbit species, establish molecular-informed core populations - squash</td>
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<td>MM (CU)</td>
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<td>1.2.2. Population genetics and GWAS analysis - squash</td>
<td>UR (WVSU), ZF (BTI)</td>
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<td>MM (CU)</td>
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<td>(b) Obj. 2. Genomic assisted breeding for disease resistance</td>
<td>Screen for resistance (Se), develop populations (P), phenotype (F), sequence (S), QTL map (Q)</td>
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<td>PF</td>
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<td></td>
<td>MM</td>
<td>PFQ</td>
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<td>2.1.4 Squash</td>
<td>MM (CU), LWB(UPR)</td>
<td>RM</td>
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<td></td>
<td>MM (CU)</td>
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<td>2.2 Marker development and verification:</td>
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<td>2.3 Introgress resistance into advanced breeding lines:</td>
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1.2.1. GBS of cucurbit species, establish molecular-informed core populations - squash

The Cucurbita collection includes three commercially important species and several wild species, yet is one of the smaller crop focused collections in cucurbits. We plan to perform GBS on most of genus. In *C. maxima*, 165 accessions have already been genotyped by sequencing with PstI. About 319 accessions of *C. moschata* and 800 accessions of *C. pepo* are being prepared for DNA extraction. Of the wild species, we have performed GBS on *C. okeechobeensis* and are preparing *C. ecuadorensis* and *C. lundelliana*. All species other than *C. maxima* will be genotyped using ApeKI digests. While using different restriction enzymes will confound comparisons between species, the most interesting accessions will be resequenced and thereafter comparative studies will be unaffected.
Powdery mildew resistance in squash

2.2.4.1 Marker development and verification (Mazourek lab – K. LaPlant)

Powdery mildew is a major fungal disease on squash and pumpkins (*Cucurbita spp*) in the USA and throughout the world. Genetic resistance has been achieved in cultivated species through the introgression of a major resistance gene from the wild species *Cucurbita okeechobensis* subsp. *martinezii*. Today, this gene, *Pm-0*, is used extensively in breeding, and is found in nearly all powdery mildew-resistant *C. pepo* and *C. moschata* commercial cultivars. The region containing the resistance allele was refined to a final candidate interval of 76.4 kb. Studies are currently underway to validate markers in this region and explore gene candidates by exploring gene expression in powdery mildew challenged materials using RT-PCR and QuantSeq.

We have shared a CAPS marker in the presumed causative locus and the sequence polymorphism of course could be converted to other platforms (Holdsworth et al., 2016). This marker has been reported to predict resistance perfectly in other breeding programs within *Cucurbita pepo* but was not effective for some parties in *C. moschata*. Though greenhouse inoculations, we confirmed that the marker does predict resistance effectively in *C. moschata* when the petioles of the plants are evaluated. We cannot rule out the possibility that other groups are using alternative genetics.

Virus resistance in squash

2.1.4.3, 2.1.4.4. Mapping resistance (M. Mazourek lab – K. LaPlant)

We are using an introgression mapping approach similar to that for powdery mildew resistance, above, to identify introgressions from the sources of resistance. Initially, we were focused on the *C. moschata* sources of resistance, ‘Nigerian Local’ and ‘Menina’, however resistance may also be derived from *C. ecuadorensis*. Accordingly, we are looking more broadly for shared introgressions and are aware that introgressions may be from divergent sources. Germplasm in the CU and UPR breeding programs has focused on the *C. moschata* sources of resistance and may be increasingly important as we seek to characterize resistance from these sources for support of the UPR germplasm and bolster the limited numbers of cultivars with resistance that are available for evaluation. We have identified and genotyped 43 cultivars with reported resistance to ZYMV, 27 cultivars with reported resistance to WMV, 13 with reported resistance to PRSV, and 10 with reported resistance to CMV. We will bolster these populations with historical materials from our breeding programs and classical genetic populations.

2.3.4.3, 2.3.4.4. Introgress resistance into advanced breeding lines (L. Beaver, A. Linares labs – M. Miranda, W. Seda)

Populations for studies of inheritance of PRSV and ZYMV resistance and for development of potyvirus resistant lines:

We have continued to develop PRSV and ZYMV resistant x susceptible populations (F1, F2, and some backcrosses). Cultivars of tropical pumpkin (*C. moschata*) widely used in Puerto Rico are being used as susceptible parents. Nigerian Local and Menina are being used as resistant parents. We have generated sufficient seed of F2 populations with the local cultivars Verde Luz
and Taina Dorada, breeding line TP411, and temperate cultivar Waltham. The F1, F2 and BC populations of Nigerian Local x Soler and Menina x Soler still need to be made and additional F1 seed is needed of some populations. Sufficient F2 seed of the resistant x resistant cross of Nigerian Local x Menina has been made with the purpose of determining if these two sources of resistance are allelic.

Study of inheritance of PRSV resistance:
We have initiated the study of inheritance of PRSV resistance in the F2 population Nigerian Local x Taina Dorada. A total of 100 F2 plants have been evaluated using a number of scoring techniques. Although those data have not been analyzed, we used symptom severity as a basis to select a group of the most resistant and most susceptible F2 plants to transplant to the field for self-pollination. Because of weather conditions, we were only able to self-pollinate five lines, including two that showed few symptoms as they matured in the field. We noted that no F2 plant was as symptom-free as the resistant parent Nigerian Local. Another group of 100 F2 plants from this same population are currently being tested. Again, we have not observed an F2 plant as resistant as Nigerian Local. The most resistant F3 or F4 lines derived from this and other populations will later be used to validate resistance markers that are expected to be developed by other members of the Squash Team.

Methods for evaluating potyvirus (PRSV and ZYMV) resistance in C. moschata:
Ideally the plant breeder would like to be able to reliably phenotype a seedling for disease resistance as soon as possible after inoculation with ZYMV or PRSV. Results from a greenhouse-based test for resistance must also be strongly correlated with field results. Since carrying out a small preliminary study that was reported on at Cucurbitaceae 2016 (Wessel-Beaver et al., 2016) we have conducted a series of experiments focusing on the development of reliable protocols for phenotyping for potyvirus resistance.
Using ELISA, we measured virus titer in the first 4 leaves of inoculated seedlings (cotyledons at approximately 5 days post seeding), evaluating leaf samples as each leaf expanded. The genotypes used had a known range of resistance from highly resistant (Menina and Nigerian Local) to intermediate resistance to highly susceptible. Four separate runs were conducted (in different months). There was a very marked difference in results for PRSV and ZYMV. Differences among genotypes could be differentiated for ZYMV very early on: in either the 1st or 2nd expanding leaf. We also noted that visual symptoms of virus susceptibility (mosaic, mottling, leaf deformation) could also usually be observed, especially by the time the 2nd leaf was expanding. The situation for PRSV was very different. Genotypes could not usually be differentiated until sampling the 4th leaf (sometimes in the 3rd). Very vague symptoms (light mottling) could sometimes be observed earlier in susceptible genotypes, however symptoms were much more obvious and easier to classify once the 4th leaf emerged.

In March to July 2016 we conducted a trial that began in the greenhouse by inoculating seedlings (PRSV, ZYMV) of genotypes with a range of known resistance or susceptibility. Plants were evaluated for virus symptoms and ELISA in the greenhouse (3 weeks post-seeding), then transplanted to the field at 4 weeks. The individual plants were monitored for virus symptoms.
and sampled for ELISA at 10 and 14 weeks post-seeding (6 and 10 weeks post-transplant). In general, Spearman (rank) correlations between ELISA results at different dates were lower than expected. For PRSV, \( r = 0.48 \) (\( p<0.001 \)) between ELISA readings in the greenhouse and 5 weeks later (10 weeks post-seeding) for the same plants in the field. The correlation was not significant between the greenhouse readings and field readings taken 14 weeks post-seeding (\( r = 0.26 \) \( p=0.063 \)). Results for ZYMV were similar: \( r = 0.54 \) (\( p<0.001 \)) and \( r = -0.14 \) (NS) between readings in the greenhouse and the 1st and 2nd field ELISA tests, respectively. We are currently repeating this study (transplanted to the field Feb 2017).

We also wanted to determine if leaves from multiple apices from a single plant should be sampled when evaluating resistance at the field level. We sampled two newly-expanded leaves from plants that had been inoculated in the greenhouse. We conducted ELISA tests on 10 and 14 week old plants (6 and 10 week post-transplant). At 10 weeks plants were flowering. At 14 weeks many plants had fruits beginning to mature. For both PRSV and ZYMV rank correlations between the two samples from a plant were high (\( r = 0.81 \) for PRSV and \( r = 0.66 \) for ZYMV, both with \( p<0.001 \)). We are currently repeating this study as well and intend on taking more samples per plant.

**Evaluation of resistant parents Nigerian Local and Menina:**

Although our impression is that Nigerian Local and Menina have been used in the commercial seed industry as sources of resistance to PRSV and ZYMV, the reality is that there is little or no documentation in the literature about these genotypes except concerning their performance in seedling tests. We conducted a field trial in spring-summer 2016 to study how well plants of Nigerian Local and Menina, inoculated with either PRSV or ZYMV, maintain their resistance when transplanted to the field. In the case of plants inoculated with either PRSV or ZYMV, Nigerian Local remained symptom-free in the field and produced ELISA readings similar to the non-inoculated control plants. Menina also remained symptom-free, but in some cases plants inoculated with PRSV produced ELISA readings similar to other genotypes with symptoms and intermediate levels of resistance/susceptibility. In the case of ZYMV, ELISA readings for Menina were similar to the non-inoculated control plants. In general, both of the genotypes are excellent sources of resistance to both PRSV and ZYMV. We are currently repeating this trial and expect to document what, if any, impact PRSV and ZYMV have on yield of the two resistant genotypes. In a greenhouse study we found that even though potyvirus-inoculated Menina and Nigerian Local remain symptom-free and have low virus titer (as determined by ELISA), side-by-side observations of inoculated and uninoculated 3-week-old plants clearly show an effect of virus infection. Inoculated plants (especially of Menina) were slightly more chlorotic and less developed than control plants.
Phytophthora blight resistance in butternut squash

2.1.4.2 Mapping resistance and breeding new butternut squash with resistance to Phytophthora blight and 2.3.4.2 Introgress resistance into advanced breeding lines (M. Mazourek)

We are self-pollinating F1’s between a mildew resistant bush butternut breeding line and Phytophthora blight resistant C. moschata accessions PI 211996, PI 483347. Crosses with PI 634693 and this breeding line were unproductive and this population has been put on hold accordingly. This summer we will screen F2 individuals to found them to be asymptomatic when inoculated with P. capsici on the blight farm. We will create F2:3 populations for QTL analysis by crossing them with a powdery mildew resistant bush butternut developed by Mazourek. Phytophthora resistant, bush squash will be crossed to a high quality parent for further breeding and validation of QTL from the initial F2:3 populations.
Economics Team

Team members:
Marco Palma (Texas A&M Univ.)
Lius Rivera (Texas A&M Univ.)

(b) Obj. 3. Economic impact analyses, disease control information

<table>
<thead>
<tr>
<th>3.1 Perform economic analysis, cost of production/disease control</th>
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<tr>
<td>3.1.1. Define, parameterize, simulate, validate production variables</td>
<td>LR (TAMU), MP (TAMU)</td>
<td>X</td>
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<tr>
<td>3.1.2. Estimate the potential economic impacts to the cucurbit industry</td>
<td>LR (TAMU), MP (TAMU)</td>
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</table>

3.1 Perform economic analysis, cost of production/disease control
3.1.1. Define, parameterize, simulate, and validate production variables based on cucurbit production crop budgets

Completed:
- Macro and micro economic variables were collected to develop the economic model, such as interest rates, input costs, production windows and existing crop budgets.
- Graduate students were selected to work on the project and were trained on how to collect data to develop representative farms.
- Faculty and graduate students have IRB clearance to collect information from producers.
- Developed 11 representative farms in California (3 watermelon and 3 cantaloupe), Florida (3 watermelon) and Texas (1 watermelon and 1 cantaloupe)

In progress:
- Identify facilitators to develop representative farms in the Northeast region (Spring 2017)
- Develop and validate all representative farms (Summer/Fall 2017)
**Extension/Outreach Team**

**Team members:**

*Jonathan Schultheis (N. Carolina St. Univ.)*  
Mary Hausbeck (Michigan St. Univ.)  
Angela Linares (Univ. Puerto Rico)  
Jim McCreight (USDA, ARS)  
Lina Quesada (N. Carolina St. Univ.)  
Chris Smart (Cornell Univ.)  
Linda Wessel Beaver (Univ. Puerto Rico)

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(b) Obj. 3. Economic impact analyses, disease control information

3.2.1. Develop a centralized cucurbit disease website  
| JS (NCSU) | X | X |

3.2.2. Develop and post diagnostic resources and disease control information in English and Spanish; prepare diagnostic poster  
| LQ (NCSU), MH (MSU), CS (CU), ALR (UPR) | X | X | X | X |

3.2.3. Provide disease alerts and forecasting tools  
| X | X | X | X | X |

3.2.4. Provide diagnostic and disease management assistance.  
| LQ (NCSU), MH (MSU), CS (CU) | X | X | X | X |

3.2.5. Field days and demonstration plots  
| Crop and extension teams | X | X | X | X |

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3.2. Provide readily accessible information to facilitate disease control

As reported in the initial report, the extension component of this grant will be used to communicate the grant’s goals, progress, results, and its applications. The extension component reaches beyond those directly involved in the grant, such as breeders, seed company personnel, allied industry partners, growers, and other interested persons. Leadership for extension by commodity is provided mainly by Mary Hausbeck (cucumber), Lina Quesada (watermelon), Chris Smart (squash), and Jim McCreight (melon). The focus is on aspects related to disease. Linda Wessel-Beaver and Angela Lineares are the lead persons that will provide translation of documents from English to Spanish. Jonathan Schultheis complements these lead plant pathology PIs with pertinent cultural management information. He is also providing leadership with respect to the development of Cucurbit CAP webpage.

Many extension activities actively incorporate both stakeholders and extension personnel via field days, extension workshops, and commodity meetings at the local, state, national, and international levels. Specifically, the information which follows provides updates for June 2016 through March 2017 regarding the objectives and their associated results or outputs.

**3.2.1 Develop a centralized cucurbit disease website.**

A website developer and manager was hired in fall 2016 and the website has been under development for the past several months. There have been inputs during the website’s development from CucCAP team leaders, the CucCAP Extension team, and specific input from the Project Leader, Rebecca Grumet and Lina Quesada. Quesada designed the logo for the project which is featured on the website. The Webpages on the CucCAP website include pages
describing the overall CucCAP project, work of each of the seven teams, contact information for CucCAP team members and their lab or institution, links to the Cucurbit Genomics Database (http://www.icugi.org) housed at Cornell University, links to forecasters including the Cucurbit Downy Mildew IPMpipe (http://edm.ipmpipe.org/) and Melcast (http://melcast.ceris.purdue.edu/), links to diagnostic labs at Land Grant Universities, links to Plant Pathology & Extension factsheets or webpages for disease diagnosis, links to vegetable production guides, links to crop field trials, links to commodity organizations, and links to other disease management resources. Time sensitive information and location specific information including disease alerts, upcoming meetings or events, pesticide information and current articles about cucurbit diseases will be available in menus on the main page and footer of the site. The website is ready to be launched with a permanent address in March 2017. The main website address will be www.cuccap.org. Additional addresses to the site are www.cucurbitcap.org and www.cucurbitaceaecap.org. After review by the entire CucCAP team, the site will go public in April 2017. At that time, search engines will start indexing the website and analytics will be reported for purposes of understanding and optimizing usage of the CucCAP website.

3.2.2. Develop and post diagnostic resources and disease control information

Publications
3.2.3 Provide disease alerts and forecasting tools

Weekly conference calls, NCSU Vegetable Team (Quesada): These calls began June 7 and continued through September 27.

Weekly conference calls, Cucurbit ipmPIPE (Hausbeck, Quesada, Smart): These calls began in May and continued through August and will include plant pathologists from the eastern US.

Smart has active facebook and twitter accounts, and is also active in the Cornell Vegetable alerts blog (which sends messages to vegetable extension educators). As soon as diseases of cucurbits are first reported in NY, she alerts growers through these avenues. Additionally, any new advances made through CucCAP are also shared through these methods.

3.2.4 Provide diagnostic and disease management assistance.

Since the project started, Quesada has provided diagnostics and disease management recommendations for 12 cucumber, 33 watermelon, 7 melon, 12 squash, and 9 pumpkin samples submitted to the NCSU Plant Disease and Insect Clinic. Since the last report, Quesada has provided diagnostics and disease management recommendations for 3 cucumber, 22 watermelon, 5 melon, 8 squash, and 5 pumpkin samples submitted to the NCSU Plant Disease and Insect Clinic. Quesada has also been involved in providing disease management recommendations through oral presentations and generating disease management resources such as the NC Agricultural and Chemicals Manual and the Southeastern US 2016 Vegetable Crop Handbook.

Smart diagnosed over 100 samples during the 2016 growing season, in addition to over 100 disease issues diagnosed via photo through email or text message. She also provides management recommendations through oral presentations and production guides (both conventional and organic).

Production guides

3.2.5 Field days and demonstration plots.

Quesada recruited a part-time graduate student (Nicholas Noel) that evaluating commercial watermelon varieties for anthracnose resistance. Quesada and Noel are collaborating with Wehner, also on the watermelon team, who has a graduate student focusing on the genetic basis of disease resistance to anthracnose in watermelons. Quesada also supported demonstration plots to evaluate fungicides for disease control and combinations of tolerant varieties and fungicide applications.

Smart has yearly demonstration plots at the Phytophthora blight farm with variety trials for squash and other vegetables. She also has cucurbit downy mildew trials on research farms in
Geneva NY.

Schultheis was involved in several cucurbit variety studies in 2016; 2 zucchini squash, 2 butternut squash, 2 watermelon, 1 melon, 2 parthenocarpic pickling cucumber and 1 pumpkin. These trials were open to the industry and extension agents to evaluate for yield, quality and potential diseases. Representatives from multiple seed companies visited the studies and interacted.

A 1.5 day agent in-service training organized by Schultheis and focused on butternut squash and watermelon crops in August in Salisbury, NC. Cultural and disease management were featured at this in service training.

**Publications from demonstration plots**

**Oral and Poster Presentations**

**Extension and Industry Venues**

**Invited seminars**

**Invited talks**


**Oral presentations**


17. Smart, C.D. Understanding and controlling diseases of cucurbits. Crop Consultant Meeting, December 1, 2016 Syracuse NY.

18. Smart, C.D. Why is the Phytophthora blight from important? New York State Ag Experiment Station Task Force, October 10, 2016.


20. Smart, C.D. Vegetable disease management (1.5 hour discussion with growers and educators). Willsboro, NY, Aug 4, 20116


22. Smart, C.D. How the NY Farm Bureau helped established the Phytophthora blight farm. Midwest Farm Bureau visit to NYSAES, June 24, 2016.


Posters

Webinars
1. Smart, C.D. Vegetable Diseases (for beginning growers), March 15, 2017. This was a 1.5 hour webinar.


3. Smart, C.D. Vegetable Diseases (for beginning growers), March 16, 2016. This was a 1 hour webinar.
Cumulative CucCAP

PUBLICATIONS,

RESOURCE MATERIALS

and

PRESENTATIONS
Refereed Publications


Conference Proceedings


Cucurbitaceae 2016, XIth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae, Warsaw, Poland.


**Book/Book Chapters**

Many members of CucCAP have contributed chapters to:


**Other**

EXTENSION and OUTREACH RESOURCES and PRESENTATIONS

DISEASE CONTROL and EXTENSION RESOURCES


Web Content
Anthracnose of cucurbits: http://content.ces.ncsu.edu/anthracnose-of-cucurbits
Cucurbit downy mildew: http://content.ces.ncsu.edu/cucurbit-downy-mildew
Cucurbit powdery mildew: http://content.ces.ncsu.edu/cucurbit-powdery-mildew
Fusarium wilt of watermelon: http://content.ces.ncsu.edu/fusarium-wilt-of-watermelon
Gummy stem blight of cucurbits: http://content.ces.ncsu.edu/gummy-stem-blight-and-phoma-blight-on-cucurbits

Webinars
Smart, C.D. Vegetable Diseases (for beginning growers), March 15, 2017. This was a 1.5 hour webinar.
Smart C.D. Managing cucurbit downy mildew in organic systems in the northeast. December 6, 2016. 1.5 hour webinar with 135 participants.
Smart, C. D. Vegetable Diseases (for beginning growers), March 16, 2016. 1 hour webinar.
EXTENSION and OUTREACH PRESENTATIONS
Grumet, R. 2015. Update on resistance to Phytophthora capsici in cucumber. PPI Annual Meeting October 30, 2015, Fort Worth, TX


Hausbeck, M. 2017. A smorgasbord of vegetable diseases is on today’s menu. MSU Extension and AgBioResearch State Council Meeting, Lansing, MI, Mar. 30 attendees.


Kousik, C.S. 2016. Progress and challenges in managing Phytophthora fruit rot of watermelon. Indiana Horticultural Congress, Indianapolis, IN. January. 45 attendees (at the talk)

Kousik C.S. 2016. Managing Phytophthora fruit rot of watermelon. Georgia Watermelon Association, St. Simmons, GA. January. Over 100 attendees

Kousik, C.S. 2016. Breadth of resistance of USVL developed Phytophthora fruit rot resistant germplasm lines to *Phytophthora capsici* isolates from across USA. Watermelon Research and Development Group meeting. San Antonio, TX. Feb >50 attendees


McCreight, J.D. 2015. Melon host plant resistance to powdery mildew and CYSDV. Fall Desert Crops workshop, sponsored by the University of California ANR Cooperative Extension, Imperial County, and University of Arizona Cooperative Extension, Yuma County. El Centro, CA, Oct.


McGregor, C.E. 2016. Advances in Watermelon Breeding. Southeast Regional Fruit & Vegetable Conference, 8-10 January 2016, Savannah, GA.


\((\text{Citrullus lanatus})\) yield and quality. Watermelon Research Group, Mobile, AL. Feb. 2017

Smart, C. 2015. Disease problems common during the 2015 growing season. Twilight meeting, Eden Valley, NY.


Smart, C.D. 2016. Understanding and controlling diseases of cucurbits. Crop Consultant Meeting, December 1, Syracuse NY.

Smart, C.D. 2016. Why is the \textit{Phytophthora} blight from important? New York State Ag Experiment Station Task Force, October 10, 2016.


Smart, C.D. 2016. Vegetable disease management (1.5 hour discussion with growers and educators). Willsboro, NY, Aug 4, 2011


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