CUCURBITACEAE 2022

ABSTRACT BOOK

October 30 - November 2, 2022
Naples, Florida, USA

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UF IFAS UNIVERSITY of FLORIDA

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CUCURBITACEAE 2022

OCTOBER 30 - NOVEMBER 2, 2022

Naples Grande Beach Resort

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WELCOME COLLEAGUES,

Thank you for joining us for Cucurbitaceae 2022!

Welcome to Cucurbitaceae 2022 in Naples Florida, USA! Cucurbitaceae 2022 brings together scientists from around the world for an in-depth exploration of new frontiers in cucurbit research and development. This year's conference will consist of six themed sessions, a flash and formal poster session, four crop-specific meetings, a Cucurbits Crops Germplasm Committee meeting, a Cucurbit Genetics Cooperative meeting and a field tour of southwest Florida vegetable industries. The conference will cover 62 contributed oral and poster presentations in cucurbit genetics, genomics and germplasm resources, floral & fruit development, production and quality, resistance to pest and diseases, tolerance to abiotic stress and cucurbit pathology/entomology.

We are grateful to have an in-person meeting on the heels of a global COVID pandemic and the recent hurricane that affected communities across Florida.

Thank you all for attending. We wish you an enjoyable and fulfilling conference!

On behalf of the Planning Committee and the Conference Organizing Team, we welcome you to Cucurbitaceae 2022!

Sincerely,

GEOFFREY MERU

Conference Co-chair
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ABSTRACT COMPILATION

Abstracts are listed in alphabetical order by presenter **last name**.

DESIGNING OF A RECOMBINASE POLYMERASE AMPLIFICATION-BASED LATERAL FLOW TEST FOR RAPID DETECTION OF CUCURBIT LEAF CRUMPLE VIRUS

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Cucurbit leaf crumple virus (CuLCrV), a member of the genus *Begomovirus,* is a whitefly transmitted virus that is an economically important viral pathogen affecting cucurbits production in the U.S. Various methods like PCR, quantitative PCR (qPCR), and LAMP have been applied to detect CuLCrV. These techniques are highly specific with some limitations including PCR and qPCR restricted to lab-based detection, qPCR mandate an expensive instrument, and all of the above methods require well-trained personnel to conduct the assays. This project aims to establish a simple and rapid field-based diagnostic assay, specifically a lateral flow test (LFT) system using recombinase polymerase amplification (RPA-LFT). The RPA-LFT assay created a CuLCrV-specific primer and probe labeled with fluorophores for high specificity of the system (a reverse primer labeled with Biotin and probe labeled with Fam). The RPA-LFT detects CuLCrV within 30 mins without requiring DNA extraction and need for an instrument. The sensitivity of the RPA-LFTs assay was examined using a serially diluted plasmid and resulted in a detection limit of up to 10^2 copies. To further determine the sensitivity and specificity of RPA-LFT, symptomatic pumpkin, squash, and watermelon RNA samples collected from research and commercial fields were used. The RPA-LFT detected CuLCrV in all samples with the presence of the virus and did not react with other virus-containing samples, healthy and buffer control. PCR and qPCR assays confirmed the presence of CuLCrV, confirming the RPA-LFT result. The established RPA-LFT assay successfully detected CuLCrV from a small set of virus-infected samples tested so far (n=6). The results indicate that the RPA-LFT assay has the potential to be used for field-level detection of CuLCrV. The long-term goal of this project is to continue development and standardization of the RPA-LFT strip for the detection of CuLCrV, which can be used by untrained personnel under field conditions without the need for an instrument.

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LIGNIN BIOSYNTHESIS GENE EXPRESSION IS ASSOCIATED WITH AGE-RELATED RESISTANCE OF WINTER SQUASH TO *PHYTOPHTHORA CAPSICI*

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The oomycete plant pathogen, *Phytophthora capsici*, is a serious fruit rot disease of winter squash (*Cucurbita* spp.) that limits production. Some *Cucurbita moschata* cultivars develop age-related resistance (ARR) whereby fruit develop resistance to *P. capsici* 21 days post pollination (dpp). Previous studies showed thickened exocarp in resistant fruit and that wounding negates ARR. Here we uncover the genetic mechanisms of ARR of two *C. moschata* cultivars 'Chieftain' and 'Dickenson' that exhibit ARR at 14 and 21 dpp, respectively, using RNA sequencing. The sequencing was conducted using RNA samples from fruit at the following dpp: 7, 10, 14, and 21 from 'Chieftain' and 'Dickenson'. Differential gene expression analysis led to the identification of 3326 and 1120 upregulated genes in resistant fruit peel 14 dpp and 2047 and 1482 in 21 dpp of 'Chieftain' in contrast to susceptible 7 and 10 dpp respectively, while in 'Dickenson, 4644, 3577, and 1284 upregulated genes were identified in resistant fruit peel 21 dpp in contrast to susceptible 7, 10, and 14 dpp respectively. Subsequent gene set enrichment analysis revealed an overrepresentation of upregulated genes in functional categories relevant to cell wall structures biosynthesis, cell wall modification/organization, transcription regulation, and metabolic processes. Pathway enrichment of *C. moschata* orthologous genes detected upregulated genes in cutin, suberin monomer, and phenylpropanoid biosynthetic pathways. Further analysis of the expression profile of genes in those pathways revealed upregulation of genes in monolignol biosynthesis and lignin polymerization in the resistant fruit peel, such as the cinnamyl alcohol dehydrogenase, cinnamoyl-CoA reductase, ortho-methyltransferases, phenylalanine-ammonia-lyase, and peroxidase. Our findings suggest a shift in gene expression toward the physical strengthening of the cell wall associated with ARR to *P. capsici*. These findings provide candidate genes for the development of *Cucurbita* varieties with resistance to *P. capsici* and in turn improvement of winter squash and pumpkin fruit rot management.

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RESEARCH UPDATES ON DEVELOPING STRATEGIES FOR MANAGEMENT OF *XANTHOMONAS CUCURBITAE*, INCITANT OF CUCURBIT BACTERIAL SPOT

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Bacterial spot of cucurbits, caused by *Xanthomonas cucurbitae*, is an emerging disease. Evaluation of isolates of *X. cucurbitae* in the greenhouse showed that *X. cucurbitae* isolated from Illinois, Michigan, Kansas, Ohio, and Wisconsin were more virulent than the reference ATCC 23378 strain. In a four-year rotation with nonhost crops, development of bacterial spot was delayed only by two weeks. The pathogen survived for more than 24 months in infected pumpkin debris buried in the field, and the bacterium was viable. *X. cucurbitae* was also isolated from asymptomatic weeds in pumpkin fields. In greenhouse inoculation of weeds with *X. cucurbitae*, however, bacterial spot developed only on leaves of bur cucumber (*Sicyos angulatus*) and velvetleaf (*Abutilon theophrasti*) weeds. *X. cucurbitae* carried on and in the seeds was eradicated by hot-water and HCl treatments. However, the importance of seed-borne aspects of the pathogen, has not been documented. Although applications of some chemicals reduced incidence and severity of leaf and fruit infection under field conditions, no spray program provided effective protection of plants against the pathogen. We screened 81 commercial cultivars of gourds, pumpkins, and squashes, as well as 300 *Cucurbita* spp. accessions for their resistance to *X. cucurbitae* under greenhouse and field conditions. In the greenhouse studies, all commercial cultivars and most of the accessions developed typical symptoms of bacterial spot disease, while some of the accessions developed none or fewer lesions. In the field studies, all commercial cultivars developed bacterial spot. Based on the results of the greenhouse and field tests 9 and 21 accessions were classified as resistant and less resistant, respectively. Resistant and less resistant accessions belong to the species *Cucurbita maxima*, *C. maxima* subsp. *maxima*, *C. maxima* subsp. *andreana*, and *C. okeechobeensis* subsp. *martinezii*. This is the first report of potential resistance to bacterial spot of cucurbits.

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PREVALENCE AND INCIDENCE OF VIRUS DISEASES IN CUCURBITS IN GEORGIA, USA

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Surveys were conducted in the fall of 2019 and 2020 and spring of 2021 in the cucurbit production region in southern Georgia. High throughput sequencing of small RNAs from symptomatic samples followed by bioinformatics analysis was used to identify the viruses present. A larger number of samples were analyzed by conventional methods like PCR and RT-PCR to determine the distribution of viruses identified by HTS. Whitefly transmitted viruses (WTVs), cucurbit leaf crumple virus (CuLCrV) and cucurbit yellow stunting disorder virus (CYSDV) were consistently detected along with the newly identified cucurbit chlorotic yellows virus (CCYV; genus Crinivirus, family Closteroviride) in the fall season in the region. All three viruses were more widely distributed in Georgia than previously assumed and were detected in squash, cantaloupe, and cucumber. CCYV was detected in 60%, CuLCrV in 76%, and CYSDV in 43% of the total samples (n = 820) tested. The level of mixed infections of these viruses was also very high, with most samples tested being infected with at least two viruses. Wild radish (*Raphanus raphanistrum* L.), a common weed in the southeastern USA, was found to harbor and transmit CCYV to cucurbit hosts. WTVs were not detected in the spring-grown cantaloupe and watermelon although these crops get infected WTVs when grown in the fall. However, watermelon crinkle leaf-associated virus 1 (WCLaV-1) and three persistent, asymptomatic RNA viruses were identified on melons in Georgia: cucumis melo endornavirus (CmEV), cucumis melo amalgavirus (CmAV1), and cucumis melo cryptic virus (CmCV). Biological information, including vector relations, is unknown for WCLaV-1. Further studies are also required to understand the biology and impact of this virus on watermelon production and other crops if any.

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EXTREME-PHENOTYPE GENOME-WIDE ASSOCIATION STUDY OF POWDERY MILDEW RACE2W RESISTANCE IN THE USDA CITRULLUS GERMPLASM COLLECTION

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Powdery mildew outbreaks, caused by *Podosphaera xanthii*, lead to reduced watermelon yields from premature leaf senescence. Fruit quality can also be affected from sun scalding due to the reduced leaf canopy. Resistance sources have been identified by screening the USDA Citrullus germplasm collection for disease response to multiple races of *P. xanthii* but there is limited knowledge of the genetic basis of resistance. Here we used historical data from the USDA-Germplasm Resource Information Network for an extreme-phenotype genome-wide association study (XP-GWAS) of resistance to *P. xanthii* race 2W in Citrullus accessions (N=1,148). XP-GWAS combines a bulked-segregant analysis of diversity panels with next-generation sequencing data to identify genomic regions associated with the trait of interest. Whole-genome resequencing of 45 individuals bulked from each extreme (resistant and susceptible) resulted in 301,059 high-quality biallelic SNPs. Four SNPs (from three regions) were significantly associated with *P. xanthii* race 2W resistance in the bulks and were used to design Kompetitive Allele-Specific PCR (KASP) markers. The KASP markers were validated by genotyping 186 accessions from the extremes of the Citrullus collection. Analysis of variance determined that all markers were significantly associated, each explaining 21-31% of the variation in powdery mildew resistance. Haplotype analysis showed non-additive interaction between the resistance QTL.

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CHARACTERIZING GENETIC DIVERSITY OF PSEUDOMONAS STRAINS CAUSING FOLIAR DISEASES OF CUCURBITS IN THE SOUTHEASTERN US

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Pseudomonas syringae is a plant-pathogenic bacterium that causes disease on a diverse range of economically important hosts, including bacterial leaf spot of cucurbits (BLS). BLS causes necrotic foliar lesions on affected hosts, which may lead to slower transplant growth and delayed maturity of plants and fruits, thereby affecting the timely harvest during peak market price window. Fifty-one bacterial strains associated with BLS-like symptoms on cucurbits in the Southeastern United States were collected from 2020 to 2022 to better understand the causal agents of the disease. The strains were compared to strains collected from the prior widespread outbreaks of the 2013 to 2014 production season in Florida and Georgia. Strains were first characterized using traditional laboratory assays, including fluorescence, the LOPAT scheme for identifying fluorescent plant-pathogenic bacteria, colony morphology, and pathogenicity to watermelon and squash. Promising strains then underwent whole genome sequencing, average nucleotide identity to available *Pseudomonas* reference strains, and multi-locus sequence analysis of housekeeping genes to determine their identity, relation to each other, as well as their relation to strains from the 2013-14 BLS outbreaks. Analysis indicates that, while some collected strains are genetically similar to those collected from previous outbreaks, others belong to different *Pseudomonas* species entirely. This suggests that not only strains that fall into *P. syringae* sensu lato, but a diverse range of other *Pseudomonas* species are involved in BLS in cucurbit production in the Southeastern US. This new insight may prove useful for understanding the biology and epidemiology of foliar diseases caused by *Pseudomonas* species.

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GENETIC ARCHITECTURE OF *DM4.1* MAJOR-EFFECT QTL FOR DOWNY MILDEW RESISTANCE IN WI7120 (PI 330628) CUCUMBER

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Downy mildew (DM), which is caused by the obligate oomycete biotroph *Pseudoperonospora cubensis*, is among the most destructive diseases in cucumber production. The high DM resistance in PI 330628 (WI7120) and PI 197088 is controlled by multiple QTL including *dm4.1*. Previous studies proposed that *dm4.1* in PI 197088 consisted of three subQTL: *dm4.1.1, dm4.1.2*, and *dm4.1.3*. Here we report investigation of the genetic architecture of the *dm4.1* locus in WI7120. Development, phenotypic and molecular characterization of near isogenic lines (NILs) and NIL-derived segregating populations also suggested the presence of multiple subQTL at *dm4.1* locus in WI7120 including *dm4.1.1*, *dm4.1.2A, dm4.1.2B*, and *dm4.1.3*. Both WI7120 and PI 197088 carried the same resistance alleles at the *dm4.1.2A* and *dm4.1.3* loci, which encode a receptor-like kinase (CsLRK10L2), and the amino acid permease 2A (CsAAP2A), respectively. The newly detected *dm4.1.2B* subQTL from this study exhibited both anti-chlorosis and anti-sporulation effects against *P. cubensis* infection at the early stage of inoculation, which was consistent with DM pathogen growth assessed with qPCR in NILs. Fine genetic mapping delimited *dm4.1.2B* into a 37.9kb region with three annotated genes. Multiple lines of evidence supported *CsGy4G017730* as a possible candidate for *dm4.1.2B* which encodes the NADH-cytochrome b5 reductase 1 (CsCB5R1). Sequence variant analysis identified several SNP and InDel polymorphisms inside the promoter region, while no sequence variation was found in the coding region between NILs of *dm4.1.2B*. Transcriptome profiling of NILs in response to *P. cubensis* inoculation identified differentially expressed genes that were significantly enriched in photosynthesis and phenylpropanoid biosynthesis pathways. Evaluation of DM inoculation responses in NILs carrying different combinations of the four sub-QTL in both growth chamber and field experiments revealed additive effects of DM resistance among these subQTL. To summarize, our work suggests that *dm4.1* locus in WI7120 and PI 197088 harbors four subQTL: *dm4.1.1*, *dm4.1.2A, dm4.1.2B*, and *dm4.1.3* with *CsCB5R1* as the most probable candidate for *dm4.1.2B*. The DM resistance conferred by *dm4.1.2B* seems associated with the salicylic acid pathway. The subQTL *dm4.1.1* is probably present in both lines, which, however, may have a relatively small contribution to overall DM resistance conferred by *dm4.1*.

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EVALUATION OF MELCAST SYSTEM IN THE MANAGEMENT OF IMPORTANT WATERMELON DISEASES IN NORTH FLORIDA

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Three important foliar diseases that affect watermelon production in North Florida are powdery mildew, gummy stem blight, and downy mildew. In the spring of 2020, a field trial was conducted at the North Florida Research and Education Center in Live Oak, FL to evaluate the use of the MELCAST advisory program for timing fungicide applications for control of powdery mildew. The trial was repeated in the spring of 2021 and 2022 but expanded to include management of downy mildew and gummy stem blight. The spring 2020 trial consisted of six treatments (i.e., spray programs): a standard program that targeted powdery mildew, a standard program that included secondary fungicides targeting downy mildew and gummy stem blight, and three MELCAST-driven programs with fungicides being applied when the sum of the Environmental Favorability Index values exceeded 30, 35, and 40 or at 14 days – whichever came first – and a water sprayed control. The 2021 and 2022 trials consisted of eight treatments: a standard program that targeted powdery mildew, a program that targeted downy mildew, a program that targeted gummy stem blight, a program that targeted the diseases observed in the field, and three MELCAST-driven programs as described above that also target the observed diseases. Disease evaluations were made using the Horsfall-Barratt scale, and disease progress was calculated using the area under the disease progress curve (AUDPC). Statistical analyses of the 2020 AUDPC data indicated weekly application of the standard with the secondary fungicide provided statistically better control of downy mildew than all other treatments ($P<0.05$). Statistical analyses of 2021 and 2022 data indicated the weekly standard programs and MELCAST-driven programs provided statistically better disease control when compared to the water control ($P<0.05$). This study shows the potential of using MELCAST for reduced use of fungicides for effective watermelon disease management in North Florida.

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IDENTIFICATION OF A NEW MECHANISM OF RESISTANCE TO POTYVIRUS INVOLVING INTRACELLULAR VESICLE FORMATION IN MELON.

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Cucurbits are susceptible to many viral diseases. Recessive resistance based on the modification of a protein required for the viral infection cycle is a major way for protecting plants. Thus, modifications of the initiation translation factor provide resistance to ZYMV in watermelon and to MNSV in melon. Endosomal sorting complexes required for transport (ESCRT) play a key role in membrane trafficking in plants and participate in regulating entry of several plant RNA viruses. Our objective was to demonstrate the role of the ESCRT protein CmVPS4 as a new susceptibility factor to the potyvirus watermelon mosaic virus (WMV) in melon. Using a worldwide collection of melons, we identified three different alleles carrying non-synonymous substitutions in *CmVps4*. Two of these alleles were shown to be associated with WMV resistance. Using a complementation approach, we demonstrated that resistance is due to a single non-synonymous substitution in the allele $CmVps4^{P30R}$. The fact that polymorphisms in *VPS4* orthologs have been also shown to co-segregate with resistance to two additional potyviruses, ZYMV and BCMV, in cucumber and in common bean, respectively, clearly demonstrates that this family of host factors required for virus infection is a new generic target for resistance.

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POLLEN PRODUCTION, GERMINATION AND VIABILITY OF MELON GENETIC RESOURCES

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To better understand the mechanisms that contribute to the attractiveness of flowers to pollinating insects in melon, we assessed pollen production as well as pollen viability and germination as these are key characteristics for fruit production and pollen is the main source of nitrogen for bees. Thus 140 melon accessions, that belonged to 16 botanical groups (*acidulus, agrestis, ameri, cantalupensis, chandalak, chate, chito, conomon, dudaim, flexuosus, inodorus, kachri, makuwa, momordica, reticulatus* and *tibish)* were used. Pollen production were assessed with a hemacytometer, pollen germination was determined in vitro with a medium prepared with 15% sucrose, 1% agar and 5 mg/L boric acid, and pollen viability was determined with tetrazolium salts (TTC method). Pollen production in male flowers ranged from 19 500 to about 100 000 pollen grains per flower, i.e. a 5-fold variation. Interestingly, the lowest pollen production was determined in the Humaid 95-1, WM18, and Cuba2 accessions, which all belong to the *agrestis, chito* and *kachri* groups. In contrast, the highest pollen production was recorded in the PI 216030, Golden Beauty Casaba, and Pourrières-1 genotypes, which belong to the groups *inodorus* and *chandalak*. Pollen germination ranged from 56% to 100%. It was below 70% in the genotypes Pourrières-1, Korgoho, WM38, Persia Small Type, PI 164797, Delicious 51, Kok Kalja Pos, Vert Olive d'Hiver, and 100% in the genotypes Valenciano 67 and Jacumba. A significant correlation (r = 0.80) was found between the amount of pollen in male flowers and the fruit weight. In other words, we showed that there is more pollen in the male flowers of the accessions with large fruits. This result suggest that the breeding process contributed to a higher pollen production in the melon plants. These studies are continuing for a second year by examining more flower characteristics and pollen viability.

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A SUPER-PANGENOME OF CULTIVATED AND WILD WATERMELON SPECIES

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Sweet watermelon (*Citrullus lanatus* subsp. *vulgaris*) is an important cucurbit crop and among the most consumed fresh fruits. Besides the direct wild progenitor of sweet watermelon, *C. lanatus* subsp. *cordophanus*, the *Citrullus* genus includes six other extant wild species, *C. mucosospermus*, *C. amarus*, *C. colocynthis*, *C. ecirrhosus*, *C. rehmii* and *C. naudinianus*. Effective utilization of these wild relatives is key for genetic improvement of sweet watermelon that has a narrow genetic base. To maximize the capture of genome variations within and among *Citrullus* species and to identify novel loci underlying agronomically important traits, we have been constructing pan-genomes of different *Citrullus* species through development of reference-grade genome assembly for each *Citrullus* species and deep genome resequencing of various *Citrullus* accessions. A super-pangenome of the *Citrullus* genus has been then constructed by integrating species-specific pan-genomes based on orthologous gene relationships. Presence-absence variation (PAV) analysis of genes in the super-pangenome reveals that many disease resistant genes present in wild watermelons are lost in sweet watermelon and its wild progenitor, while genes related to fruit quality have been positively selected during domestication. Furthermore, we have been *de novo* assembling and annotating reference-grade genomes for ~130 watermelon accession spanning all seven extant *Citrullus* species. A graph-based super-pangenome is being constructed, which allows for comprehensively exploring all genetic elements and facilitating variant discovery in wild and cultivated watermelons. The *Citrullus* (graph-based) super-pangenome provides a novel and valuable resource that enables us to explore genes in the wild relatives that underlie disease resistance and other important horticultural traits to enhance the efficiency of watermelon breeding.

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DEVELOPMENT AND APPLICATION OF MOLECULAR MARKERS FOR THE HULL-LESS SEED TRAIT IN PUMPKIN

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Hull-less seeded pumpkin is desirable for pumpkin oil production and direct use in food industry. The occurrence of the natural mutation of hull-less seeded pumpkin was first found in *C. pepo* and later in *C. moschata*. The hull-less trait is controlled by a single recessive gene, (designated *n*), and other potential modifying loci. In the current study, the QTLSeq approach was used to detect the genomic position for the hull-less trait in an F_2 population (n= 143) between Kakai (nn) and Table Gold Acorn (NN). A single QTL (*Qtlhull-less-C12*) was detected on chromosome 12. Two SNP markers were significantly associated ($p < 0.05$) with the hull-less trait in the segregating population and accessions of diverse background. A NAC domain-containing gene (*Cp4.1LG12g04350*) reportedly involved in lignin biosynthesis in plants was found within the confidence interval of *Qtlhull-less-C12*. Current efforts aim at marker-assisted selection of the hull-less trait into diverse genetic background of *C.* pepo, as well as interspecific introgression into *C. moschata*. For interspecific introgression into *C. moschata*, a three-way cross involving Cream of the Crop (NN; *C. pepo*), Kakai (nn; *C. pepo*) and a SS333 UF bridge line (NN; 68% *C. moschata* + 32% *C. pepo*) was made in the greenhouse. Heterozygous (Nn) individuals were selected using molecular markers and selfed to generate F_2 seed. The F_2 seed was genotyped to identify *nn* individuals with hull-less/ semi-hulled phenotype. Similar efforts are currently ongoing to introgress the hull-less trait into *C. maxima* background.

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MAPPING AND VALIDATION OF QTLS IMPARTING *FON* RACE 2 RESISTANCE IN WATERMELON

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Fusarium wilt on watermelon is caused by the fungus *Fusarium oxysporum* f. sp*. niveum (Fon)*. While Fon races 1, 2 and recently 3 are found in the U.S., *Fon* race 2 is considered one of the most important threats to U.S. watermelon production by the National Watermelon Association. Screening of the USDA watermelon (Citrullus) accessions for resistance to *Fon* race 2 resulted in the identification of two *Citrullus amarus* accessions, USVL-246 and USVL-252, with high levels of tolerance to the pathogen. Intraspecific crosses were made between USVL-246 and USVL-114 (a *Fon* race 2 susceptible *C. amarus* accession), and $F_{2:3}$ families were utilized for genetic mapping. The objectives of this study were to map *Fon* race 2 resistant QTLs and develop Kompetitive allele specific polymorphism (KASP) markers flanking QTLs in the USVL-246 x USVL-114 RIL population and to validate these markers in an inter-specific population developed between USVL-246 and 'Sugarbaby' (*Citrullus lanatus*). The RIL population (N=200) was phenotyped for *Fon* race 2 resistance in two tests with two replications per test consisting of five plants per line. Mapping identified one major QTL on chromosome 9 and three minor QTLs on chromosomes 1, 6 and 8. All together explaining 30% of phenotypic variation. KASP markers were developed for all the QTLs. 160 F_2 individuals from the USVL-246 x 'Sugarbaby' cross were genotyped with Fon race 2 KASP markers. 160 F_3 families (20 plants per family) were phenotyped for *Fon* race 2 resistance to validate the markers and their utility for introgression. F_3 families with heterozygous alleles for chromosomes 9 and 1, and 'Sugarbaby' alleles for the remaining two QTL had the lowest disease severity, while families with the highest disease severity were developed from F_2 segregating for major QTL and with 'Sugarbaby' alleles at the minor QTLs. Only markers on chromosome 1 (one SNP) and 9 (two SNPs) were significant and together explained only 15% of phenotypic variation.

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IDENTIFICATION AND CHARACTERISATION OF A *CUCURBITA PEPO* DWARF MUTANT THAT IS DEFECTIVE IN FEMALE FLOWER OPENING

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A mutant has been identified in *Curcurbita pepo* which has two distinct phenotypes: dwarfness (df) and absence of female-flower opening. The dwarf phenotype was found to be controlled by a major recessive gene *df*. While the male floral organs developed normally, the female petals of mutant plants grew at a slower rate and reached senescence without elongation and maturation. In absence of flower opening the ovary of *df* showed a parthenocarpic development to an average of 230 mm long & 27 mm wide 33 days after female flowers were marked at 4 mm. In response to the application of auxins, GA3, AVG and ACC, only GA3 application produced elongation of internodes and pedicel but only for time being, indicating that a deficiency in GA could be responsible for dwarfness. Moreover, female petal maturation and flower opening were rescued by exogenous application of methyl jasmonate in some treated flowers, although the treatment did not alter fruit set. Given that jasmonic acid (JA) is the main regulator of flower opening, these data indicate that *df* has a deficiency in JA in the female flower but not in the male flower. In accordance with the specific role of ethylene for triggering JA production and female flower opening in squash, we found that the content of ACC in female flowers was lower in *df* compared with WT, and that the peak of ethylene production around anthesis was delayed in the mutant female flower. We are currently using a BSA-seq approach to detect the causal mutation of the *df* phenotype. Meanwhile, all the data seem to indicate that a deficiency in GA and ethylene (and consequently in JA) are the main responsible for the mutant phenotype.

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DROUGHT TOLERANCE IN A RIL POPULATION DERIVED FROM TGR-1551

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Climate change causes serious concerns to growers and breeders, exacerbating drought in many parts of the world, increasing its frequency, severity and duration. Due to the economic relevance of melon, the development of new melon cultivars adapted to this abiotic stress, with high quality standards, is required by global markets. Although plants can be affected by drought at any time of their life one of the most critical stages is during seedling growth. A melon Recombinant Inbred Line Population ($F_{8:9}$) obtained from an original cross between TGR-1551 (tolerant to drought) and the drought susceptible Spanish cultivar 'Bola de Oro', has been tested and characterized attending to several morphological traits easily-measured at seedling stage. Eight-nine plants/genotype were grown in nursery trays (5 cm diameter x 5 cm tall cells), which were placed in an insect-free glasshouse. Trays were watered daily until the seedlings reached the stage of two or three true leaves. Seedlings were then subjected to two consecutive water-stress periods as follows: trays were placed in a water container for 2 minutes and no watering was applied for 4 days, when they were again placed in a water container for two 2 min, and left without watering until the end of the experiment. Drought tolerance evaluations were done on the fourth and the seventh day after the second water stress period, by careful examination of each individual seedling for their drought-induced injury symptoms on each accession following a scale (0=no symptoms to 7= wilting). Three replications were observed. Significant differences have been observed between the parents; the F1 performed like 'Bola de Oro'. Several RILs behaved as TGR-1551 and several genomic areas associated to drought tolerance have been localized. These findings may provide valuable information about potential crosses in future breeding programs to develop drought-tolerant commercial varieties.

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PHENOTYPIC AND GENETIC ANALYSIS OF FRUIT MORPHOLOGICAL TRAITS FOR THE USDA CUCUMBER CORE COLLECTION

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Diversity in cucumber fruit morphology provides valuable variation for breeding programs targeting consumer preference, shipping and storage quality. In this work, we utilize the cucumber core population developed by the CucCAP project (https://cuccap.org) to develop photographic and quantitative phenotypic databases for cucumber fruit traits and identify genomic regions contributing to variation. The cucumber core collection of 399 accessions is designed to capture >95% of the allelic diversity contained in the United States National Plant Germplasm System (NPGS) collection (1,234 accessions), along with accessions providing key disease resistances, fruit quality, and agronomic traits. Portions of the core collection were grown under field conditions at the MSU Horticulture Teaching and Research Center (HTRC) in 2019 and 2020 and the full collection was grown in 2021. Fruit were harvested during exponential fruit growth (5-8 dpp) and at maturity and assessed for fruit quality external and internal traits such as: skin color, length, diameter, shape, netting, spine color, spine density, seed cavity size, flesh thickness and flesh color. The resultant photographic and quantitative data will be incorporated into Cucurbit Genomics Database (CuGenDB) version 2. Genome wide association studies (GWAS) are being performed to identify potential quantitative trait locus (QTL) associated with the traits measured and provide markers for fruit quality characterization.

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COMMERCIAL PUMPKIN BIN YIELD PREDICTION MODEL BASED ON FRUIT SIZE AND PLANT AREA

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Pumpkins are commercially sold in standard pallet-sized bins which are categorized by the size of the fruit within the bin. The objective of this study was to create a yield prediction model for the quantity and category of pumpkin bins based on plant area. This would enable growers to optimize land use and accurately prepare for harvest. This study was completed in Laurel Springs, NC, in 2020 and 2021. 'Kratos' pumpkins were grown in four plant areas, 0.9 m^2, 1.8 m^2, 2.7 m^2, and 3.6 m^2. The field was laid out in a randomized complete block design. Fruit length and diameter data were collected on the mature pumpkins from the inner 25% of each plot. Additionally, the length and diameter of fruit in typical commercial bins were measured at several North Carolina pumpkin producers. For results, fruit length and diameter increased significantly as plant area increased. Fruit number per hectare significantly decreased as plant area increased. Average length and diameter for fruit grown in the 0.9 m^2 planting area was 27.5 cm and 29.8 cm. For 3.6 m^2, fruit measured 31.9 cm by 35.5 cm. The 0.9 m^2 planting area produced an estimated 12,663 pumpkins per hectare compared to 6,562 pumpkins from the 3.6 m^2 planting area. For commercial bins with 30 fruit per bin, the average fruit size was a length of 29.6 cm and diameter of 30.3 cm. For commercial bins with 50 fruit per bin, fruit measured on average 24.6 cm in length and 26.7 cm in diameter. Further analysis on the bin quantity and category for each planting area to be completed. In conclusion, growers can use planting area to influence the size and quantity of pumpkins. This cultural management practice can also be correlated to projected yield in category and quantity of commercial bins.

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DEVELOPMENT OF GENOMIC RESOURCES FOR *X. CUCURBITAE*, THE CAUSAL AGENT OF BACTERIAL SPOT OF CUCURBITS

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Xanthomonas cucurbitae is the causal agent of bacterial spot disease of cucurbits, which is a major problem in cucurbit-growing areas worldwide. In the Midwestern region of the United States, pumpkin and squash fields can have greater than 90% infected fruits, leading to high yield losses. We recently assembled a reference-quality genome sequence for the strain ATCC_23378, which was isolated in New York in 1925. While analysis of this genome has provided useful information about the genomic architecture and genetic composition of this bacterium, we reasoned that sequencing additional genomes from contemporary isolates could provide greater understanding about the current genetic diversity of this pathogen.

We selected five representative *X. cucurbitae* isolates collected from the Midwestern region in 2012-2013. The genomes of these isolates were sequenced using a combination of short-read and long-read sequencing, with sequences assembled using Flye and annotated using the NCBI Prokaryotic Genome Annotation Pipeline. The genomes are comparable in genome size, % GC content, and number and type of genes, with all genomes having >99% nucleotide identity. Additionally, using multi-locus sequence typing we observed that the six isolates can be separated into three pairs, with each pair having greater than 99.9% sequence identity.

Next, we performed disease assays using five cucurbit species. Plants were spray-inoculated with the different bacterial isolates and symptoms were observed over two weeks. While virulence of the ATCC_23378 strain was significantly reduced compared to the other isolates, there were no differences in virulence between the five Midwestern isolates. Additionally, the Midwestern isolates caused greater disease symptoms on pumpkin and winter squash plants as compared to muskmelon and cucumber, with the fewest symptoms observed on watermelon plants. Together, these results suggest that minimal genetic diversity exists between isolates collected 85+ years apart and from different regions of the United States.

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ROOTSTOCK EFFECTS ON FLOWER DEVELOPMENT IN GRAFTED TRIPLOID WATERMELON (*CITRULLUS LANATUS*)

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Watermelon (*Citrullus lanatus*) grafting is practiced in many countries and is increasingly used by U.S. growers for disease resistance and yield improvement. However, delay in flowering has been often observed for grafted watermelon, which presents an obstacle to further adoption. To further investigate the effects of different rootstocks on flowering, a study was conducted using a triploid watermelon scion 'Fascination' self-grafted and grafted to two commercial rootstocks: an interspecific hybrid squash (*Cucurbita maxima* x *C. Moschata*) 'Super Shintosa' and a citron melon (*Citrullus amarus*) 'Carolina Strongback'. Un-grafted 'Fascination' plants were included as control. Dissections to identify flower primordia were performed on three plants of each treatment immediately and one week post healing. The remaining plants were transplanted in pots filled with calcined clay and grown in a randomized complete block design in a glass greenhouse (25.5±3.37 °C/22.6±1.19 °C day/night temperatures), with ten blocks. Vines were trained vertically and drip irrigated with nutrient solution and initial flower positions recorded. The nodal position of the first flower primordium identified during post-healing and at transplanting dissections was similar regardless of treatment (4.5 in average). However, nodal position of the first open flowers was higher than those identified in dissections and was significantly affected by rootstock or grafting. Observed nodal position for the first female flower was 3.7-4.5 nodes higher with 'Super Shintosa' and 'Carolina Strongback' compared to un-grafted 'Fascination' but was not different compared to self-grafted 'Fascination'. Similarly, the first male flower on plants with 'Super Shintosa' and 'Carolina Strongback' rootstocks was 2.9-3.0 and 4.6-4.9 nodes higher compared to self- and un-grafted 'Fascination', respectively. However, date of anthesis was not significantly affected by treatments. These results suggest that certain rootstocks arrest the development of previously initiated flowers. Future research will examine the nutrient status effects on grafted watermelon flower development.

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IDENTIFICATION AND CHARACTERISATION OF A SQUASH ABA INSENTIFIVE MUTANT AFFECTED IN PROTEIN PHOSPHATASE 2C

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Abscisic acid (ABA) is a key hormone involved in plant response to multiple abiotic and biotic stresses, but also in vegetative and reproductive plant development. ABA is a sesquiterpene component synthesized in the carotenoid pathway via oxidative cleavage. In presence of ABA, the signal transduction starts with abscisic acid receptors PYR/PYL/RCAR, which inhibit the activity of Protein Phosphatase 2C (PP2C), a repressor of ABA signaling pathway. PP2Cs can also function as regulators in other signal transduction pathways. Up to date, no mutation in the ABA biosynthesis or signaling pathway has been reported in *Cucurbita pepo*. Using a massive phenotypic screening of a *C. pepo* EMS mutant collection at germination stage, we identified several mutant lines with altered response to ABA. BSA-seq analysis of one of the selected EMS lines (2087) allowed the identification of the causal mutation of the ABA insensitive phenotype as a G>A transition in the gene Cp4.1LG02g07080 of chromosome 2. The gene encodes for an H type PP2C that share a high homology with Arabidopsis AT1g47380. The mutation results in the amino acid substitution E414K, a residue that is highly conserved in PP2C enzymes. We assessed the germination pattern of WT and *pp2c* seeds in response to 500 µM of ABA, demonstrating that the mutant is partially insensitive to ABA. The seed of *pp2c* plants germinated earlier (average germination time = 231 h) than that of heterozygous (average germination time = 245 h) and homozygous WT plants (average germination time 262 hours). We have also tested the effect of *pp2c* mutation on plant vegetative development and the ability of the *pp2c* mutant plants to respond to different environmental stresses.

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BIOFUNGICIDES FOR ORGANIC MANAGEMENT OF POWDERY MILDEW IN WINTER SQUASH

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Organic growers have limited options for effective chemical control of cucurbit powdery mildew. Biofungicides are possible alternatives or additions to spray programs which need additional testing to determine efficacy. Field trials were conducted in New York with 11 treatments in 2021 and 14 treatments in 2022. Active ingredients included biological agents, plant extracts and oils, and copper hydroxide (as a non-bio-pesticide grower standard). Treatments were applied to susceptible bush delicata plants prior to the first observation of powdery mildew on plants and applied weekly for six weeks. Plants became infected with powdery mildew from natural inoculum. Area under the disease progress curve was calculated from weekly ratings of percent disease severity, averaged per plot. In 2021, Kocide 3000-O was the most effective at suppressing disease symptoms. While Theia and MBI-121 were numerically the next best treatments, they were statistically similar to all other treatments except Timorex Act. There was no difference between Timorex Act, Tril-21, Howler, AVIV, and the untreated control. There was also no difference in the number and weight of harvested fruit across treatments. Results from the 2022 field trial will also be reported.

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ALLELIC VARIATION IN *CLSUN25-26-27A* ASSOCIATED WITH WATERMELON FRUIT SHAPE

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Watermelon (*Citrullus lanatus*) is an economically important horticultural crop, with US production valued at over $574 million in 2020. Watermelon possesses a diverse phenotypic diversity in fruit size. This trait is essential for consumer preference and shipping logistics, making fruit size a key selection trait in watermelon breeding. Only one candidate gene, *ClSUN25-26-27a* (*Cla011257*), has been identified as a significant contributor to fruit shape. *ClSUN25-26-27a* is a member of the *SUN* gene family, which has been extensively studied in relation to fruit morphology in tomatoes. Currently, three alleles of *ClSUN25-26-27a* are known to be associated with fruit shape variation in watermelon: the wild-type, a SNP, and a 159bp deletion in the 3rd exon resulting in variations in fruit shape. This study aims to identify additional allelic variation for *ClSUN25-26-27a* in the wider *Citrullus* gene pool and determine the effect of such novel alleles on fruit shape. This study has identified and sequenced four novel alleles with nonsynonymous SNPs in the coding region of *ClSUN25-26-27a* across three *Citrullus* species. To determine their effect on fruit shape, the introgression of the novel alleles into a common genetic background with the assistance of KASP marker assays is being carried out. The resulting NILs will be phenotyped in the field to determine the effect of the alleles on fruit shape. The results of this study will contribute to the understanding of the genetic mechanisms contributing to watermelon fruit shape.
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GENOME-WIDE ASSOCIATION STUDY FOR COLD STRESS TOLERANCE IN CITRON WATERMELON

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Watermelon is an important vegetable crop of tropical origin. It is widely grown and consumed around the world for its hydration and nutritional quality values. Cold stress can affect early planting, seedling establishment, and expansion of crop production to new areas. A collection of 122 citron watermelon accessions were obtained from the USDA's National Plant Germplasm Repository System gene bank in Griffin, Georgia, United States. The accessions were genotyped with single nucleotide polymorphisms (SNPs) markers and screened for four cold stress tolerance associated traits including shoot biomass, vine length, chlorophyll content, and maximum quantum efficiency of photosystem II. Correlation analysis revealed presence of positive relationships among traits. Broad-sense heritability for all traits ranged from 0.35 to 0.73 implying presence of genetic contributions to the observed phenotypic variation. Genomic regions underlying these traits were located on chromosomes Ca02, Ca04, Ca05, Ca06, and Ca08. Four cold stress tolerance related candidate genes collocated with the peak SNPs on the above chromosomes. These genomic regions and marker information will be useful in molecular breeding of watermelon for cold stress tolerance.

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IDENTIFYING AND CONFIRMING RESISTANCE TO WHITEFLY-TRANSMITTED CUCURBIT LEAF CRUMPLE VIRUS IN WATERMELON USING INFECTIOUS CLONES

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Whitefly-transmitted cucurbit leaf crumple virus (CuLCrV) is routinely detected in watermelon and other cucurbit crops in the southeastern United States. If watermelon plants are infected during early stages of crop growth, severe stunting of plants and crumpling of leaves is evident. Phenotyping watermelon germplasm for resistance in the field did not yield consistent results across years, largely due to natural variation in the field and confounded results due to multiple virus infections. Hence, we developed Agrobacterium-mediated infectious clones of CuLCrV (DNA A & B) capable of inducing symptoms on susceptible watermelon plants to phenotype cultivars and germplasm for resistance. Initially 48 cultivars were evaluated for their reaction to CuLCrV clones by injecting a suspension through the hypocotyl. Variation in symptom expression among watermelon varieties was observed, but majority were highly susceptible to CuLCrV. Based on these results, 16 cultivars with a range of susceptibilities, including highly susceptible, moderate, and resistant, were reevaluated in two more trials. Results showed that commercial cultivars Crimson Sweet, Scarlet Princess and All Sweet were highly susceptible. In contrast, the pollenizer SP-6, germplasm accession PI 386015 and an advanced line USVL531-MDR were resistant to CuLCrV. QIAcuity digital PCR (dPCR) was used to determine actual copy numbers of CuLCrV coat protein in growing tips of inoculated plants using primers based on the coat protein sequence of CuLCrV. Significantly greater copy numbers of CuLCrV were detected in All Sweet (>4000 copies / 0.1 ng total DNA) compared to very low numbers in SP6 and USVL531-MDR (<200 copies) and PI 386015 (<10 copies). Significant (≤0.0001) correlations between disease ratings and CuLCrV copy numbers was also observed. Using this strategy, we aim to phenotype the watermelon germplasm collection available with the USDA ARS germplasm resources network (ars-grin.gov) to develop CuLCrV resistance sources for use in breeding programs.

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SCREENING FOR POWDERY MILDEW RESISTANCE IN *CUCURBITA MOSCHATA* DUCH IN LAJAS, PUERTO RICO

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Powdery mildew, caused by *Podosphaera xanthii* (Castagne) Braun y Shishkoff in Puerto Rico, is an important pathogen causing plant defoliation and significant yield reduction in cucurbits. A study was conducted to evaluate powdery mildew resistance in local and temperate genotypes of *Cucurbita moschata* Duch in field and greenhouse conditions in January 2022. Eleven *C. moschata* genotypes were planted in a randomized complete block design with three repetitions at the Lajas Research Substation of the University of Puerto Rico. The greenhouse trial had a randomized complete design with five repetitions. Data collected for agronomic traits included: days to flowering, days to harvest, number of fruits and yield. Powdery mildew incidence and severity were scored for each genotype at 7, 14, 28 and 42 days after inoculation. A scale from 0-5 was used to score disease severity, where 0 = free from infection and 5 = <76% of mycelium in leaf surfaces. For field trial results, flowering had an average of 52 to 62 days after sowing for male flowers and 54 to 65 for female flowers. Harvest days varied between 93 to 105 after sowing. Number of fruits per hectare was significantly higher in 'Taína Dorada' and 'Soler' and yield was significantly higher in 'Taína Dorada' genotype when compared to the remaining genotypes ($p<0.05$). For powdery mildew evaluations, incidence was highest in 'Waltham' (50%) and 'Taína Dorada' (50%), while severity was highest in 'Waltham' (2), 'Dickinson' (1) and 20-1716-02x1720 (1) genotype ($p<0.05$). Greenhouse evaluations indicated incidence (80-89%) and severity (4) was highest in 'Ponca' and all temperate genotypes ($p<0.05$). Therefore, temperate genotypes are susceptible under high pressure of powdery mildew and local genotypes demonstrate low susceptibility under tropical screening conditions. Further evaluations should be carried out on local genotypes given its potential in withholding novel resistance genes against *P. xanthii*.

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DIFFERENTIAL SET FOR DETERMINATION OF VIRULENCE VARIATION (RACES) OF CUCURBIT DOWNY MILDEW (*PSEUDOPERONOSPORA CUBENSIS*)

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Cucurbit downy mildew (CDM), caused by *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev (1903), is highly destructive pathogen causing economically important problems of cucurbit vegetables production world-wide. Host-parasite interactions between Cucurbitaceae and *P. cubensis* exhibit significant variation on both the individual and population level. Two approaches are developed to characterize the virulence variation of cucurbit downy mildew: 1) on the level of pathotypes (variation in level of host genera and species) and, 2) races (variation in intraspecific level). The concept of pathotypes identification, introduced in 80th of 20th century was further elaborated. An improved differential set of 12 genotypes of six cucurbit genera (*Benincasa*, *Citrullus*, *Cucumis*, *Cucurbita*, *Lagenaria*, and *Luffa*) developed by Lebeda and Widrlechner (2003) to characterize pathotypes among *P. cubensis* isolates is broadly used. Recently, the differential set of 22 genotypes of *Cucumis melo* was developed for determination of races of another obligate biotrophic parasites - cucurbit powdery mildews (CPM), caused by *Podosphaera xanthii* and *Golovinomyces orontii s.l.*) (Lebeda et al., 2016, 2021). Most recent research of virulence variation of *P. cubensis* population in the Czech Republic showed very broad spectrum of virulence patterns on *Cucumis melo* demonstrating existence of huge number of races by this pathogen, and suitability of this differential set also for the determination of races of *P. cubensis*. After long-lasting study of CDM and CPM virulence variation, recently this research yielded the first comprehensive and internationally (globally) applicable differential set and system for CDM, as well as CPM, virulence description and denomination as a background for better understanding of pathogen population structure, communication and breeding of melon and other cucurbits for resistance to both groups of pathogens. The differential set is publically available for utilization, and open for future enlargement and development (Lebeda et al., 2021).

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EFFECTS OF HIGH-TEMPERATURE AND SOIL MOISTURE CONDITIONS ON THE PHYSIOLOGICAL RESPONSE OF CUCUMBER

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The objectives of this study were to determine the effects of a combination of air temperature and soil moisture content on growth, physiological response, and yield of cucumber (*Cucumis sativus* L.). These experiments were conducted in a typical plastic house (one side open and the other side installed ventilation fans) with a gradient air temperature (maximum different value of air temperature: 6°C). The deficit irrigation (DI) treatment commenced 65 days after transplanting. As a result, regardless of the soil moisture conditions, the growth of the plants decreased at high air temperature (ambient + 3°C and ambient + 6°C). The height of plant was 301cm and 272cm, respectively, in the ambient and ambient + 3°C, and the number of leaves was the least in the ambient + 6°C. Furthermore, the leaf area decreased significantly with the DI treatment. The net photosynthesis rate of the full irrigation (FI) treatment was 12.38 μmol $CO_2m^{-2}\cdot s^{-1}$, the highest among all the treatments; however, the photosynthesis rate of the EHT treatment decreased by 39% (4.81 μmol $CO_2m^{-2}\cdot s^{-1}$). The effects of air temperature treatment were more pronounced on the physiological disorder rate and yield. The physiological disorder rate was similar between treatments. The yield of the AFI (ambient air temperature with full irrigation) treatment was 12,862 kg/10a, the highest among all the treatments; however, the yield of the EHT treatment with DI and FI decreased by 16% and 39%, respectively.

We assumed that foliar application treatment would improve heat stress tolerance in cucumber. Thus, we investigated the effects of ascorbic acid, salicylic acid and spermidine on the growth, photosynthetic properties, and antioxidant enzyme activity of cucumber under high-temperature stress conditions. The foliar application of biostimulants reduced physiological damage and enhanced the activity of the antioxidant enzymes, thereby improving heat stress tolerance in cucumber.

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EVALUATION OF FUSARIUM WILT RACE 2 RESISTANCE AND DEVELOPMENT INBRED LINES IN WATERMELON (*CITRULLUS* SPP.)

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In summer, watermelon is often suffered from the plant wilting due to high temperature with deficient water uptake through the root. Fusarium wilt is a global plant disease, which is caused by soil-borne pathogen, *Fusarium oxysporum* f. sp. *niveum*. The pathogen invades through the root and blocks the vessel, leading to severe damage before harvest. Grafting technique is used to prevent the invasion of the pathogen which is watermelon-specified. However, new pathogen, *F. oxysporum* f. sp. *lagenaria*, has been recently reported which penetrates guard root and pumpkin rootstock affects adversely the fruit quality. In addition, although there are several breeding lines and cultivars resistant to Fusarium wilt race 0 and 1, it is not to race 2 which is more virulent. Therefore, the purpose of this study is to develop breeding lines resistant to Fusarium wilt race 2, together with high fruit quality. A total of 183 watermelon germplasm were evaluated with disease resistance by root dipping method. Disease index of each seedling was scored according to the disease scale ranging from 1(resistant) to 5(susceptible). Homozygosity of the germplasm was evaluated with survived seedlings after inoculation. The genotype on 196 SNPs was analyzed through Fluidigm system, calculating the ratio of homozygous SNPs. As a result, a total of 10 germplasm were more resistance to the others. A total of 5 germplasm showed more than 95% of homozygosity, which can be introduced to breeding program per se. The selected breeding lines will be utilized to develop new cultivars resistant to Fusarium wilt. This study was supported by a grant (Project No: PJ01421402) from National Institute of Horticultural Herbal Sciences, Rural Development Administration.

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QTL MAPPING FOR YOUNG FRUIT RESISTANCE TO *PHYTOPHTHORA CAPSICI* IN CUCUMBER

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Phytophthora fruit rot caused by *Phytophthora capsici* is one of the most challenging diseases for Midwestern cucumber growers. Young fruits are especially susceptible. The goal of this research is to identify QTL associated with young fruit resistance (YFR), originally found in PI 109483, and develop molecular markers to assist breeding and introgression. QTL-seq performed on bi-parental populations derived from a cross between the PI 109483-derived resistant doubled haploid line, DH A4-3, and the susceptible pickling cucumber line, Gy14 identified multiple QTL , suggesting that YFR is a quantitative trait. Kompetitive allele specific PCR (KASP) markers were designed to target the QTL on chromosomes 5 and 6. The phenotypic and allelic effect data from a selected F_2 population showed that YFR was correlated with the frequency of DH A4-3 allele for the QTL on chromosome 5 (YFR5.1). The effect of YFR5.1 was further verified in a second genetic background from a slicing cucumber variety, Pointsett 76. To narrow the YFR5.1 region, a set of F_4/F_5 recombinant inbred lines (RIL) was developed from screening an F_2 population. Multiple KASP markers spanning the 5-Mb QTL interval narrowed the QTL to 1 Mb. Additional F_3 recombinant families (n=33) are being genotyped and phenotyped to confirm the narrowed region. The cucumber core collection established from the CucCAP project was also screened to identify potential QTL. The core collection was grown in the field in 2019-2021 and phenotypic data collected from the three years is being used for genome wide association study (GWAS) analysis. To further verify GWAS results, accessions with extreme phenotypes were grown in 2022 for Extreme-Phenotype GWAS (XP-GWAS) analysis.

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GENOME WIDE ASSOCIATION STUDY OF C. PEPO EVALUATED FOR WHITEFLIES AND THEIR TRANSMITTED VIRUSES

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Whiteflies (*Bemisisia tabaci* Genn.) and their transmitted viruses are a major pest of summer squash (*Cucurbita pepo* L.) resulting in severe yield losses. Management mainly relies on insecticides, however, this is only indirectly managing the viruses at a high cost to the growers. The optimal way to protect squash from whiteflies and their transmitted viruses is to plant resistant cultivars. Unfortunately, to date, no resistant cultivars of summer squash have been identified. Previous work evaluated squash germplasm to identify resistance to whiteflies, *Cucurbit leaf crumple virus* (CuLCrV), *Cucurbit yellow stunting disorder* (CYSDV), and *Cucurbit chlorotic yellows virus* (CCYV) under field conditions in Florida and Georgia over two years, 2019 and 2020. The germplasm was assessed for visual virus symptoms and quantitative PCR was performed to determine the viral load of CuLCrV, CYSDV and CCYV. The objective of this study is to use genotyping by sequencing data generated by the CucCap project to perform a genome wide association study. Initially, the program Structure has been used to identify two distinct groups within the *C. pepo* germplasm. To identify single nucleotide polymorphisms (SNPs) associated with the phenotypes previously evaluated, the Gapit function within R was used. With the minor allele frequency set to 0.01 and the population structure imported from Structure, a Blink analysis was run. SNPs were only identified for CYSDV and CCYV viral load. Nine SNPs were identified for CYSDV and five for CCYV, all of which were associated with the susceptible phenotype. Three of the SNPs associated with each virus load were nonsynonymous mutations. These findings may provide targets for selection during resistance breeding and elucidate susceptibility mechanisms for CYSDV and CCYV in *C. pepo*.

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CUCURBITS OF THE WORLD NETWORK

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As the demographic of the United States continues to diversify, we have an increasing need to expand crop diversity to meet the needs of our communities. Many of these crops have major regional importance but, there is a gap in making these crops more accessible in diverse communities. The Cucurbits of the World Network is an initiative to help bridge this gap. The goal of the network is to improve accessibility and preserve the diversity of these crops in the US. The current stage of the initiative is building the network to allow for nation-wide trialing with extension specialists, farmers, researchers, and consumers. One of the major hurdles in making these crops more accessible is improving knowledge accessibility. The trialing network aims to gather species and cultivar specific data on crop yield, pest and disease resistance, and adaptability to each growing region of the US. The data will be collected from each partner and compiled into user friendly materials on our website, including interactive maps and growing guides. Currently commercial and USDA GRIN germplasm is being evaluated for the Northeastern US. Based on 2021 field trials, four focus crops were chosen: *Trichosanthes cucumerina* (snake gourd)*, Benincasa hispida* (wax gourd/winter melon)*, Lagenaria siceraria* (bottle gourd)*,* and *Momordica charantia* (bitter melon)*.* These crops are being trialed at Cornell University's Vegetable Research Farm in Freeville, NY as well as a partnering farm, Norwich Meadows in Norwich, NY. This community driven approach will be complimented with on campus efforts to characterize commercial and USDA GRIN germplasm including plant and fruit phenotype data, genotyping, and mapping important traits identified by the network.

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NOVEL ETHYLENE-JASMONATE CROSSTALK TRIGGERING FLOWER OPENING AND UNFERTILIZED FRUIT ABORTION IN *CUCURBITA PEPO*

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Jasmonic acid (JA) has been found to be a relevant hormone on flower development in numerous species, but its function in cucurbit floral development and sex determination is unknown. Crosstalk between JA and ethylene (ET) in the differential regulation of male and female flower development was addressed by using the novel JA-deficient mutant *lox3a*, and the ET-deficient and -insensitive mutants *aco1a* and *etr2b* of *Cucurbita pepo*. The *lox3a* mutation suppresses male and female flower maturation and opening, inducing the development of parthenocarpic fruit. A BSA-seq and fine mapping approach allowed the identification of *lox3a* mutation in *CpLOX3A*, a *LYPOXYGENASE* gene involved in the JA biosynthesis. The reduced JA-biosynthesis and -signaling gene expression, the JA content in male and female flowers of *lox3a*, and the rescue of *lox3a* phenotype by external application of methyl jasmonate (MeJA), demonstrated that JA controls petal elongation, maturation, and flower opening, as well as the fruit abortion in the absence of fertilization. Furthermore, MeJA treatment was also able to rescue the phenotype of ET mutants *aco1a* and *etr2b*, which are specifically defective in female flower opening and fruit abortion, indicating that these mutants are also deficient in JA. The results suggest that ET, the sex determining hormone of cucurbits, is induced in female flowers towards anthesis, up-regulating JA biosynthesis and signaling and promoting the faster aperture of the female flower and the abortion of the unfertilized ovary. The phenotypes of ET and JA mutants demonstrate that these two hormones play a negative role in fruit set and development. Given the close association between flower opening and fruit abortion, we propose for the first time a model in which flower opening mature and senescent petals in open flowers can serve as remote signals that activate ovary/fruit abortion in the absence of fertilization.

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RESISTANCE TO *CUCUMBER MOSAIC VIRUS* IN MELON: LOCALIZATION OF THE VACUOLAR PROTEIN SORTING 41

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The melon resistant gene *cmv1* encodes a Vacuolar Protein Sorting 41, involved in intracellular trafficking to the vacuole. In the melon lines carrying *cmv1* CMV strains of subgroup II are restricted to the bundle sheath cells and do not enter the phloem, whereas those of subgroup I overcome this restriction. The viral virulence factor that communicates with *cmv1* is the Movement Protein (MP). The viral MP somehow may communicate with CmVPS41to use it for the viral intracellular trafficking

We have studied the cellular localization of CmVPS41 from PS (susceptible) and SC (resistant) genotypes by agroinfiltration the GFP-tagged genes in *Nicotiana benthamiana* cells. They show significant differences in their localization pattern, with structures such as nuclear speckles, membrane dots and transvacuolar bridges that are numerous in CmVPS41PS, but scarce in CmVPS41SC. These CmVPS41 characteristic structures co-localize with the late endosome. Moreover, CmVPS41 from exotic resistant melon accessions are similar to those of SC and the structure that most correlates with susceptibility is the presence of transvacuolar strands. Those patterns do not change in the presence of overexpressed MP. However, during a real infection those patterns change dramatically, with transvacuolar strands disappearing. This suggests that those structures are involved in susceptibility to CMV and that at late stages of infection, the transvacuolar strands would not be needed and could disappear.

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ADVANCEMENT OF CYSDV-RESISTANT MELON USING MARKER-ASSISTED SELECTION

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Cucurbit yellow stunting disorder virus (CYSDV: Crinivirus; *Closteroviridae*), has become a major production constraint of melon in the western United States. Another crinivirus, cucurbit chlorotic yellows virus (CCYV), which produces similar foliar symptoms emerged in U.S. melons in 2014 and often co-infects with CYSDV. Both viruses are transmitted in this region by whitefly *Bemisia tabaci* (MEAM1, aka biotype B). Host plant resistance is a strongly attractive, economic, and ecofriendly way to manage CYSDV and CCYV. We previously developed a F2:3 Top Mark (susceptible) x PI 313970 (CYSDV-resistant) population and found resistance to be controlled by a single, recessive Mendelian gene and were introgressing CYSDV resistance to western U.S. shipping type melon using naturally-infected field tests. The emergence of CCYV and common co-infection with both viruses, which produce identical symptoms confounded field-selection for CYSDV resistance. QTL analysis of relative virus titer in the F2:3 subjected to natural infection in an open field test identified a QTL on chromosome 5 for resistance to CYSDV between markers S5-20880639 and S5-21356819. Our objective in this study was to re-evaluate the most resistant, i.e., lowest virus titer, F2:3 families in order to refine molecular markers for marker assisted introgression of CYSDV resistance to western U.S. shipping type melon. Ten to twenty plants from eight F2:3 families were inoculated with CYSDV using 50 whiteflies per plant (48 h acquisition access, 48 h inoculation access periods), treated with imidacloprid, moved to a greenhouse for symptom development, monitored for virus titer using single-step RT-qPCR, and probed with QTL flanking markers for association with disease reactions. The eight F2:3 families presented all combinations of parental flanking markers, virus titer, and foliar symptom expression. Plants with favorable combinations were self-pollinated for advancement.

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THE ROLE OF *QFD2.2* IN CONTROLLING RIND THICKNESS AND FRUIT DIAMETER IN WATERMELON

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Rind thickness (RTH) and fruit diameter (FD) are important traits in watermelon breeding due to their role in shipping durability and consumer preference. We have previously found high correlations between RTH and FD and co-localization of a major QTL for RTH with a major QTL associated with fruit diameter (*QFD2.2*). To decouple RTH from FD, the rind thickness percentage (RRP) = [RTH/FD]*100 was calculated. QTL for RRP colocalized with *QFD2.2*, *QFSI3.1,* and *QRTH5.1.* Interactions were identified between *QRTH5.1* and *QFD2.2*, where *QRTH5.1* only had an effect when *QFD2.2* were homozygous for the increased FD allele. We show that selecting for the desired allele combinations at these three loci can double RRP. Further fine mapping narrowed down the region of *QFD2.2* to a 728 kb interval containing 53 genes. KASP marker assays were developed for *QFD2.2* and *QRTH5.1* and will be a valuable resource for selection of RRP in watermelon.

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A NEW WORKING GROUP TO ADDRESS EMERGING VIRUSES IN CUCURBITS

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During the past 25 years, viruses infecting cucurbits have emerged in the cucurbit-producing areas of the U.S. at an alarming rate. Most of these introductions have led to reduced crop yields. Whitefly-transmitted viruses, including cucurbit leaf crumple virus, cucurbit yellow stunting disorder virus, squash vein yellowing virus, and cucurbit chlorotic yellows virus, which were introduced between 1998 and 2014, can now be found in most cucurbit-producing areas of the U.S. The seed-borne cucumber green mottle mosaic virus was detected in California in 2013; however, aggressive containment efforts have, thus far, limited its spread. Several additional viruses impact cucurbit production internationally and pose an inherent risk to the U.S. cucurbit industry. Knowledge of many cucurbit viruses remains limited, methods of detection/identification are not universally practiced across the industry, and limited communication within the industry slows the rapid identification of new viruses and the implementation of containment measures. The Emerging Viruses in Cucurbits Working Group (EVCWG) was established, with support from the U.S. cucurbit industry, to address these issues. Its mission is to improve communication and knowledge about viruses across the cucurbit industry and develop strategies to successfully identify and mitigate virus threats to cucurbit production in the U.S. EVCWG members will identify and prioritize potential risk factors for viruses affecting cucurbits, known and potential virus threats, mitigation strategies for these viruses, and methods to reduce virus threats and will determine the best approaches to address these issues. The EVCWG will work to increase knowledge and awareness of virus threats among members of the cucurbit industry through the delivery of educational presentations and the development of educational resources that will be posted to the EVCWG website. Through these efforts, the U.S. cucurbit industry will benefit from improved stability and profitability resulting from decreased virus introductions and/or spread and associated yield losses.

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RNA-SEQ REVEALS POTENTIAL DEFENSE MECHANISMS AGAINST PHYTOPHTHORA CAPSICI IN SQUASH

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Phytophthora capsici is a hemi-biotropic soil pathogen causing foliar blight, damping off, and root, fruit or crown rots in cucurbit crops worldwide. Resistance to *P. capsici* has been identified in wild genotypes of Cucurbita and transferred to *C. moschata* background, but the genes and metabolic pathways underlying resistance are currently unknown. In the current study, a University of Florida resistant breeding line (394-1-27-12), and a commercial susceptible cultivar (Butterbush) were subjected to RNAseq analysis for exploring transcriptional and metabolic changes in the genotypes post pathogen inoculation. The two genotypes were inoculated with P. capsici, and samples were collected 0, 12, 24, 72 and 120 hours post infection (hpi) with three technical and three biological replicates for each time point. On an average 90% of the genes were uniquely mapped to *C. moschata* var. Rifu reference genome. Transcript data for 394-1-27-12 and Butterbush showed strong correlation among replicates (85%) and were further selected for differential gene expression analysis (DEGs). Overall, DEG analysis revealed nearly 2,500 upregulated genes in 394-1-27-12 compared to Butterbush. Transcriptomes of resistant and susceptible lines were compared at 0, 12, 24, 72 and 120 hpi. Percentage of differentially expressed genes between resistant and susceptible genotypes was lowest at 72 hpi and highest at 12 hpi accounting for genes responsible for stress. The stress response genes namely Ethylene-responsive factors was 15-fold higher and Disease resistance protein RGA2 was 7-fold higher in expression level in 394-1-27-12 at 12 hpi. While the number of DEGs varied among hpi, overall expression of genes involved in defense response pathway was highest at 12 hpi. Ongoing effort include gene ontology enrichment and KEGG analysis of the DEGs to identify potential gene pathways and networks in response to P. capsici infection. The outcome of the current study lays a foundation for functional characterization of the identified genes.

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INCIDENCE AND YIELD RESPONSE OF SEEDLESS WATERMELON CULTIVARS AFFECTED WITH FUSARIUM WILT

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Fusarium wilt of watermelon, caused by the fungus *Fusarium oxysporum* f. sp. *niveum* (FON), is a widespread disease in the southeastern United States and many other parts of the world. FON can have devastating effects on yields. Planting resistant cultivars is a preferred management practice. However, there is limited resistance to races 2 and 3 of FON. Ten watermelon cultivars were evaluated in North Carolina in 2021 and 12 in 2022 for disease incidence and yield response in a FON-infested field. The ten cultivars in 2021 included: El Capitan, Embasy, Fascination, Joy Ride, Powerhouse, Shoreline, Sierra Madre, Tri-X-313, 7197HQ, and Fascination grafted to Carolina Strongback rootstock. In 2022, Joy Ride and Sierra Madre were replaced with four new entries: HMC633800, HMC633802, Syngenta-1, and Syngenta-2. Both years, a randomized complete block design with four replications was used. Entries were transplanted on 27 April 2021 and 3 May 2022. Plants were evaluated weekly (7 times) for the occurrence of symptoms caused by FON. Grafted Fascination had the lowest incidence (percentage of plants with symptoms) (0%) in the 2021 study. All other cultivars had at least 75% incidence. Marketable yields corresponded with incidence, with the grafted Fascination yielding 1.9 marketable fruit per plant and non-grafted Fascination, the second highest yielding cultivar, with 0.6 fruit per plant. All other cultivars had yields ranging from 0.4 to 0.1 fruit per plant. In the 2022 study, 10 of the 12 entries had 80% to 100% incidence. The grafted Fascination again had the lowest incidence at 8%. HMC633802 had the lowest incidence of the non-grafted entries with 50%. The grafted Fascination was the highest yielding cultivar for 2022 with 1.0 fruit over 9 lb per plant, followed by HMC633800 with 0.3 fruit over 9 lb per plant.

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A NOVEL FEMALENESS ALLELE OF THE SEX DETERMINATION GENE *CMACS11* IN MELON

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Sex determination in cucurbit crops is a critical trait that improves fruit yield and quality and contributes to more efficient cultivation. Previous studies have shown that local ethylene levels at the floral primordia determine the sexual fate of melons. It has been explained that the local ethylene levels are controlled by an orchestrated regulation mechanism of previously reported three sex determination genes (*CmACS-7*, *CmWIP1,* and *CmACS11*). In monoecious melons, the absence of *CmACS11* expression results in the expression of *CmWIP1* and the formation of male flowers on the main stem, and the expression of *CmACS11* promotes female flower formation on the lateral branch by suppressing the expression of *CmWIP1*. *CmACS11* supports the flower development and the coexistence of male and female flowers. However, we found a Japanese weedy melon line doesn't follow the mechanism. Here we have detected two significant QTLs on Chr. 8 and Chr. 3 related to sex determination and developed a marker with *CmACS11* for femaleness on the main stem. We performed quantitative trait locus (QTL) analysis of sex expression using double-digest restriction-site-associated DNA sequencing (ddRAD-Seq) technique in a total of 382 F2 segregating populations derived from a cross between an andromonoecious cultivar 'Earl's Favourite Harukei-3' and a hermaphrodite Japanese weedy melon 'UT1'. We detected two significant QTLs on Chr. 8 and Chr. 3 for femaleness. The QTL on Chr. 3 was detected near *CmACS11*. Sequence comparison of *CmACS11* between two parental lines revealed two non-synonymous SNPs in the coding region in 'UT1'. A CAPS marker was developed for the SNP, which was co-segregated with the femaleness phenotype in the F2 population. The results of this study will be helpful for better understanding the molecular mechanism of sex determination in melons and developing marker-assisted selection (MAS) for new femaleness melon cultivars.

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WHOLE-GENOME SCANNING USING QTL-SEQ AND GWAS FOR GUMMY STEM BLIGHT RESISTANCE IN WATERMELON

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Watermelon is a nutritionally and economically important crop in the U.S and worldwide. Gummy stem Blight (GSB) is one of the most devastating diseases for watermelon in the U.S. GSB is caused by three cryptic Stagonosporopsis species, which can infect most of the above-ground parts of the watermelon. In the present study, we identified important quantitative trait loci (QTLs) associated with the GSB resistance in the selfed derivatives of a MAGIC population from intercrosses involving the most resistant lines of *Citrullus amarus* and highly susceptible cultivars of *Citrullus lanatus*. The resistant and susceptible bulks were constructed by combining equimolar concentrations of DNA from 30 extreme resistant RILs and 30 extreme susceptible RILs. The two bulks were subjected to whole-genome sequencing to generate more than 1 billion reads per bulk and achieve the genome's in-depth coverage. The mapping percentage of bulks to the parental genomes ranged from 92 to 99. More than 6 million SNPs were identified from the bulks based on the resistant parental genome, while less than 2 million SNPs were identified using the susceptible parental genome. The QTLs associated with the GSB resistance were identified using single-nucleotide polymorphism-index and Gprime methods. We have identified statistically significant variants/loci associated with the GSB resistance in chromosomes 1, 2, 3, 5, 10 and 11. Several important candidate genes were assigned to GSB resistance based on the physical location of genes in the important QTL regions on these chromosomes. The significant loci associated with the GSB resistance were used to develop PACE genotype markers. Our findings will support the development of GSB-resistant watermelon cultivars.

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QTL MAPPING FOR FRUIT QUALITY TRAITS IN CUCUMBER, *CUCUMIS SATIVUS*

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Cucumber (*Cucumis sativus* L.) is an economically important vegetable crop worldwide. There is an increasing demand for cucumber varieties with better flavor which involves a combination of smell (aromas, organic volatile compounds), taste (sweetness, bitterness, acidity, astringency), and texture (firmness, crispness, juiciness). These traits are genetically complex and knowledge on their genetic basis is still limited. Here we reported QTL mapping for flavor quality-related attributes in cucumber including fruit aroma (organic volatile compounds, or VOCs), taste and fruit firmness. Segregating F2 and F2:3 populations were developed from the cross between two cucumber inbred lines, WI7633 and 9930 with contrast flavor profiles. A linkage map was developed through genotyping by sequencing (GBS) of 140 F2 plants that contained 1912 SNP loci spanning 1077 cM in 7 linkage groups. Phenotypic data for C6 and C9 VOCs, fruit firmness and other taste-related traits including sugar contents (glucose, sucrose), acidity (pH, titratable acidity) and astringency (total phenolic compounds) were collected from 140 F2 individual plants and 138 F2:3 families across three environments. QTL analysis identified 38 QTLs for 16 traits across all 7 chromosomes (Chr) that are associated with cucumber fruit flavor quality. Most of these QTLs were reproducibly detected in at least two populations or environments. Many QTLs associated with different C6 and C9 VOCs were co-localized on same chromosomal intervals. Notably, QTLs for five C9 VOCs were detected in a 7.1-Mbp region of Chr1, and QTLs for one C6 and four C9 VOCs were mapped in an 8.1-Mbp region of Chr4. A 4.9-Mbp region in Chr2 harbored major-effect QTLs for one C6 (hexanol, phenotypic variance explained or PVE = 25.6%) and four C9 VOCs, including (E,Z)-3,6-nonadienol (PVE=17.4%), (Z)-3-nonenol (PVE=8.1%), (Z)-6-nonenal (PVE=16.8%) and nonanol (PVE=16.9%). This is the first report on comprehensive genetic analysis for cucumber flavor quality attributes, which provides a framework for dissection of the genetic architecture of and marker-assisted breeding for cucumber flavor.

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THE *MF* GENE FROM *CUCURBITA PEPO* SUBSP. *TEXANA* IN COCOZELLE AND ZUCCHINI SQUASH, SUBSP. *PEPO*

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In *Cucurbita pepo* L., the recessive allele *mf* confers *multiple flowering*, specifically, the differentiation of more than one flower bud in leaf axils. This allele is fixed in *C. pepo* subsp. *texana* (Crookneck, Straightneck, Scallop, and Acorn squash) but was absent in *C. pepo* subsp. *pepo* (Cocozelle, Zucchini, and Vegetable Marrow squash and Pumpkin), which differentiates only one flower bud in each leaf axil. A cross was made between a Crookneck squash and a Cocozelle squash with the goal of introgressing this recessive allele into *C. pepo* subsp. *pepo*. Multiple-flowering F_2 plants were then backcrossed to Cocozelle squash and crossed to Zucchini squash, followed by selfing and selection for *mf/mf* plants. This procedure was repeated for six cycles in several inbreds each of Cocozelle and Zucchini in order to develop near-isogenic (BC_6-generation), *mf/mf* and *Mf/Mf* inbreds. Near-isogenic, *mf/mf* and *Mf/Mf*, hybrids were developed from some of the inbreds in order to measure what, if any, yield advantage is afforded by the *mf/mf* genotype in Cocozelle and Zucchini under typical field conditions at Newe Ya'ar (northern Israel) in two consecutive years. In both Cocozelle and Zucchini and in both years, the *mf/mf* hybrids significantly outyielded their near-isogenic *Mf/Mf* counterparts. The *mf/mf* Cocozelle hybrid produced 51% and 25% more fruits than its *Mf/Mf* counterpart in the two years. The advantage in yield was significant already by the end of the first week of harvest. The *mf/mf* Zucchini hybrid produced 23% and 14% more fruits than its *Mf/Mf* counterpart in the two years. The advantage in yield was significant from the beginning of the third week of harvest. No obvious differences were observed in fruit shape or quality between the *mf/mf* and *Mf/Mf* genotypes. Hence, the introgression of the recessive *mf* allele has the potential to markedly increase yields, under some growing conditions, of Cocozelle and Zucchini squash.

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IDENTIFICATION OF A NOVEL LOCUS (C_2) CONTROLLING CANARY YELLOW FLESH IN WATERMELONS

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The flesh color of cultivated watermelons(*Citrullus lanatus* L.) is classified into scarlet or coral red, canary yellow, and orange due to the accumulation of specific carotenoids. Canary yellow flesh(CY) is dominant to red flesh, which is determined by the *C* locus encoding LCYB(Lycopene β-cyclase) involved in the carotenoid biosynthesis pathway. However, in some inbreds homozygous for *C*, yellow flesh mixed with red(incomplete canary yellow flesh, ICY) is observed, implying that a secondary genetic factor in addition to *LCYB* may exist in determining canary yellow flesh. In this study, whole genome resequencing(WGRS) of three CY and three ICY inbreds and mapping of an F_2 population derived from a cross between CY and ICY inbred were performed to identify the locus(named C_2) associated with the ICY. The WGRS deliminated a genomic region that is conserved in DNA sequence only among three CY inbreds. This genomic region spends 27.60-28.32Mb on chromosome 2 and carries 26 genes. A total of eight SNPs from these genes were converted to the CAPS and mapped using 131 F_2 plants. In this F_2 population, CY and ICY segregated by 3:1, indicating dominant effect of single gene for CY. In linkage mapping, the C_2 was franked by two tightly linked CAPS markers. Our results show that C_2 is a new locus that regulates canary yellow flesh along with the *LCYB*. The molecular function of C_2 and its interplay with the *LCYB* in the formation of canary yellow flesh remains to be elucidated in the future.

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TOLCNDV RESISTANCE IN MELON AND CUCUMBER

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Tomato Leaf Curl New Delhi Virus disease (ToLCNDV) was first identified in 1995 in India on tomato plants. This Begomovirus, vectored by white flies (*Bemisia tabaci*) in a semi-persistent manner, soon spread all over the Far East (Indonesia, Japan, Taiwan and Thailand) infecting broad spectrum of Solanaceous and Cucurbitaceae crops (cucumber, Melon, Luffa, Momordica and eggplants). In spring 2012 zucchini fields in Murcia, Spain, were infected with a leaf curl disease that was later identified as ToLCNDV. During autumn 2012 the disease spread west toward Almeria on melon and cucumber crops (but not in tomato). Infected melon plants exhibit leaf curl, stunting, mosaic and leaves yellowing. Fruits skin can develop cracks and exhibit generally poor development under severe infection. In cucumber, symptoms observed include leaf mosaic and stunting on leaves, but generally lacking symptoms on fruit. In Almeria, Spain, in 2014, Hazera conducted screening trials of melon and cucumber aimed to identified resistant accessions in both crops. Screened material included Limagrain internal plant genetic resources, and from the screenings a few potential trait donors (resistance sources) were identified. Among them, H-MLCND-32)*Cucumis melo* subsp. *agrestis* var. *acisulus*) and CUC29 (Cucumis sativus var sativus) were chosen for furthered studies. In melon, F2, F3 and RIL mapping population studies revealed one major QTL in chromosome 11, with recessive inheritance. In cucumber, F2 and F3 QTL mapping population detected two major QTLs in chromosome 1 and chromosome 2. Inheritance in cucumber appears co-dominant. In both crops, backcrossing through Marker Assisted Backcross (MABC) were performed, preserving and moving the resistant QTL into cultivated material where the causal effect of these QTL were validated by pathology test.

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DIFFERENTIALLY EXPRESSED GENES IN MELON LINES RESISTANT TO WMV AND TOLCNDV

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Watermelon mosaic virus (WMV) and tomato leaf curl New Delhi virus (ToLCNDV) are among the most damaging viruses affecting cucurbits. Recessive resistance to WMV has previously been reported in the melon (*Cucumis melo* L.) African accession TGR-1551. The resistance gene wmv1551 was mapped to a 150 kb region in chromosome 11, where 11 predicted genes were annotated. Resistance to ToLCNDV was identified in the Indian melon accession WM-7, controlled by a major QTL in chromosome 11 and two additional modifying regions in chromosomes 2 and 12. Two RNA-seq analysis were performed to compare the response after inoculation with WMV and ToLCNDV of the susceptible cultivars and the resistant lines (research projects PID2020-116055RB C21 and C22, and RTC-2017-6023-2-AR). In the case of resistance to WMV, a consensus list of 616 differentially expressed genes (DEGs) was obtained. Three DEGs located within the interval of the major QTL were up-regulated in the resistant genotype when compared to the susceptible accession. Those DEGs coded a basic 7S globulin-like protein, a dual specificity protein phosphatase and a mediator of RNA polymerase II transcription subunit. Other DEGs involved in responses to biotic stresses were also up-regulated in the resistant line, including susceptibility and transcription factors, ubiquitination genes, as well as genes related to hormonal signaling. After ToLCNDV inoculation, ten DEGs located within the major QTL of chromosome 11. The most promising candidate gene coded a DNA-primase with down-regulated transcription in WM-7 accession. We have evaluated the functional role of these genes in melon plants infected with ToLCNDV following virus-induced gene silencing and transient Agrobacterium-mediated expression approaches. Viral accumulation was associated to DNA-primase altered expression levels in both resistant and susceptible genotypes, when compared to control plants. These results allow advancing our knowledge about the resistance candidate genes to WMV and ToLCNDV in melon.

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COMMERCIAL HARD SQUASH CULTIVARS EXHIBIT DIFFERENCES IN RESISTANCE TO PHYTOPHTHORA FRUIT AND CROWN ROT

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Crown and fruit rot, incited by *Phytophthora capsici*, limit yield and quality of hard squash grown for processing. With a low profit margin, growers limit production costs, including fungicides. We compared commercial squash cultivars for fruit and crown rot resistance. Sugar content measured as degrees Brix (°Bx) was also determined. Four butternut (*Cucurbita moschata)*, two hubbard (*C. maxima*), and six kabocha (*C. maxima)* squashes were evaluated for crown rot resistance (2020, 2021) and °Bx (2019, 2020). For the crown rot study, plants were inoculated with millet colonized by two isolates (mating types A1, A2). 'Dickinson,' 'Buckskin,' 'New England Cheddar,' and 'Ultra' had significantly less plant death at the final rating of both years (<15%) than the other cultivars. Significantly fewer 'Thunder' plants died in 2020 (59.6 %) and 2021 (76.9%) compared to 'NK-580' (≥90.0%), 'Golden Delicious' (≥88.5%), 'Sweet Mama' (>94.2%), 'Delica' (≥92.3%), and 'Sunshine' (≥90.4%). In separate experiments, when the fruits were inoculated 21 days post-pollination (DPP), the *C. moschata* cultivars exhibited age-related resistance and did not become infected. The *C. maxima* cultivars Autumn Cup and Thunder exhibited an intermediate level of fruit rot resistance 21 DPP in one study. Healthy-appearing mature fruits were harvested and analyzed for °Bx. Cultivars with moderate ('Thunder') and low ('Ultra') fruit and crown rot susceptibility had similar °Bx to the standard ('NK580') in both years. The resistant cultivars Dickinson, Buckskin, and New England Cheddar had similar °Bx in 2019 and lower °Bx than the standard in 2020. 'Thunder' and 'Ultra', appear to meet the standards for sugar content, while 'Dickinson' 'Buckskin', and 'New England Cheddar' showed inconsistent °Bx between years. Disease control can be advanced by integrating resistance demonstrated by some commercially available cultivars into an overall management program. Further evaluation is needed to evaluate yield and quality characteristics required for processing.

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INTROGRESSION LINES COMBINING CLIMACTERIC AND NON-CLIMACTERIC MELON GENOMES IN THE DISCOVERY OF VOLATILE-RELATED GENES

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Introgression Lines are powerful tools to discover new genes involved in fruit quality traits. One of the most important fruit traits in melon is aroma. The fruity aroma of climacteric melons is linked to the production of esters, whereas the fresh, cucumber-like notes of non-climacteric varieties is related to aldehydes. The biosynthetic pathways involved in volatile formation are still not fully understood. However, understanding their genetic control is of great importance in order to improve melon fruit quality. Two reciprocal introgression line collections obtained by crossing the climacteric variety '*Védrantais*' with the non-climacteric variety '*Piel de Sapo*' were used to validate volatile QTLs previously identified in a Recombinant Inbred Line population sharing the same parental lines. Fruit flesh and rind samples of the introgression lines were analyzed by Solid-phase Micro-Extraction in a Gas Chromatograph coupled to a Mass Spectrometer and compared to each recurrent parental profile. In the '*Piel de Sapo*' background, '*Védrantais*' introgressions on chromosomes 1, 5, 6 and 8 increased the ester content. Some of these regions include ripening-associated genes such as *CmNAC-NOR* on chromosome 6 and QTL *ETHQV8.1* on chromosome 8, supporting the fact that ethylene production can activate ester synthesis. In '*Védrantais*' background, '*Piel de Sapo*' introgressions on chromosomes 1, 3, 5 and 8 decreased the ester content while increasing the proportion of aldehydes. On the other hand, introgressions on chromosomes 2, 10 or 11 increased the ester production, suggesting the existence of active alleles in non-climacteric melons to produce esters. In addition, on chromosome 6, a '*Piel de Sapo*' introgression drastically reduced the production of lipid-derived alcohols and esters. Two introgression lines in the climacteric background containing '*Piel de Sapo*' fragments on chromosomes 11 and 6 were selected for a fine-mapping of the region. F2 recombinant populations were generated to dissect the QTLs on chromosome 11 and chromosome 6. On chromosome 6, two delta3-delta2 enoyl-Coa isomerases were selected as candidate genes for the degradation of the precursors of 3-hexen-1-ol (Z) and other related volatiles. These findings highlight the power of introgression lines in the identification of volatile-related genes and provide the basis for their application into melon breeding programs.

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ISOLATION AND CHARACTERIZATION OF LIPID DROPLETS IN CUCUMBER FRUIT

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Lipid droplets (LDs), which consist of a neutral core surrounded by a phospholipid monolayer with embedded proteins, are increasingly recognized as highly dynamic entities, potentially modulating intracellular metabolism and communication. LDs can vary in their composition and assist in functions such as lipid metabolism, stress response, and accumulation of specialized metabolites. Our previous studies of cucumber fruit found that LDs varied in size depending on developmental age and variety, and that the regulatory factor *CsSHINE1/WIN1* was found to be a source of natural variation for LD size. In epidermal cells, LDs are thought to transport cuticular substrates, but they continue to persist in the epidermis of cucumber fruit after cuticle deposition is largely complete, ~16 days post pollination (dpp). To examine possible functions of LDs in cucumber fruit peel, a protocol was developed to isolate and purify LDs from the cucumber peel. Peel samples were ground in a sucrose solution, centrifuged for multiple rounds using sucrose cushions to purify the LDs, and swirled on a rotary shaker to concentrate LDs for further analysis. Cucumber peel sections and the isolated LDs were stained with Sudan IV, a red lipid stain, to confirm the isolated LDs were consistent with those found in peel sections. Untargeted profiling using Ultra Performance Liquid Chromatography (UPLC) mass spectrometry was performed on extracts from the total fruit peel, isolated LDs, and the sucrose solution surrounding isolated LDs. Preliminary mass spectra for isolated LD extracts showed strong enrichment of triacylglycerols, characteristic of lipid droplets. Further analyses are in progress to identify additional compounds that are enriched in LDs. In addition, phenotyping data for lipid droplet associated traits done on a cucumber core population developed for the CucCAP project (https://cuccap.org), are being used to conduct a genome wide association study (GWAS) to identify new LD-associated Quantitative Trait Loci (QTL).

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MARKER-ASSISTED BREEDING FOR GUMMY STEM BLIGHT RESISTANCE IN WATERMELON.

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Gummy stem blight (GSB) is a major disease in cucurbits caused by three different species of *Stagonosporopsis*: *S. cucurbitacearum*, *S. citrulli*, and *S. caricae*. Cultivated watermelons (*C. lanatus*) are susceptible to GSB, but resistance has been identified in *C. amarus*, a crop wild relative (CWR) of watermelon. Five QTL for *S. citrulli* and *S. cucurbitacearum* resistance have been identified from two different *C. amarus* accessions. Our objective is pyramiding these small-effect QTL into the *C. lanatus* background. Marker-assisted backcrossing (MABC) was carried out using KASP marker assays flanking the target QTL. *C. lanatus* genome recovery is being accelerated by selection for known fruit quality-related domestication traits, including loss of bitterness (*bHLH*), Brix (*ClAGA2*, *ClTST2*), sucrose content (*ClVST1*), and red flesh color (*LYCB*). Once QTL introgression is complete, the lines will be evaluated for GSB resistance and horticultural traits. The breeding lines have potential utility in developing resistant cultivars to help watermelon growers reduce the economic losses associated with gummy stem blight.

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YIELD AND HORTICULTURAL PERFORMANCE OF TROPICAL PUMPKIN IN FLORIDA

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Tropical pumpkin (*Cucurbita moschata*), also known as Calabaza, fulfill lucrative emerging markets for the crops in the U.S. A recent consumer survey indicated fruit quality (flavor, texture, flesh color and shape) and price point as the most important factors in buying decision, while growers emphasized yield, earliness, growth habit and tolerance to pest and diseases. In the current study, the yield and horticultural performance of 6 breeding lines and 3 commercial tropical pumpkin cultivars (La Estrella, Soler and Taina Dorada) was evaluated under conventional practices at two locations in Florida (Homestead and Live Oak) U.S. Data was collected on flowering time (days to male or female flowering), fruit size, fruit shape, disease/insect tolerance, and yield. At harvest, representative fruits per plot per cultivar were processed to determine fruit quality attributes including, flesh color, flesh thickness and degree brix. Data was analyzed using the GLM procedure of SAS. The average fruit yield per plant ranged from 1.0 to 3 fruits/ plant, while the mean yield per acre across locations ranged from 6,760 lb/ acre to 41,760 lbs/ acre. Among the small size pumpkins (<6lb), UFTP8 had the highest yield followed by UFTP22, while for medium-large size pumpkins (>6lb), UFTP38, UFTP42 and Taina Dorada had superior yield. UFTP8 and UFTP22 showed the best resistance to silverleaf disorder, while Soler and UFTP57 were the most susceptible. Brix values ranged from 3.7 to 7.7, and was highest in UFTP8 and UFTP24, but least in Soler. Generally, the results from this study indicate wide variation in yield and horticultural performance among the germplasm evaluated. The work described here provides useful insights into the best cultivars for adoption by growers in Florida.

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FINE MAPPING *ETHQV8.*1 SUGGESTS A ROLE OF *ERF024* IN MELON CLIMACTERIC RIPENING

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Fruit ripening is an essential process in plant development as it allows seed dispersion at the end of the fruit development. It is also a crucial factor in fruit shelf life, making fruit ripening behavior one of the main goals in plant breeding. Ethylene is a hormone that plays a key role in fruit ripening, and together with the respiration pattern determines whether the fruit ripens as climacteric or non-climacteric. In melon (*Cucumis melo*) there are both climacteric and non-climacteric varieties, making it an ideal crop to study fruit ripening from a genomics point of view. By QTL mapping in a Recombinant Inbred Line population funded by the climacteric variety 'Védrantais' and the non-climacteric 'Piel de Sapo', we identified a major QTL in chromosome 8: *ETHQV8.1*, covering a region of 140 kb. *ETHQV8.1* was validated and fine-mapped using Introgression Lines (ILs) funded by the same parental lines and F2 populations, narrowing down the interval to a region of 5.8 Kb containing a single gene: MELO3C024520.2. Annotated as *Ethylene Responsive Transcription Factor 024* or *ERF024*, its expression was correlated with climacteric ripening behavior. In 'Védrantais', it had a peak of expression at 30 days after pollination (DAP), at the beginning of climacteric ripening, while in 'Piel de Sapo' it was barely expressed. We also observed an effect in the ILs, correlated with their ripening behavior. To validate *ERF024* as the causal gene of *ETHQV8.1*, gene editing using *CRISPR/Cas9* technique was performed. Two knock-out lines with nonsense mutations were obtained, and a preliminary phenotyping experiment suggested *ERF024* as the causal gene. In order to investigate the gene function, a DAP-seq experiment was performed to find the genes regulated by *ERF024,* together with a gene co-expression analysis based on RNA-seq data. A list of 26 genes were found being co-expressed with *ERF024* and having a binding peak in their promoter, considering them High Confident Targets (HCT). A Gene Ontology analysis of these HCTs revealed a significant enrichment in DNA structure-related terms, suggesting an important role of *ERF024* in the control of DNA structural changes associated with the transcriptomic switch happening during climacteric fruit ripening in melon.

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POPULATION DYNAMICS AND MANAGEMENT OF MELONWORM IN SOUTH FLORIDA

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Melonworm, *D. hyalinata* L., is a serious foliage-feeding pest of cucurbits, which causes huge annual economic losses to cucurbit crops. To develop effective management strategies, its biology and population dynamics were studied on four cultivars of cucurbits: yellow squash, zucchini, cucumber, and watermelon in Homestead, FL, during 2014. Population density of *D hyalinata* ranged from a maximum of 6.58 ± 0.14 larvae per 2 leaves (± SE) during September 2014, when temperatures were relatively large, to a low population density in December 2014 (1.25 ± 0.04 larvae per 2 leaves; ± SE) when temperatures were relatively low. *D. hyalinata* distributions tended to be aggregated during crop-growing periods in May 2014, June-July 2014, and September 2014, when temperatures were relatively large, but had uniform distributions in December 2014, when temperatures were relatively low. Studies on the oviposition and larval preferences of *D. hyalinata* on four cucurbit cultivars showed that yellow squash was the most preferred host and watermelon was least preferred. The preference level for yellow squash by melonworm did not differ greatly from zucchini. The preference of melonworm for different on-plant locations was also investigated and showed that the middle of the plant was most preferred for oviposition. However, largest numbers of melonworm larvae were found on the bottom of the plant, but the top was the most highly defoliated part. There were small larvae than medium or large larvae in the field of cucurbit crops. Survival of melonworm larvae was lowest when reared on yellow squash, but largest on watermelon. However, larva reared on watermelon required more time to develop than on other crops with development time shortest on yellow squash. A larger head capsule width occurred when larvae were reared on yellow squash than on watermelon. However, whole-body length, pupal weight, and pupal body dimensions showed little or no differences among melonworm larvae reared on the four host plant species. This study also developed management program for controlling this pest.

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BSA-SEQ APPROACH REVEALS CPCUC2 AS A NAC-LIKE TRANSCRIPTION FACTOR REGULATING FLOWER DEVELOPMENT IN *CUCURBITA PEPO*

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It is well known that ethylene is the main regulator of both sex determination and sex expression in cucurbit species, controlling the fate of the floral meristem towards a female or a male flower. Using a direct genetic approach, we screened an EMS mutant collection in the zucchini line MUCU16, identifying a mutant (*cuc2*) with delayed transition to female flowering and a very high number of male flowers per plant. Mutant plants also show multiple duplications of flowers (two flowers per node instead of one) and floral organs (more than five petals and sepals), as well as increased serration of leaf boundaries. Mutant plants were crossed with the scallop line VCU196, and backcrossed with MUCU16 to generate F2 and BC1S1 segregating populations. The causal mutation of the phenotype was found after resequencing two DNA bulks from WT and mutant plants in F2 segregating populations. The QTLseq analysis in F2 (MUCU16 x VCU196) allowed the identification of six candidate genomic regions on chromosomes 2, 5, 6 and 7, although the most prominent region was that on chromosome 6. After selecting EMS canonical mutations with a contrasting allele frequency in the WT and mutant bulks of the BC1S1 population, a C>T transition in the coding region of a *CpCUC2* gene (Cp4.1LG06g06610) was detected on chromosome 6, causing the substitution S230F in a very conserved residue of the deduced protein. The mutation cosegregated with the mutant phenotype in 360 segregating plants from BC1S1 generation. *CpCUC2* is highly homologous to Arabidopsis *AtCUC2*, a transcriptional activator of *NAC* gene family that are required for apical meristem formation, leaf serration and initiation of axillary meristems, regulating thereby plant architecture.
The *cuc2* mutation downregulated the expression of ethylene biosynthesis and signalling sex-determining genes, which suggests that the function of *CpCUC2* on sex expression is mediated by ethylene.

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INSECT EXCLUSION MATERIALS AND REMOVAL TIMINGS FOR MANAGING PESTS AND POLLINATORS IN SUMMER SQUASH

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Squash bug *Anasa tristis* (DeGeer) (SB), is a key pest of cucurbits. Use of crop exclusion covers is suggested for protecting summer squash crops from this pest. With this method, pollinator access must be considered and detailed information on managing pests and pollinators is limited. To address this, trials were conducted at Lane, OK to research cover materials and removal timings for summer squash. There were 6 treatments (T). Uncovered treatments were (T1) without or (T2) with insecticide (acetamiprid applied 13 July). Materials tested were 0.5 oz spunbond polyester fabric (T3 and T4) and a polypropylene mesh (T5 and T6). Removal timings were 2 weeks (T3 and T5) and 3 weeks (T4 and T6) after 50% of squash plants had a first female flower. Yellow squash *Cucurbita pepo "Lioness"* was planted 9 June 2021 and plants covered (T3-T6) once emergence was complete. On 8 July 50% flowering occurred and fruits maturity began one week later. Fruits were harvested several times weekly over 42 days and numbers of marketable fruit determined. Greatest overall yields were for T2 and T3. Delay of cover removal by a week reduced yield 22% to 35% for T4 and T6, respectively. Yields were 27% lower in T1 than in T2. Viewed over 4 ten-day harvest periods (P), for P1 only T1 and T2 produced fruits. For P2, yields were comparable for T1-T3 and T5 was 44% lower than T3. For later harvest periods, T1 yields were lowest but there were no significant differences among T2 and the covered treatments. Plant examination found that both cover types effectively excluded SB, which appeared soon after cover removal. T1 yield reductions suggest that SB is a causal factor. Fabric covers delayed fruit production without an overall reduction. Possible applications of this information to production systems will be discussed.

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DISCOVERY AND DEPLOYMENT OF GENOMIC RESOURCES FOR BREEDING RESISTANCE TO POTYVIRUSES IN SQUASH

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Aphid-transmitted potyviruses pose a major threat to squash (*Cucurbita spp.*) production worldwide. Breeding cultivars resistant to potyviruses is essential for complementing integrated disease management strategies. Resistance to Zucchini Yellow Mosaic Virus (ZYMV) and Papaya Ringspot Virus (PRSV-W) is available in unimproved accessions of *C. moschata*, Nigerian Local and Menina. Introgression of resistance into elite cultivars can be facilitated through marker-assisted selection (MAS) however; quantitative trait loci (QTLs) and efficient markers are currently unavailable readily. In the current study, the QTL-seq method was deployed to identify QTL and markers linked to PRSV-W and ZYMV resistance using three F_2 populations derived from crossing Nigerian Local (R) x Butterbush (S) and Menina (R) x Waltham Butternut (S). For PRSV-W, a single QTL associated with resistance was identified on Chromosome (Chr) 9 (*QtlPRSV*-C09) in Nigerian Local, while four QTLs were significantly associated with resistance in Menina on Chr 6 (*QtlPRSV-C06A* and *QtlPRSV-C06B*) and 12 (*QtlPRSV-C12A* and *QtlPRSV-C12B*). For ZYMV resistance in Nigerian Local, four QTLs were detected on Chr 2 (*QtlZYMV-C02*), 4 (*QtlZYMV-C04*), 8 (*QtlZYMV-C08*), and Chr 20 (*QtlZYMV-C20*). Forty-four Kompetitive allele specific PCR (KASP) markers were developed from the identified QTL regions. Seven KASP markers significantly associated with resistance to PRSV-W and ZYMV across the QTL regions were identified by testing association in the F_2 populations and accessions of diverse genetic background. These markers are currently in use to facilitate introgression of PRSV-W and ZYMV resistance from Nigerian Local and Menina into germplasm of diverse genetic background, including *C. moschata* (winter squash) and *C. pepo* (summer squash).

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GRAPH-BASED PAN-GENOME OF THE *CITRULLUS* GENUS PROVIDES INSIGHTS INTO WATERMELON EVOLUTION AND DOMESTICATION

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Breeding of watermelon (*Citrullus lanatus* subsp. *vulgaris*) has suffered from the narrow genetic base of the cultivated type. Although wild watermelons have been used in disease-resistance breeding, this is hindered by the lack of genomic information of the wild species, which further limits the discovery of molecular markers, especially structural variants (SVs), underlying important traits. To comprehensively explore all genetic elements in wild and cultivated watermelons, a graph-based pan-genome is being constructed by *de novo* assembling reference-grade genomes of over 100 accessions representing all seven *Citrullus* species. Novel genes absent from previously published genomes are being discovered by genome comparison and further investigated for their potential roles in disease resistance and fruit development. The identified SVs are further genotyped in a broader range of resequenced accessions to characterize their roles in watermelon evolution and domestication. Candidate SVs and genes associated with agronomic traits are being identified by selective sweep and genome-wide association analyses using the graph-based pan-genome as the reference. These trait-associated SVs and genes can be utilized in genomic-assisted breeding. The graph-based *Citrullus* pan-genome, novel genes, and the genetic variation map provide extensive genomic resources for future biological research and breeding. Furthermore, the divergence times between *Citrullus* species are being estimated with higher robustness enabled by the less biased sampling and the whole-genome gene tree, and the domestication event is being dated via the effective population size estimation. These results improve our understanding of watermelon evolution and domestication.

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THE US PROCESSING CUCUMBER GENOME ASSEMBLY GY14V2.0

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A genome assembly as complete as possible is essential for many biological studies and applied research. Cucumber (*Cucumis sativus* L.) is an important vegetable crop with a relatively small genome that is ~367Mbp. Among major horticulture crops, cucumber was the first with a publicly available draft genome released in 2009. Since then, genome assemblies with a few cucumber genotypes have been published with varying degrees of genome coverage, and sequence continuity. Here, we reported development of an improved genome assembly for the US processing cucumber inbred line Gy14. The genome was sequenced with the PacBio Single Molecule Real-Time (SMRT) sequencing technology at 48x coverage. From ~1.2 million reads (mean read length: 15,101 bp), 762 primary contigs were generated with the FALCON and CANU assemblers totaling 238 Mbp sequences with an N50 size of 8.6 Mbp. The contigs were oriented and anchored to the chromosomes by alignment against six high-density genetic maps. Scaffolding and super-scaffolding were achieved with BAC (bacterial artificial chromosome)-end and FOSMID-end sequencing, BioNano (optional mapping) and Hi-C technologies. Multiple rounds of polishing were performed before genome annotation. The final assembly of Gy14v2.1 contained a total of 258,543,391 bp sequences (70.3% genome coverage) in 626 scaffolds with N50 and N90 of 33.3 Mbp and 1.48 Mbp, respectively. Of the 258.5 Mbp, 231 Mbp in 22 super-scaffolds were anchored and oriented on the seven chromosomes. Appropriately 35.5% of the assembly are repetitive DNA sequences with the long terminal repeats (LTR, mainly Copia and Gypsy types) the most abundant, which account for ~17.5% of the assembly. In total, 22,626 protein-coding genes were annotated with a BUSCO (Benchmarking Universal Single-Copy Orthologs) completeness score of 97.5%. As compared with published cucumber genome assemblies, Gy14v2.1 seems to have the highest genome coverage. The additional sequences added are in centromeric or heterochromatic regions of each chromosome. In this presentation, comparison of main genome features of Gy14v2.1 and other cucumber or cucurbit assemblies will be made. Issues with current cucumber genome assemblies will be discussed. Examples will be also be given on use of the newest Gy14 assembly in various studies. The improved Gy14 genome assembly is publicly accessible (https://www.cucurbitgenomics.org/v2).

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SEASONAL PREVALENCE AND SPREAD OF WHITEFLY-TRANSMITTED VIRUSES IN WESTERN UNITED STATES MELON PRODUCTION REGIONS

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Whitefly-transmitted viruses have become an increasing problem for melon production in the western United States and are now common in all western production regions where whitefly (*Bemisia tabaci* MEAM1) populations exist. Viruses common in western regions now include cucurbit yellow stunting disorder virus (CYSDV; Crinivirus, Closteroviridae), cucurbit chlorotic yellows virus (CCYV; Crinivirus, Closteroviridae), and squash vein yellowing virus (SqVYV; Ipomovirus, Potyviridae). CYSDV, CCYV and the aphid-transmitted cucurbit aphid-borne yellows virus (CABYV; Polerovirus, Solemoviridae) which is of marginal economic concern in the region, produce identical yellowing symptoms, and mixed infections of these viruses are common making it difficult to determine which viruses are present; this impacts resistance evaluations in the field. A recently developed diagnostic system involving single-step multiplex RT-PCR and RT-qPCR has been instrumental in differentiating and quantifying virus level in plants and has facilitated studies leading to the identification of these viruses in single and mixed infections in the California Central Valley as well as determination of seasonal differences in virus prevalence within Sonoran Desert production regions where these viruses have now co-existed for eight years. Surveys of spring and fall melon crops in the Sonoran Desert from 2019-2021 have demonstrated seasonal differences in virus prevalence, with CCYV the most abundant virus during spring seasons, and CYSDV the most abundant during the fall. Incidence of SqVYV and CABYV were consistently lower compared to CCYV and CYSDV. Surveys of melon samples from 2020-2021 in the Central Valley of California revealed low incidence of CYSDV and CCYV and a much higher incidence of CABYV in 2020; however, in 2021, all fall melons sampled were found to be infected with CYSDV. Continued monitoring of virus establishment in the Central Valley will aid in determining the impact of these newly introduced viruses on this important melon production region.

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PAN-GENOME OF WILD AND CULTIVATED WATERMELONS

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Watermelon (*Citrullus lanatus* subsp. *vulgaris*) is among the most important vegetable crops in the world. Wild relatives, including the direct progenitor of cultivated watermelon, are important resources for breeding and understanding watermelon domestication. To maximize the capture of genome variations within and among *Citrullus* species and to identify novel agronomically important genes to facilitate watermelon improvement, we generated high-quality reference genomes of wild watermelons, *C. lanatus* subsp. *cordophanus*, *C. mucosospermus*, *C. amarus* and *C. colocynthis*. Structural variations were identified among the watermelon reference genomes, some of which could affect local recombination and introgression. A *Citrullus* super pan-genome was constructed by integrating species-specific pan-genomes based on orthologous gene relationships. Through gene presence-absence variations analysis, we identified disease resistant genes that were lost in the cultivated watermelon, as well as those related to fruit quality that were possibly selected during domestication. This *Citrullus* pan-genome resource will enable us to explore genes in the wild relatives that underlie disease resistance and other important horticultural traits to enhance the efficiency of watermelon breeding.

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CUCURBIT GENOMICS DATABASE (CUGENDB) V2: AN UPDATED DATABASE FOR CUCURBIT GENOMICS

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The Cucurbitaceae family (cucurbit) consists of about 965 species in more than 90 genera, which include several economically important and popular fruit and vegetable. Currently, cucurbit genomes have been generated for more than 20 species, and during the past several years, variome and transcriptome profiling data have been rapidly accumulated for cucurbits. To efficiently mine, analyze and disseminate these large-scale datasets, we have developed an updated version of Cucurbit Genomics Database (CuGenDB) using the improved Tripal toolkit. The updated database, CuGenDBv2, currently hosts 33 genomes from ~25 cucurbit species/subspecies. Protein-coding genes from these genomes have been comprehensively annotated by comparing their sequences to various public protein and domain databases. Synteny blocks between any two and within each of the 33 genomes, representing a total of 561 pair-wise genome comparisons, have been identified and can be visualized in CuGenDBv2. In addition, all publicly available RNA-Seq raw reads of cucurbit species have been retrieved from NCBI Sequence Read Archive (SRA), processed and aligned to the corresponding genomes to derive gene expression values. An updated "Expression" module has been developed to facilitate the retrieval of these gene expression data. Furthermore, a novel "Genotype" module has been developed to mine the variome data (functionally annotated SNPs and small indels) from large-scale genome resequencing and genotyping-by-sequencing projects. CuGenDBv2 can be accessed via http://cucurbitgenomics.org/v2, and will be updated regularly.

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USING POLLEN EMS MUTAGENESIS TO GENERATE MUTATION FOR CUCURBIT GENETICS，GENOMICS AND CROP IMPROVEMENT

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Due to long-term cultivation and selection for yield and fruit quality traits, cultivated cucurbit crops are low for genetic diversity. More genetic variation is needed to enable the development of innovative products for sustainable production. Ethyl methane sulfonate (EMS) is most widely used for creating genetic mutation and variation. Although many attempts have been made to mutagenesis cucurbits by conventional EMS treatment of seed, no saturated mutation library has been created. In this study, we developed a pollen EMS mutagenesis protocol for cucurbit crops. Using the optimized protocol we constructed high-quality mutation libraries of watermelon, butternut squash and cucumber, and obtained a large number of mutants with phenotypic variation for each crop. In the case of watermelon, G42, an elite watermelon inbred line, was selected for EMS mutation library construction because of its high-quality fruits with early maturity, durable rind, stable fruit set under diverse environments and available telomere to telomere gap-free genome sequence. Over 200,000 M1 seeds from G42 were generated. A total of 223 observable phenotypic mutants were identified with the mutation frequency of 3.64% in 6,126 M1 plants. Among 507 M2 families, 78 (15.38%) had observable phenotypic changes. Phenotypic variation occurred across all watermelon developmental stages. To analyze the genome-wide distribution of EMS-induced mutations, 39 individuals, including seven wild-type, 20 M1 and 12 M2 plants, were sequenced. The results show that the average density of mutation is 1 SNP/1.69 Mb and 1 indel/4.55 Mb per M1 plant, and 1 SNP/1.08 Mb and 1 indel/6.25 Mb per M2 plant. Genes of an elongated fruit shape and a dominant male sterility were quickly identified using the gap-free genome. Different type of mutations was also observed and are being evaluated in squash and cucumber. The mutants generated by pollen EMS mutagenesis will greatly enhance cucurbit genetics, genomics and improvement.

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IDENTIFICATION AND CHARACTERIZATION OF GENETIC RESISTANCE TO CUCUMBER GREEN MOTTLE MOSAIC IN CUCUMBER AND WATERMELON

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Cucumber green mottle mosaic virus (CGMMV) in the genus Tobamovirus causes significant damage to cucurbit crop productions around the world. CGMMV is a seed-borne pathogen, stable in the environment and is highly contagious in the field and in greenhouse conditions, through plant handling and mechanical means. CGMMV infection causes leaf mottling and fruit decay on infected cucumber or watermelon plants, resulting in serious crop losses. Breeding for resistance is the most effective approach to manage the CGMMV infection. Our present study focuses on screening and identification of genetic sources of resistance to CGMMV in cucumber and watermelon for genetic evaluation and genomic analysis. Three of the 50 cucumber inbred lines that we screened were tolerant to CGMMV infection. Segregating populations derived from crosses with these three lines are being phenotyped for CGMMV tolerance. Disease symptoms were scored using severity disease index, with a rating of 0-4, with 0 being healthy with no symptom and 4 being severely infected with prominent symptoms (mosaic, mottling). Serological tests (enzyme-linked immunosorbent assay) were used to assess the virus infection on asymptomatic plants. Similarly, watermelon population was developed between a previously identified susceptible and resistant parent of *Citrullus colocynthis*. Subsequent F1 and F2 populations were assessed for CGMMV infection and phenotyped using the standard rating of 0-4. Bulk segregant analysis (BSA) was conducted to identify potential SNPs associated with tolerance to CGMMV infection. The putative SNPs identified using BSA are being validated in segregating populations derived from crossing the tolerant accessions with susceptible cultivars.

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