

Cucurbitaceae 2018
Conference abstracts

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ABSTRACTS

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Conference Session Topics

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Biotic Stress (BS)

Biotic Stress (BS)

Aphid-Triggered Immunity in Melon / Key Determinants for Durable Resistance to Virus and Aphids

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The *Vat* gene in melon is unique in conferring resistance to both *A. gossypii* and the viruses it transmits. This double phenotype is controlled by a cluster of genes including a CC-NLR which has been characterized in detail. Copy-number polymorphisms (for the whole gene and for a domain that stands out in the LLR region) and single-nucleotide polymorphisms have been identified in the *Vat* cluster. The *Vat* gene structure suggests a functioning so called effector-triggered immunity (ETI), with separate recognition and response phases. During the recognition phase, the VAT protein is thought to interact (likely indirectly) with an aphid effector introduced by aphid salivation within the plant cells. A few hours later, several miRNAs are upregulated in *Vat* plants. Peroxidase activity increases, and callose and lignin are deposited in the walls of the cells adjacent to the stylet path, disturbing aphid behavior. In aphids feeding on *Vat* plants, the levels of miRNAs are modified. At the plant level, resistance to aphids is quantitative (aphids escape the plant and display low rates of reproduction). 'Aphid-ETI' has qualitative and local effect against non-circulative viruses *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV) and but quantitative effect against circulative virus such as *Cucurbit aphid-borne yellows virus* (CABYV). Durability of ETI is highly variable. At the population level, 'Aphid-ETI' reduces aphid density and genetic diversity, and durability of the 'Aphid-ETI' strongly depends on the agro-ecosystem. Some clones are adapted to *Vat* resistance, putatively either by introducing a polymorphic effector not triggering ETI, or by adapting to the defenses they triggered. Several ways to enhance 'Aphid-ETI' durability will be proposed. 'Aphid-ETI' against viruses decreases the intensity of CMV and CABYV epidemics. Laboratory experiments strongly suggested that non circulative viruses cannot adapt to 'Aphid-ETI' and therefore durability of 'Aphid-ETI' against CMV is predicted long. Field experiments combined to modelling suggested that highly durable resistance against CABYV could be obtained by combining within a same melon genotype the *Vat* gene with recessive genes conferring resistance to CABYV. Extension of those results to any other circulative viruses will be discussed.

Session Topic

Biotic Stress (BS)

Biotic Stress (BS)

Genomic Characterization of *Xanthomonas cucurbitae*, the Causal Agent of Bacterial Spot Disease of Cucurbits

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Plants in the Cucurbitaceae family comprise several economically-important vegetable and fruit crops including pumpkin, squash, zucchini, cucumber, melon, and watermelon. In 2017, world production of pumpkins, squash, and gourds exceeded 24 million tons worth more than \$4 billion, and U.S. production of pumpkins, squash, and gourds was worth \$370 million. Illinois farmers are responsible for 40% of total pumpkin production and 85% of the processing pumpkins grown in the U.S., making it a top vegetable commodity in Illinois. Economically important diseases, including bacterial spot on cucurbits, are a problem globally and particularly in Illinois where pumpkin fields can have both high disease incidence and extensive (up to 90%) yield losses. Bacterial species in the genus *Xanthomonas* can cause bacterial spot disease on a variety of plants. In recent years, bacterial spot disease caused by *X. cucurbitae* has spread throughout the U.S. and globally, and has become an important bacterial diseases of cucurbit plants. Large-scale genomic studies of other *Xanthomonas* species has increased our general understanding of virulence mechanisms, evolution, and host specificity factors of these phytopathogens. However, as bacterial species in this genus have many host-specific factors essential to their virulence functions, and as our current genetic and genomic knowledge of *X. cucurbitae* is limited, a high-quality reference genome would significantly aid in characterizing this particular bacterial species. Since the *X. cucurbitae* strain ATCC 23378 has been utilized in many previous studies, we used this strain to create a *X. cucurbitae* reference genome. We employed Oxford Nanopore long-read sequencing technology along with Illumina paired-end short-read sequences to generate a high-quality hybrid genome assembly. The assembly consists of a single, circular, 4.6 Mbp chromosome and one 14 kb plasmid. Initial gene prediction using Prokka estimated approximately 4,000 genes, and characterization of several genes of interest, include one TAL (transcription activator-like) effector, are currently in progress. Additionally, we are using RAD-seq to evaluate population genomics of field isolates from the Midwest region, and we plan to expand our analysis to include strains from different regions of the U.S. isolated from different cucurbit host plants.

Session Topic

Biotic Stress (BS)

Biotic Stress (BS)

Cucurbit Rootstocks Resistant to Fusarium Wilt of Watermelon Retain Resistance When Co-Infected by Southern Root-Knot Nematode

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The interspecific hybrid squash (*Cucurbita maxima* × *C. moschata*) rootstock ‘Carnivor’ is resistant to *Fusarium oxysporum* f. sp. *niveum* (FON) but susceptible to *Meloidogyne incognita*, the southern root-knot nematode. A new citron (*Citrullus amarus*) rootstock ‘Carolina Strong Back’ is resistant to FON and *M. incognita*. The objectives of this study were to determine if an interaction between *M. incognita* and FON race 2 occurred on ‘Carnivor,’ ‘Carolina Strong Back,’ or watermelon ‘Fascination,’ which is susceptible to FON race 2. In 2016 and 2018 field experiments, plants of non-grafted ‘Fascination’ and ‘Fascination’ grafted onto ‘Carnivor’ and ‘Carolina Strong Back’ were inoculated with one of four pathogen treatments: no pathogens, *M. incognita* alone, FON alone, or both pathogens. In both years, 20 g wheat grain colonized by FON was added to the transplanting holes, while in 2018, an additional 10 g was applied per 0.3 m of row in infested plots. *M. incognita* was applied as 2000 eggs (2016) or eggs plus juvenile nematodes (2018) to seedlings before transplanting. After 9 weeks, incidence of Fusarium wilt and area under the disease progress curve did not differ when hosts were inoculated with FON alone or with FON and *M. incognita* together. Plants not inoculated with FON did not wilt. Fusarium wilt was greater on nongrafted watermelon (78% incidence) than on both grafted rootstocks and lower on ‘Carnivor’ (1%) than on ‘Carolina Strong Back’ (12%) ($P \leq 0.05$). In 2016 after 16 weeks, ‘Carnivor’ had a greater percentage of the root system galled than the other two hosts, and watermelon had more galling than ‘Carolina Strong Back.’ In conclusion, cucurbit rootstocks that are susceptible and resistant to *M. incognita* retain resistance to FON when they are co-infected with *M. incognita*. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Biotic Stress (BS)

Biotic Stress (BS)

Cucurbit Powdery Mildew Population Virulence Variation – a Complex View from a Global Perspective

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Cucurbit powdery mildew (CPM) is mainly caused by two obligate ectoparasites, *Golovinomyces orontii* s.l. (Go) and *Podosphaera xanthii* (Px), that are highly variable in virulence. Various independent systems of CPM race determination and denomination have been used in recent decades. We recently developed new tools to enhance research of CPM virulence variation. Diversity models were applied to analyses of virulence variation of Go and Px populations (115 Czech isolates) from 2010 through 2012. Diversity within and distances between Go and Px populations and each other in spatio-temporal context and with regard to original host plant species were analyzed, based on virulence patterns of individual isolates (races) on a set of 21 melon (*Cucumis melo* L.) race differentials. Significant differentiation among the Go and Px pathogen populations was revealed. The results clearly demonstrate that the set of differential *C. melo* genotypes is well composed because of significant differentiation capacity for both species. There were no significant differences between Go isolates from different host plant species due to high variability, but there was significant host-specific differentiation among Px isolates. This approach was used to evaluate virulence variation of CPM populations in other European countries, South Africa and Asia (Thailand). Preliminary results obtained for Px showed enormous virulence variation in other European countries outside of Czech Republic, as well as in South Africa. Px isolates from Netherlands that originated from wild *Cucumis* species, grown in greenhouse however expressed lower virulent variation than the isolates from other countries. A Px isolate from *Momordica charantia* in Thailand differed in virulence compared with isolates from other countries. The approach used in this study provides revealed complex virulence structures of CPM populations of diverse origins, and when completed by race determination and denomination on melon, it may serve as a base to understand virulence variation of both CPM species on a global perspective.

Session Topic

Biotic Stress (BS)

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Pathotypes and Races of *Pseudoperonospora cubensis* – Different Concepts of Virulence Differentiation

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Host-parasite interactions between Cucurbitaceae and *P. cubensis* exhibit significant variation. This contribution reviews the current state of knowledge regarding characterization of *P. cubensis* virulence variation on the level of pathotypes (variation in host genera and species host-range) and races (variation in intraspecific level). However, our knowledge of the interactions between *P. cubensis* isolates and the most important genera and species of cultivated cucurbits is limited. The concept of pathotypes identification was introduced in 1980s with some modifications later. The former concept was missing some crucial requirements to be applied and comparable internationally. It was the reason why an improved differential set of six cucurbit genera and 12 genotypes (*Benincasa*, *Citrullus*, *Cucumis*, *Cucurbita*, *Lagenaria*, and *Luffa*) was developed to characterize pathotypes among *P. cubensis* isolates, and is broadly used. This concept allows application of various mathematical approaches for virulence comparison. Long-lasting research of interactions between cucurbits (*Cucumis* spp., *Cucurbita* spp.) and *Pseudoperonospora cubensis* demonstrated frequent expression of race-specific reaction patterns. The differential set of 21 genotypes of *Cucumis melo* was developed for determination of cucurbit powdery mildew races. 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. The approach of how to determine races of *P. cubensis* is discussed and demonstrated. Utilization and combination of both approaches (establishment of pathotypes and races) are important for science as well as for practical application in cucurbit resistance breeding.

Session Topic

Biotic Stress (BS)

Biotic Stress (BS)

Resistance to Fungicides in Cucurbit Powdery Mildew in Europe

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One hundred cucurbit powdery mildew (CPM) isolates (41 *Golovinomyces orontii* s.l. [Go], 59 *Podosphaera xanthii* [Px]) from the Czech Republic (2012 to 2015), were screened for fungicide efficacy to the six frequently used fungicides (quinoxifen /Atlas 500 SC/, propiconazole /Bumper 25 EC/, fenpropimorph /Corbel/, dinocap /Karathane LC/ azoxystrobin /Ortiva/) and penconazole /Topas 100 EC/). Fungicide efficacy was determined by a modified leaf-disc bioassay with three concentrations. Highly susceptible *Cucumis sativus* 'Stela F₁' was used for preparation of leaf discs. Efficacy of fungicides towards screened CPM isolates varied significantly. There were observed also differences in efficacy of some fungicides between both CPM species, and as well as, among years. In the case of quinoxifen, propiconazole, fenpropimorph and penconazole, there have been no reports from Czech Republic since the year 2012. Fenpropimorph was 100% effective and showed some phytotoxicity to *C. sativus* 'Stela F₁' leaf discs. Propiconazole was also highly effective; the same phenomenon was recorded for penconazole, however only for Go during the studied period. Nevertheless, efficacy of penconazole has decreased since 2014 when frequency of Px strains with moderately resistant reactions to the lower and recommended concentrations increased. The highest number of various reaction patterns of CPM populations was observed in response to quinoxifen. There was recorded decreased efficacy, except the year 2015, when a majority of CPM isolates were controlled by recommended concentration. Dinocap showed high efficacy and the majority of screened CPM isolates expressed sensitive reaction to recommended concentration. However, there were also observed occurrence of strains (Go, Px) with moderately resistant or resistant responses to screened concentrations. This phenomenon reflects the situation in Czech CPM populations from 2001 to 2011. Azoxystrobin showed low efficacy, this situation started in 2007 (1st year of study), when a continual shift towards prevalence of azoxystrobin-resistant strains in Czech CPM populations was observed (Lebeda et al. 2010a,b; Sedláková et al. 2017).

Session Topic

Biotic Stress (BS)

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Cucumber Green Mottle Mosaic Virus: Seed Transmissibility, Seed Health Assays and Screening Watermelon Germplasm for Disease Resistance

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Cucumber green mottle mosaic virus (CGMMV), a tobamovirus in the family *Tobamoviridae*, seriously affects cucurbit crop productions around the world, with its epidemics in Asia and Europe, and as an emerging disease in North America and Australia. This seed-borne virus is very contagious and poses serious threat to all types of cucurbit crops, including cucumber, melon, watermelon, and squash. Planting clean and certified CGMMV-free seeds is an important measure in disease prevention. Currently, there is no standard method of seed health assay and no known resistant genetic materials that are available in watermelon. In the present study, we investigated the nature of seed transmissibility and compared various molecular and serological methods for CGMMV detection. In an effort to breed watermelon for resistance, we evaluated the USDA watermelon germplasm (1,486) for resistance to CGMMV. Using a Canadian isolate of CGMMV (in Asian genotype), through mechanical inoculation on seedlings and symptom observation, plants from seven accessions with putative resistance (tolerance) to CGMMV were selected. A repeat screening was conducted using seedlings generated through self-pollination of selected plants, resistance (tolerance) to CGMMV was confirmed on those *Citrullus colocynthis* lines, but not *C. lanatus* lines. Those CGMMV-tolerant *C. colocynthis* plants had no apparent visible symptom, but virus-titers could be detectable using lab tests. The genetic materials from those advance-selected *C. colocynthis* lines could be useful for breeding watermelon cultivar or rootstock with resistance to CGMMV. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic
Biotic Stress (BS)

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Molecular Diagnosis and Characterization of *Cucumber green mottle mosaic virus* (G: Tobamovirus, F: Virgaviridae) Infections on Bottlegourd (*Lagenaria siceraria* L.)

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During the year 2017-18 the symptoms of green mottling on leaves, vein banding, leaf distortion and green patches were notified on bottlegourd fruits with 60-70% incidence. Further, the infected leaf samples of bottlegourd were collected; diagnosed and characterized through Transmission Electron Microscopy (TEM), Atomic Force Microscopy and RT-PCR analysis. The TEM results confirmed the presence of rigid rod virus particles. It was suspected that the pathogen might be *Tobamovirus*. RT-PCR analysis was conducted with *Tobamovirus* specific primer and results confirmed the infections by *Cucumber green mottle mosaic virus* (CGMMV) on bottlegourd. The sap transmission (Mechanical transmission) method was followed to screen the bottlegourd genotypes and to identify the host ranges of CGMMV. Under glass house conditions 27 genotypes of bottlegourd were screened for resistance against CGMMV; none of them found resistant. Host range studies revealed that CGMMV was easily transmitted to squash, pumpkin and cucumber. Currently, CGMMV is designated as global pathogen under quarantine perspective due to its seed borne and highly contagious nature. Hence needs immediate attention to nab the pathogen at national level through quarantine measures and integrated disease management approaches.

Session Topic

Biotic Stress (BS)

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Role of Antioxidant Molecule Melatonin in Plant-Host Resistance and Pathogen Suppression in Cucurbits

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Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring low molecular weight indole-based metabolite and serves as an antioxidant molecule in various plants and animals. Melatonin, as an animal neurohormone has multi-regulatory effect on patients suffering from insomnia, cancer, Alzheimer's and other neurobiological disorders. In plants, melatonin plays a wide and diverse range of cellular and physiological functions including plant growth and development, and is inducible in response to diverse biotic and abiotic stresses. However, studies on the direct role of melatonin in disease suppression and as a signaling molecule in host-pathogen defense mechanism are lacking. Our study provides insight into the conserved nature of the biosynthetic pathway of melatonin in watermelon (*Citrullus lanatus*) and how exogenous application of melatonin, an environmental-friendly immune inducer, can boost plant immunity and suppress pathogen growth. Melatonin (1mM) applied as a spray suppressed powdery mildew development on various cucurbit leaves and *Phytophthora* fruit rot development on cucumber. Watermelon plants transformed with the melatonin biosynthetic gene SNAT (serotonin N-acetyl transferase) from a powdery mildew resistant plant also helped reduce PM development compared to non-transformed controls. Increased melatonin levels in plants were found to boost resistance against the foliar pathogen *Podosphaera xanthii* (powdery mildew). Our data also suggests there is subcellular exchange/flow of melatonin intermediates between cytoplasm and chloroplast during melatonin biosynthesis in watermelon. Further, transcriptomic data on melatonin sprayed (1mM) watermelon leaves, suggests that melatonin alters the expression of genes (*Pathogenesis related protein PR1a*, receptor kinases, NAC domain TFs, Elicitor-responsive protein 3) involved in both PAMP (pathogen-associated molecular pattern) and ETI (effector-triggered immunity) mediated defenses in a salicylic acid (SA) dependent pathway. Developing strategies by using CRISPR/Cas9 genome-editing method to increase melatonin levels in specialty crops such as watermelon and other cucurbit crops can have dual effect: (a) enhanced disease resistance against diverse plant pathogens, (b) serve as a source of natural antioxidant molecules for human nutrition. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic
Biotic Stress (BS)

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Inhibitory Effects of Cucumber Age-Related Resistance to *Phytophthora capsici* Manifest Within 24 Hours of Inoculation

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Cucumber (*Cucumis sativus*) fruit are largely susceptible to infection by *Phytophthora capsici*. However, some cucumber cultivars develop a fruit surface-associated age-related resistance (ARR) to *P. capsici*. Young, rapidly growing fruit are highly susceptible, but become resistant as they complete exponential growth [~16 days post-pollination (dpp); 2-3 weeks prior to ripening]. Prior transcriptomic and metabolomic comparisons of peel of ARR expressing and non-expressing uninoculated fruit identified changes associated with resistance possibly functioning as preformed defenses. Here we performed scanning electron microscopy and transcriptomic analyses of inoculated fruit at resistant (16 dpp) and susceptible (8 dpp) ages, providing a unique opportunity to examine compatible and incompatible interactions in the same genotype. Strong transcriptional changes were observed at 4 hours post inoculation (hpi), with approximately 2000 genes differentially expressed in either age. Gene Ontology term enrichment analysis of upregulated genes at 4 hpi revealed both common (including response to wounding, response to oxidative stress, defense response) and unique responses. In addition to the mutually upregulated defense genes at 4hpi, several other resistance-associated genes were uniquely upregulated in resistant fruit. At later time points the transcriptional responses markedly diverged. At 24 and 48 hpi, susceptible 8 dpp fruit continued to mount defense along with strong downregulation of genes involved in photosynthesis, cell wall synthesis and modification, lipid and cuticle biosynthesis, cell division and growth. In contrast, resistant 16 dpp samples largely downregulated defense responses while upregulating photosynthesis and other biological processes. Further investigation of the transcriptome network dynamics and pathogen development during the first 24 hours of infection (0, 2, 4, 8, 12, 18, 24 hpi) is underway. Scanning electron microscopy of resistant peels showed evidence for infection failure as early as 4 hpi, including aberrant long germ tubes, and ungerminated, deflated and/or disintegrated spores and hyphae, that were not observed on susceptible fruit. Together these results suggest that in ARR-expressing fruit, a successful defense is mounted within the first 24 hours.

Session Topic

Biotic Stress (BS)

Biotic Stress (BS)

Diversity and Pathogenicity on Watermelon of Six New Fungal Species in the Family Stachybotriaceae

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The fungal family Stachybotriaceae includes 210 species in 33 genera with high genetic diversity. Although most species from this family are saprophytic, some are plant pathogens with relatively wide host ranges. One member of this family, *Myrothecium roridum*, has been reported to cause leaf lesions on watermelon, but due to a lack of molecular identification, the species may have been misidentified. A total of 92 isolates from lesions on watermelon leaves was collected in five fields in South Carolina throughout three seasons from 2015 to 2017. In fall 2016 symptomatic leaves were found in 5 of 11 fields, and leaf lesions caused by Stachybotriaceae were the most prevalent disease in two fields (present on 68 and 43% of leaves). For all isolates, partial gene sequences were determined for *cmdA*, ITS and *tub2*. Based on these sequences, six species, *Albifimbria verrucaria*, *Gregatothecium humicola*, *Paramyrothecium foliicola*, *Paramyrothecium humicola*, *Xenomyrothecium tongaense* and *Xepicula leucotricha*, were identified. *G. humicola* was most common, followed by *P. foliicola*, whereas the other species occurred infrequently. In phylogenetic trees based on Bayesian inference, the South Carolina isolates grouped with the corresponding species reported previously (Lombard et al., 2016, *Persoonia* 36:156). In a greenhouse pathogenicity test, plants of watermelon 'Tri X-313' were inoculated with suspensions of 1×10^6 conidia/ml prepared for one isolate of each species. Water-sprayed plants were used as controls. Plants were held at approx. 95% RH and 25°C for four days and then transferred to greenhouse benches. Disease incidence and severity were rated after seven days. The test was repeated once with twice the inoculum concentration. All six species caused leaf lesions on watermelon and were recovered successfully. There were few significant differences in disease severity among the six species, with the exception that *G. humicola* and *X. tongaense* were significantly more virulent than *X. leucotricha* ($P = 0.05$). *A. verrucaria* and *X. leucotricha* were only slightly pathogenic. This study is the first report of more than one species in Stachybotriaceae causing disease on watermelon, which might be an emerging disease under humid fall environments in the southeastern United States.

Session Topic
Biotic Stress (BS)

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Detection of the Gummy Stem Blight-Causing Pathogens (*Stagonosporopsis* spp.) in Watermelon, Using Three Species-Specific LAMP Assays

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Gummy stem blight (GSB) is one of the most important diseases of watermelon in the southeastern U.S. GSB is caused by three cryptic *Stagonosporopsis* species; *S. citrulli*, *S. cucurbitacearum* and *S. caricae*, which can infect most of the above ground parts of the watermelon plant. Since there is not currently resistance to GSB in commercial watermelon cultivars, its management relies on cultural practices and costly preventative fungicide applications. Furthermore, a difference in sensitivity/resistance for the most common fungicide chemistries among the three species imposes a serious challenge for its management. Efficient methods for pathogen detection and diagnosis are therefore required for appropriate management decisions. In this study, three species-specific loop-mediated isothermal amplification (LAMP) assays were developed for the GSB-causing pathogens. Species-specific LAMP primer sets were designed in the mating type (*MAT1-1-1*) gene of the different *Stagonosporopsis* spp. to target single nucleotide polymorphisms (SNPs) different among species. LAMP assays show specificity for the intended targets and sensitivity tests are currently underway to determine their detection thresholds using gDNA and GSB pathogen spore suspensions. The LAMP assays have potential application for point-of-care diagnosis, allowing growers and county agents to make informed decisions depending on the presence and type of GSB species at any given time. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic
Biotic Stress (BS)

Biotic Stress (BS)

Understanding the Diversity of Cucumber Infecting Begomoviruses in Pakistan

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The cucumber (*Cucumis sativus*), of the family Cucurbitaceae is commonly known as cuke. Cucumber leaf curl disease complex (CuLCD) is one of the major factor for threatening cucumber crops in the Pakistan. Symptoms of CuLCD include severe upward and downward leaf curl with cup-shape, yellowing and stunted plant growth. This disease is caused by begomoviruses (single-stranded DNA viruses (family *Geminiviridae*) that are transmitted by whiteflies). The begomoviruses are either bipartite (with two genomic components known as DNA A and DNA B), monopartite (with a genome homolog of DNA A component of bipartite begomoviruses) or monopartite associated with DNA satellites (mainly betasatellites). All three types of begomoviruses are main player in CuLCD complex. Begomoviruses associated with shortening of leaves, vein swelling and enations in *Cucumber*, was cloned and sequenced. The preliminary results showed that clone MU6 and MU7 have highest nucleotide sequence identity of 97% and 98% to *Tomato leaf curl Palampur virus* (ToLCPMV) DNA-A and DNA-B isolated from tomato in India. Maximum likelihood phylogenetic analysis grouped MU6 (DNA-A) and MU7 (DNA-B) into well-supported clades together with ToLCPMV. Thus, both clones: MU6 and MU7 are new variant of ToLCPMV from Pakistan. *Agrobacterium*-mediated inoculation of the partial repeat construct of ToLCPMV clone obtained in this study to *Nicotiana benthamiana* induced severe upward leaf curl symptoms and flow of viral DNA were detected in infected plant leaves. To our knowledge this is the first report of ToLCPMV infecting *Cucumber* in Pakistan. . In another other sample we also cloned Tomato leaf curl new Delhi virus from cucumber. In This presentation I will discuss about the geographical distribution and management of CuLCD infecting Begomoviruses in Pakistan

Session Topic

Biotic Stress (BS)

Biotic Stress (BS)

Evaluating Cucurbit Rootstocks for their Utility in Preventing Disease Caused by the Soilborne Pathogens *Pythium aphanidermatum* and *Pythium myriotylum*

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Root rot and damping-off of watermelon caused by the soilborne pathogen *Pythium* is a problem in watermelon production around the world. Grafting to resistant cucurbit rootstocks has been used to control other soilborne pathogens affecting watermelon. The objectives of this study were to assess grafting as a non-chemical control method against *Pythium*. In 2017 and 2018, a survey was conducted to find the most abundant species of *Pythium* associated with root disease in cucurbits in South Carolina. The two most common species identified were *P. aphanidermatum* and *P. myriotylum*. First, inoculum produced from one isolate of both species was mixed together and used to inoculate 21 non-grafted cultivars of watermelon (*Citrullus lanatus*), citron (*Citrullus amarus*), bottle gourd (*Lagenaria siceraria*), and interspecific hybrid squash (*Cucurbita maxima* × *C. moschata*) in the field. Second, five rootstocks representing the previous three rootstock species were grafted with a Tri-X 313 watermelon scion and inoculated with the same inoculum mixture in the field. Non-grafted Tri-X 313 was included as a control. Third, 17 of the 21 cultivars were further challenged in a growth chamber against each *Pythium* species at a constant temperature of 30°C. Field experiments were conducted for 33 days and growth chamber experiments for 7 days. Area under the disease progress curve (AUDPC) was calculated from disease incidence for all three experiments. Based on AUDPC data from the non-grafted field trials, watermelon and bottle gourd cultivars were significantly more susceptible to *Pythium* disease than squash. In the grafting experiment, the non-grafted Tri-X 313 control was significantly more susceptible than any of the grafted rootstocks. In the growth chamber, the genus *Citrullus* was more susceptible to both *Pythium* species at 30°C than *Cucurbita* and *Lagenaria*. There was no significant difference in AUDPC between the two *Pythium* species. In conclusion, the genus of cucurbit rootstock has a significant impact on *Pythium* disease incidence and grafting watermelon to resistant rootstocks is a viable strategy to control *Pythium* diseases.

Session Topic

Biotic Stress (BS)

Biotic Stress (BS)

Screening of the Melon Germplasms Resistant to Phytophthora Rot Caused by *Phytophthora capsici*

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Phytophthora blight, caused by *Phytophthora capsici* is one of the most devastating disease affecting melon (*Cucumis melo*) production worldwide. *P. capsici* can infect the host plants at any growth stage, causes necrosis on root, stem, leaves, crown and fruits. The infected plants can hardly overcome. Combinations of cultural and chemical prevention measures are commonly used to reduce the damage of *P. capsici* on melon production. However, food safety problems caused by chemical pesticides are causing more and more social panic. The use of host resistance to manage *P. capsici* is a more economical and environmentally friendly strategy, but the majority of current melon cultivars are susceptible to *P. capsici*. Moreover, very limited numbers of resistant melons have been reported, and no genes for resistance to this diseases have been fine mapped or cloned. In this study, 166 melon accessions were evaluated for resistance to *P. capsici* using the root-irrigating method. The tested accessions included 41 *C. melo* subsp. *agrestis*, 104 *C. melo* subsp. *melo*, 15 were *C. melo* var. *agrestis*, and 6 were wild relatives. Melon PI 140637, PI 165514 and PI 381772 were highly resistant to *P. capsici*. Sixty-six percent of the tested melons were at least at a middle resistant level, while, 98% were susceptible or highly susceptible to *P. capsici*. Correlation analysis revealed that the resistance level of *C. melo* subsp. *melo* was significantly higher than that of *C. melo* subsp. *agrestis*. The survey date of melon fruit showed that the single-fruit weight of 14 accessions was more than 1 kg, and the central soluble solid content of 8 accessions was more than 10%. The resistant germplasms with high-yield or high soluble solid content could be used for genetic research like gene mapping or cloning, and could also be used as parent lines to transfer the resistance gene(s) into the lines which owned good agronomic characters but with poor resistance.

Session Topic

Biotic Stress (BS)

Biotic Stress (BS)

Cucurbit chlorotic yellows virus, a New Crinivirus Infecting Cucurbits in California

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During the summer of 2018, melon (*Cucumis melo* L.) plants from a germplasm diversity study in Imperial Valley, California were found infected with *Cucurbit chlorotic yellows virus* (CCYV; genus *Crinivirus*, family *Closteroviridae*). Nearly all melon accessions in the study exhibited interveinal yellowing and chlorotic spot symptoms similar to those caused by *Cucurbit yellow stunting disorder virus* (CYSDV; genus *Crinivirus*), which has been prevalent in the region since 2006. However, nucleic acid extracts of two strongly symptomatic plants tested negative for CYSDV by RT-PCR. Subsequent RT-PCR evaluation of extracts of these two plants with primers specific for RNA-dependent RNA polymerase (RdRp) and coat protein (CP) gene sequences from CCYV RNA1 and RNA2, respectively, resulted in amplification. The 753 nt full-length CP gene and 1515 nt full-length RdRp gene of these isolates were sequenced, and each shared 99% sequence identity with the same regions of CCYV isolates from China, Greece, and Taiwan. Archived RNA extracts from Imperial Valley melon plants stored at -80C, and collected over the course of 9 years (2010-2018), were assayed for CCYV and CYSDV in order to determine how long CCYV may have been present in the region. Nineteen of 23 samples collected from 2014 to 2018 tested positive for CCYV, and many contained mixed infections of CCYV with CYSDV and/or the ipomovirus, *Squash vein yellowing virus* (SqVYV). Eighteen archived samples collected from 2010 to 2013 tested negative for CCYV, but CYSDV, the virus originally identified in the samples was successfully amplified from these archived extracts. Therefore, CCYV most likely emerged in the Imperial Valley in 2014, about the same time that SqVYV was first observed in California, but remained undetected due to similarity in symptomology on cucurbits to CYSDV. CCYV is transmitted efficiently by the whitefly, *Bemisia tabaci* MEAM1, which is common throughout the region. Preliminary observations suggest CYSDV resistance may not be effective for control of CCYV. Further studies will be necessary to evaluate epidemiology of CCYV in the southwestern U.S. desert crop production region, and to determine its impact on melon production and development of crinivirus-resistant melon cultivars. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Biotic Stress (BS)

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Novel Loci *fsd6.1* and *csgl3* Regulate Ultra-high Fruit Spine Density in Cucumber

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Fruit spine density, a domestication trait, largely influences the commercial value of cucumbers. However, the molecular basis of fruit spine density in cucumber remains unclear. In this study, four populations were derived from five materials, which included three with low fruit spine density, one with high fruit spine density, and one with ultra-high fruit spine density. Fruit spine densities were measured in 15 environments over a span of six years. The distributions were bimodal, suggesting that fruit spine density is controlled by a major-effect QTL. QTL analysis determined that the same major-effect QTL, *fsd6.2*, is present in four populations. Fine-mapping indicated that *Csgl3* is the candidate gene of the *fsd6.2* locus. Phylogenetic and geographical distribution analyses revealed that *Csgl3* originated from China, which has the highest genetic diversity for fruit spine density. One novel minor-effect QTL, *fsd6.1*, was detected in the HR and HP populations derived from the cross between 65G and 02245. In addition, GWAS identified a novel locus that coincides with *fsd6.1*. Inspection of a candidate region of about 18 kb in size using pairwise LD correlations, combined with genetic diversity and phylogenetic analysis of *fsd6.1* in natural populations, indicated that *Csa6G421750* is the candidate gene responsible for ultra-high fruit spine density in cucumber. This study provides new insights into the origin of fruit spine density and the evolution of high/ultra-high fruit spine density during cucumber domestication.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Genomics-Enabled Genetic Mapping and Marker Development of Disease Resistance Loci in Melon and Watermelon

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Incorporation of disease resistance into elite cultivars is one of the main goals of most crop breeding programs. Development of markers for disease resistance loci can facilitate crop improvement through gene pyramiding, selection for resistance to multiple diseases in a single generation, large-scale germplasm screening, and potentially, gene editing. Marker development in plants is a multi-faceted process which includes resistance germplasm discovery, robust and accurate phenotyping, genotyping, development of segregating populations, narrowing of resistance-associated genetic regions, and finally validation of markers in actual breeding schemes. Here we present a combination of techniques (QTL-mapping, GWAS, QTL-seq, and marker development) that we are using to identify disease resistance loci (primarily for Fusarium wilt) in watermelon and melon by leveraging genome-level data. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Identification and Mapping of a Dwarfness Related Gene in Watermelon (*Citrullus lanatus*) by Steps Mapping

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Dwarf habit or dwarfism is a desired agronomic trait in watermelon, as it allows higher plant density than is feasible for the standard indeterminate, trailing type, and thereby increases yield as well as the decreasing soil disease and labor required to cultivate. Dwarf growth habit in watermelon is controlled by the simply inherited, recessive gene (*dw-1*). The plant hormone gibberellin (GA) regulates plant growth, including stem elongation. Numerous genes are involved in gibberellin and gibberellin precursor biosynthesis pathway, which is distributed across the watermelon genome. We used a new approach, "Steps mapping" to map *dw-1*. This approach consists of conventional mapping followed by using the reference genome and gene annotation information for fine mapping. The candidate *dw-1* was mapped in a F2 watermelon population (n=309) derived from inbred watermelon lines KK-6939 (trailing growth habit) and TH-15974 (dwarf growth habit). Molecular mapping was conducted using single nucleotide polymorphisms (SNPs) markers. A genetic map was constructed consisting of 56 SNP loci in 11 linkage groups (chromosomes). Linkage analysis placed *dw-1* at the top of watermelon chromosome 9. Molecular marker "WMSNP-9-88" co-segregated with *dw-1* and was 24.4 cM away from *dw-1*. The high-density genetic map was used to identify genomic regions highly associated with *dw-1*, and a new marker, "WMSNP-0002780," was tightly linked to *dw-1*, with a genetic distance of 0.8 cM, and physical map distance ca. 1.9 Mb. Two candidate genes including Cla015407 and Cla015408, located on chromosome 9 with a physical map distance ca. 1.8 Mb. were detected. Both genes encode a protein predicted to be a gibberellin 3-beta-hydroxylase protein, which is a catalyst in gibberellin biosynthesis III (early C-13 hydroxylation) pathway. For the controls, GA20 was metabolized to GA1, which is the active form that affects stem elongation in plants.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Evaluation of Watermelon Germplasm in Texas, a Genotype-By-Environment Study on Yield and Path Analysis on Associated Traits

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In 2017, Texas was one of the top five watermelon producing states with 0.36 Mt harvested, but it lagged behind other top producing states in productivity (40 t ha⁻¹). The objectives of this study were to 1) evaluate 42 germplasm lines, including 6 commercial checks, comprising of Texas A&M (TAM) and selected U.S. National Plant Germplasm System at College Station, TX and Uvalde, TX in 2018, and 2) understand the effect of yield components on yield. Phenotypic traits measured were total yield (TY), sugar content (SC), rind thickness (RT), fruit length (FL), number (FN), circumference (CIR), weight (FW), and firmness (FRM). An analysis of variance showed significant interaction ($P < 0.05$) between genotype and environment for TY, SC, RT, FN, and FRM. In a “Which Won Where” genotype, genotype-by-trait (GGT) biplot, TY, FL, CIR, and FW were grouped together in the ‘Jubilee’ sector. Within that sector there were F₁ hybrids and purelines; ‘Chubby Gray’, ‘Sunshade’, ‘Charleston Gray’, and ‘Calhoun Gray’. The GGE biplot analysis for TY showed that, TAM2 and ‘Pathfinder’ (F₁) were superior germplasm in Uvalde, whereas ‘Desert King’ and ‘Big Striped’ (F₁) were superior germplasm in College Station. A “Mean vs. Stability” GGE biplot showed ‘Desert King’ and ‘Jubilee’ as the most unstable, while ‘Chubby Gray’ and ‘Charleston Gray’ as stable. For SC, TAM 4 and ‘Dixielee’ performed superior in College Station and Uvalde, respectively. Path analysis showed a positive direct effect from FN (0.62), FL (0.65), CIR (0.46), and FW (0.22) on TY. This indicates that although one may select for FN, other traits such as CIR, FL, and FW will also influence TY. The path analysis also showed a negative direct effect from SC (-0.17), indicating that solely selecting for SC may deter the increase of TY. This information will aid in selecting germplasm adapted to Texas for parental use in cultivar development.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Mission Melon: Improving Qualitative traits in *Cucumis melo* using Phenomics

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Muskmelon is a diploid, monoecious, annual species in the Cucurbitaceae family with origins in India, primarily cultivated for its numerous culinary usages and nutritional benefits. The United States ranks fourth internationally for melon production, with a 300-million-dollar market and 90-thousand acres farmed yearly. The objective of this study was to address producer and consumer needs' in a dynamic market by developing melon hybrid cultivars with enhanced fruit quality and yield through the breeding of F1 hybrids from elite inbred lines. Nineteen hybrids and twenty-three elite inbred lines were evaluated in Uvalde, Texas under irrigated (control) and drought conditions. Range of qualitative traits evaluated: weight (lbs.), firmness (N), and percent total soluble solids (% TSS) were from 1.8 – 14.2 lbs., 17.3 – 134 N, and 4.8 – 15.6 % TSS under the control treatment; 2.1 – 14.3 lbs., 11 - 131 N, and 6.7 – 15.1 % TSS under the drought treatment. Experiments for this study were analyzed using statistical software (JMP Pro 13.0.0.), with a REML model = $\sigma_{\text{family}} + \sigma_{\text{environment}} + \sigma_{\text{rep}[\text{environment}]} + \sigma_{\text{family} \times \text{environment}} + \sigma_{\text{error}}$, to find the variance for each trait. These variances were then used to calculate the broad-sense heritability ($\sigma_{\text{family}} / \sigma_{\text{family}} + \sigma_{\text{family} \times \text{environment}} + \sigma_{\text{error}}$). Measured heritability estimates for qualitative traits were relatively low: a*, 0.4584; b*, 0.2506; weight, 0.2513; size, 0.2204; color, 0.2454. An analysis determined there is a biological explanation for the positive correlation in qualitative traits, as well as identified a useful hybrid (BL 110 x BL 109). Under the control treatment, % TSS high-parent heterosis ranged from -35.78 to 16.83; drought treatment % TSS heterosis ranged from -16.16 to 18.67. Due to the lack of a complete factorial experimental design, specific combining ability was not determinable. This study was supported by United States Department of Agriculture-NIFA-SCRI- 2017-51181-26834 through the National Center of Excellence for Melon at the Vegetable and Fruit Improvement Center of Texas A&M University.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Study on the Development of Tubercles and Spines in Cucumber

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The cucumber (*Cucumis sativus* L.) fruit contains tubercles and spines on the surface, which is an extremely valuable quality trait affecting the selection of customers. Cucumber fruit tubercles are derived from an increase in cell numbers by division of several layers of cells that lie near the fruit spine-base cells. Cucumber fruit spines are multicellular, non-glandular trichomes developed from epidermal cells of the fruit skin, and are similar in shape and structure to leaf trichomes. The tubercles and spines affect the cleaning, packaging, transportation, and storage of cucumber fruits. Therefore, study of the development and regulation of tubercles and spines will improve breeding and enhance the economic value of cucumber production. In past few years, we isolated three genes involved in development of cucumber tubercles and spines by map-based cloning, including tuberculate fruit gene (*Tu*), trichome-less (*Tril*), and Micro-trichome (*Mict*). The genetic analysis showed that *Tu*, a single dominant gene, controls the development of tuberculates. *Tu* encodes a transcription factor with a single C2H2 zinc finger domain. Our results suggested that *Tu* probably caused the fruit tubercles formation by promoted CTK biosynthesis in fruit warts. *The mict*, a micro-trichome mutant, is controlled by a single recessive gene in the nucleus. *Mict* encodes a class I homeodomain-leucine zipper (HD-Zip) transcription factor involved in multicellular trichome development. The *tril* is a mutant which has no spines on the surface of cucumber fruit. Genetic analysis revealed that *Tril* is inherited as a dominant trait at a single locus and controls the initiation of cucumber trichomes/fruit spines. *Tril* encodes Protodermal factor (PDF) which is a member of class IV homeodomain-leucine zipper (HD-Zip IV) family. *Tril* not only could control the initiation of cucumber trichomes/spines, but also involved the later development of trichomes/spines including the aspect of density and structure. The genetic analysis showed that *Tril* is recessive epistatic to the *Mict* and *Mict* is recessive epistatic to *Tu*. Next, we will analyze the regulation relationship among *Tril*, *Mict*, and *Tu* and how they regulate coordinately the development of tubercles and spines in cucumber.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Production and Characterization of Bitter Gourd Derived From Intraspecific Hybridization

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Bitter gourd (*Momordica charantia* L.) is an important vegetable crop in Pakistan. It is consisting of two famous local varieties, *M. charantia* var. *charantia*, which produces large fusiform fruits, and *M. charantia* var. *muricata*, a wild variety with small and round fruits. However, these two varieties have not fulfilled the required demand of farmers due to the lack of disease and pest resistance, and low yield. Many companies have imported seed from other countries, but they were not suitable our local climate and created many new production problems. This problem can be solved by the intraspecific hybridization in bitter gourd. Therefore, we made the intraspecific crosses among different bitter gourd genotypes. The maximum number of seed per fruit was found in the combination of MC27 x MC23. While, the highest seed yield was observed in the combination of MC23 x MC27. Hybrid combination MC24 x MC23 had the maximum number of pistillate flower node and number of fruit per plant, and the fewest days to maturity. The fruit length and width were maximum in hybrid MC24 x MC27, while ovary length and width were maximum in hybrid MC27 x MC23. Furthermore, the fruit weight and yield per plant was highest in combination MC24 x MC1. So, this hybrid has improved the sustainable production of Bitter gourd. It has also increased the food supply and reduce the hunger in the Pakistan.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Dissecting Multiflower Male Truss Character in Melon (*Cucumis melo* L.)

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Male flowers in melon are usually found alone or in groups of 1-3 flowers per node. However, Zimbabwean melon line TGR-1551 has been identified to exhibit multiflower male trusses. In a previous study, one major QTL on linkage group 6 associated with the character in a poorly saturated region of a map of a RIL population derived from a cross between TGR-1551 and the Spanish melon cultivar 'Bola de Oro'. Now, a dissection of the character through the construction of a high-density map and the addition of one more phenotypic character has been carried out. High resolution QTL analysis was performed using genotyping by sequencing (GBS) of the same RIL population derived from the cross between TGR-1551 and 'Bola de Oro'. Genotypic data included approximately 1600 SNP markers and phenotypic data were based on the presence or absence of multiflower male trusses in 2012, 2013, and 2014 evaluations. In 2014, the number of flowers/node in the RIL population was also recorded. A major QTL on chromosome 6 (LOD score 12.89 for 2012, 19.9 for 2013, and 22.7 for 2014), which could explain up to 51.7 % of the phenotypic variability observed for multiflower male truss has been confirmed; the same QTL has been also identified for the number of flowers per node (LOD score 6.64 and R^2 17.4). This QTL collocated with the QTL described in our previous study although this time the level of phenotypic variation explained was higher and the stability of the character across three years has been confirmed. In addition, other two minor QTLs on chromosomes 6 and 4 were detected, explaining 7.1% and 9.5% of the phenotypic variance respectively. While the candidate gene MELO3C006888 had already been detected in our previous study, three novel candidates genes (MELO3C006880, MELO3C006860, and MELO3C006940) have been identified in the same QTL region. Further research through gene sequencing and gene expression profiling is needed to confirm the exact role of the found candidate genes on the character.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Genome-Wide Association Studies of Important Agronomic Traits in Watermelon

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Watermelon is an important economic crop throughout the world. Molecular assisted breeding is a powerful approach to facilitate the variety improvement. Compared with field crops, knowledge of genomic loci associated with important horticultural traits in watermelon is limited. We performed genome-wide association studies (GWAS) of seven agronomic traits, including flesh sweetness, flesh bitterness, flesh color, seed color, rind color, rind stripe and the resistance to *Fusarium oxysporum* race 1, using a population containing 415 watermelon accessions. A genome region on chromosome 2 was identified to be significantly associated with flesh sweetness, and a tonoplast sugar transporter gene in this region was identified as the candidate gene. For flesh bitterness, a region on chromosome 1 was identified, which contains the candidate gene encoding a BHLH transcription factor. For flesh color, a region on chromosome 4 and a lycopene beta-cyclase gene in this region were identified. For *Fusarium oxysporum* race 1 resistance, a significantly associated region was identified on chromosome 1 containing a potential candidate gene encoding an acidic endochitinase. In addition, genome regions highly associated with seed color, rind color and rind stripe were also identified through our GWAS analyses. The identified genome regions and the candidate genes highly associated with important agronomic traits provide valuable resources for molecular assisted breeding in watermelon.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Physiological and Anatomical Responses of two Contrasting Pumpkin Genotypes Under Drought Stress

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In this study, we investigated drought stress tolerance of pumpkin genotypes. PEG 6000 (6%) was applied to Hoagland nutrient solution to determine the effects of osmotic stress on drought tolerant and susceptible, C-26 genotype and C-27 genotypes, respectively. Different parameters including MDA content, H₂O₂ accumulation, OH sweeping activity, antioxidant enzymes like SOD, APX, CAT, POX, GR and GPX activities and anatomical structure of roots and leaves were examined. It was determined that under PEG 6000-induced drought stress, MDA content increased in both the experimental genotypes; however, greatest increase was observed in C-27 genotype. Moreover, H₂O₂ level and the OH radical sweeping activities of both pumpkin genotypes were increased under drought stress. However, both genotypes showed major differences in antioxidant enzyme activities under drought stress.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Confirmation of Significant Parent-of-Origin Effects in Cucumber

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Cucumber is a useful plant to study organellar effects on growth and development because chloroplasts are maternally and mitochondria paternally transmitted. Multiple doubled haploids (DH) were produced from four divergent cucumber populations, reciprocal crosses were made in a diallel mating scheme, and sizes of seeds and cotyledons and weights of plants approximately 25 days after planting were measured. General (GCA) and specific (SCA) combining abilities and reciprocal effects were highly significant. Fresh and dry weights were significantly different for specific reciprocal hybrids with identical nuclear genotypes, and revealing significant parent-of-origin effects. Reciprocal hybrids from crosses among multiple DHs extracted from the same cucumber cultivars were not consistent for early plant growth, indicating that parent-of-origin effects were specific to individual DHs and not to the population *per se*. These results further support reciprocal crossing of cucumber DHs and inbreds to identify the best performing hybrids.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Analysis of Physiological Characteristics and Chloroplast Ultrastructure of a New Leaf Color Mutant in Melon

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The aim of the study was to study the differences in growth, physiological characteristics and chloroplast development between leaf color mutant (MT) and wild type (WT). Agronomic traits, photosynthetic parameters, photosynthetic pigment content, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activity and MDA content and chloroplast ultrastructure of MT, WT, F1(MT×WT) and rF1(WT×MT) were investigated. The weaker growth of MT resulted in a delayed growth stage. There was no significant change in the photosynthetic rate. With hybridization F1, rF1 could go back to the normal growth. The leaf color of MT changed with the growth and developed from yellow to yellowish green, but the veins were still green. The chlorophyll content of MT leaf was significantly lower than that of WT. The Chlorophyll and carotenoid content were reduced by 25.69% and 21.26%, respectively, during the fruiting stage. However, the ratio of chlorophyll a to chlorophyll b was significantly higher in the MT leaves than in WT leaves. SOD, POD and CAT enzyme activities and MDA content were higher in MT than in WT and then were increased by 65.45%, 13.91%, 3.23%, 15.14% in the stage, respectively. The observation of transmission electron microscopy (TEM) showed that the stacking of grana was irregular in the chloroplast structure of MT leaf and the grana lamellae were deranged with a line shape, indicating differences in the chloroplast structures between MT and WT. In the whole growth stage, the shorter height, lower chlorophyll content in WT than in MT and different chloroplast structure between them. There is no significant difference in photosynthetic rate, suggesting a basically intact function of photosynthetic apparatus. The antioxidant enzyme activity and was significantly higher in the MT than in the WT during fruiting stage and the MDA content was also significantly in the MT higher than in the WT during the whole growth stage. However, there was no significant difference among F1, rF1, and WT.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Introgression Mapping of Wild Species-Derived Resistance to Viruses in *Cucurbita*

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Viruses are major threats to global cucurbit production, capable of causing severe economic losses through plant stunting, reduced yield, and distortion of the fruit. Two of the most important viruses that are capable of infecting squash are papaya ringspot virus (PRSV) and cucumber mosaic virus (CMV). These viruses are transmitted by aphids in a non-persistent manner, making management of the diseases through vector difficult and ineffective. Breeding for resistance to these viruses is the most effective method of control; however, phenotypic selection for virus-resistant individuals by manual inoculation and inspection is laborious and slow. In addition, there are no identified sources of resistance to these viruses in *Cucurbita pepo*. Historically, virus resistance has been transferred to *C. pepo* from related species, such as *Cucurbita ecuadorensis*, and incorporated into the virus-resistant cultivars currently available. In order to elucidate the genes controlling these traits and improve breeding for virus resistance, we have evaluated the level of resistance to PRSV and CMV in a panel of *C. pepo* cultivars and historic Cornell breeding lines. These lines and *C. ecuadorensis* lines were genotyped using genotyping-by-sequencing. Using an introgression mapping approach, we have identified an approximately 2 MB introgression from *C. ecuadorensis* shared by the PRSV resistant lines. We are developing a mapping population with 'Whitaker', a cultivar resistant to PRSV and CMV, and 'Success PM', a susceptible cultivar. We will employ an F_{2:3} mapping approach to validate the PRSV resistance-associated region identified via introgression mapping and provide increased power for mapping CMV resistance. Mapping and validation efforts will lead to the development of markers for virus resistance loci and will be applied in marker-assisted selection.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Validation of KASP™ Assays for Three Alleles of a SUN gene Controlling Fruit Length and Shape in Watermelon (*Citrullus lanatus*)

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Fruit shape in watermelon (*Citrullus lanatus*) is a major concern for breeders and producers as it can influence consumer choice at point of sale in addition to impacting ease of shipping. Watermelon has a wide range of fruit shapes, but until recently, the genetic mechanisms underlying fruit length and shape have been unknown. While fruit phenotypes can be influenced by environmental factors, fruit length (FL) and shape (FSI) are believed to be controlled primarily by a SUN gene, *Clao11257*, located on chromosome 3 in a QTL associated with the traits. A 159-bp deletion (DEL) in a coding region, resulting in the absence of 53 amino acids, has been shown to be responsible for causing an elongated phenotype. We identified a new allele in Klondike Black Seeded (KBS) with a G to A point mutation in the third exon of *Clao11257*. KASP™ assays were developed for the three alleles of the gene and used to determine genotype-phenotype associations in five segregating populations. Phenotyping of three of the five populations and a diverse cultivar panel were grown in the field and phenotyped digitally with Tomato Analyzer (TA). TA phenotyping revealed relationships between the genotype and FL, fruit shape index (FSI), and the distal fruit end angle (DAN). The KASP™ assays for each allele of *Clao11257* were significantly associated with three traits in the cultivar panel: FL (p-value < 0.0001), FSI (p-value < 0.0001), and DAN (p-value < 0.0001). Based on our results, the wild-type (WT) allele was associated with round fruit (FSI = 1.07 ± 0.02), and a wide distal end shape ($152.25^\circ \pm 0.98^\circ$). The DEL allele, resulting from the 53 amino acid deletion, is associated with long fruit with the highest FSI (2.00 ± 0.02) with a narrow distal fruit end shape ($98.55^\circ \pm 1.12^\circ$). The KBS allele is associated with an intermediate phenotype (FSI: 1.59 ± 0.02 ; DAN: $120.98^\circ \pm 1.24^\circ$). These KASP™ assays could be powerful tools in molecular watermelon breeding programs for marker assisted selection for fruit length and shape. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Molecular Mapping of a New Dominant Resistance Gene for *Zucchini yellow mosaic virus* (ZYMV) in Squash (*Cucurbita pepo*)

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Zucchini yellow mosaic virus (ZYMV) is one of the most destructive viruses that badly reduce the production of squash (*Cucurbita pepo*) all over the world. Resistance is the best approach to control the disease. Squash inbred line 'BS12' showed a high-level of ZYMV resistance in our germplasm evaluations over several years. The genetic basis of the resistance in 'BS12' was elucidated through an inheritance study and molecular mapping. A total of 1172 F_{2:3} lines from the crosses of BS3, a susceptible parent, with BS12 were tested with ZYMV-CH, a highly predominant ZYMV strain in China, under the controlled greenhouse conditions, and bulked segregant analysis was carried out to identify SSR and EST-SSR markers linked to this resistance gene. Results indicated that resistance to this virus observed in 'BS12' was controlled by a single dominant gene, and closely flanked by SSR markers ZY-140 and ZY-157 at a genetic distance of 0.4 and 2.6 cM, respectively. These markers were subsequently validated for detection of ZYMV resistance gene among 30 varieties: 10 squash genotypes were resistant to ZYMV, and 20 genotypes showed the susceptible banding pattern. The MAB strategy with these two markers enabled the development of homozygous (BC₄F₂) ZYMV-resistance lines with the smallest introgressed region and 96.9% of the recurrent parental genome, which proved valuable for ZYMV resistance gene transfer in future squash breeding.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Genetic Mapping Reveals Markers for Fruit Shape and Yellow Skin in Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai)

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Watermelon has rich diversity in fruit shape and skin color, which are the major objectives of watermelon breeding. However, the candidate gene and the underlying genetic mechanism for such important trait in watermelon were unknown. In this study, we identified a locus on watermelon chromosome 3 controlling fruit shape and a locus on chromosome 4 for yellow skin. Segregation analysis in F₂ and BC₁ populations derived from a cross between two inbred lines 'Duan125' (elongate fruit) and 'Zhengzhouzigua' (spherical fruit) suggested that fruit shape of watermelon was controlled by a single locus and elongate fruit (OO) was incompletely dominant to spherical fruit (oo) with the heterozygote (Oo) being oval fruit. A segregation analysis in F₂ and BC₁ populations derived from a cross of two inbred lines '94E1' (yellow skin) and 'Qingfeng' (green skin) suggested that skin color is a qualitative trait. BSA-seq mapping in the F₂ population showed the locus was located on chromosome 3, and the candidate gene was mapped to a region of 46 kb for fruit shape. There are only four genes present in the corresponding region in the reference genome. Sequencing of four candidate genes in this region showed that the CDS of *ClA011257* had a 159 bp deletion, which resulted in the deletion of 53 amino acids in elongate watermelon. An indel marker was developed based on the deletion to test the F₂ population and 105 watermelon germplasms. The results showed that *ClA011257* cosegregated with watermelon fruit shape. BSA-seq mapping confirmed the locus for skin color in the F₂ population, which was detected on chromosome 4 by GWAS among 330 varieties. Several major markers were designed to delimit the region to 59.8 kb region. Utilizing the two populations consisting of 10 yellow and 10 green skin watermelons, we found a tightly linked functional SNP marker for the yellow skin phenotype. The application of these markers as selection tool in breeding programs will help to improve the breeder's ability to make selections at early stages of growth, thus accelerating the breeding program.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Chromosomal Locations of Four Loci Controlling Watermelon Seed Coat Color

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Global consumers associate watermelon with sweet, juicy fruits that are often consumed during the hot summer months. However, in some parts of Asia and Africa the lipid and protein rich watermelon seeds are the primary products from watermelon production. Seed coat color plays an important role in consumer preference for edible watermelon seeds and are therefore an important consideration during watermelon breeding. Watermelon seeds have diverse seed coat colors including black, stipple, red, green and white. A four gene model (R , T , W and D) was developed in the 1940s to explain the inheritance of these different phenotypes. In this study we re-examined the four gene model and identified the associated chromosomal loci. We utilized QTL-Seq and linkage mapping to map the four loci in three segregating F_2 populations: Sugar Baby (stippled) x PI 482379 (green), Charleston Gray (stippled) x PI 189225 (red) and Charleston Gray (stipple) x UGA147 (clump). Our results confirmed that stipple (R_+) is monogenically dominant over green (rr). The stipple x red population segregates for two genes, with the R gene being dominantly epistatic to the T gene. This epistatic interaction deviates from the expectation of the four gene model and we propose to name the latter locus T^1 . In the stipple x clump population, the D gene is recessively epistatic to W , as predicted by the model. The location of the R , T^1 , W and D loci were identified on chromosomes 3, 5, 6 and 8, respectively. KASP™ assays were developed for SNPs linked to the four loci and will facilitate marker assisted selection for watermelon seed coat color. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Increasing Shelf Life of Perishable Produce Using Patented Gene Technology

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Post-Harvest Loss (PHL) in crops and food wastage pose major challenges to attaining sustainable standards for food security. Each year, 1.3 billion tonnes of food are globally wasted (FAO) representing a value close to \$1 Trillion. Fruits and vegetables contribute to the highest rates of wastage. It's estimated that every year about 45% of globally produced fruits and vegetables is wasted without consumption. Extending shelf-life of perishable produce is one of the mechanisms to minimize food wastage and increase food availability to feed the growing population. Agribody Technologies, Inc. (ATI) utilizes a combination of genome editing and a highly validated pair of conserved gene targets to significantly delay post-harvest senescence, while increasing resistance to abiotic (low nutrients, drought, heat, cold, salt, etc.) and biotic (fungal & bacterial) stresses, and increasing seed and biomass yield in crops. The efficacy of this technology has consistently been proven in multiple crops under both greenhouse and field conditions. Two years of replicated field trial data in alfalfa have shown an unprecedented 20-45% yield increase in an elite commercial variety with no loss of quality. Similarly, field trials in banana and greenhouse studies in tomatoes and flowers have increased shelf-life by 2-to 3-fold. Currently, several licensing and co-development projects are underway in potatoes, coffee, canola and rice. Results from these studies will be discussed.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Development of Melon Cultivars for Organic Farming

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Melon (*Cucumis melo* L.) has been cultivated in Spain since at least Roman times, both non-sweet melons (snake-shape, *flexuosus* type), locally known as alficós, and sweet melons. Snake melons were already cultivated in the first century, but the introduction of sweet melons from Central Asia probably occurred later, during the Middle Ages. Preferences of Spanish farmers and consumers during centuries favored the selection of varieties adapted to diverse agro-climatic conditions. Cultivars of six main groups of sweet melons, all belonging to the Inodorus group (non-climacteric, casaba types as Piel de Sapo, Rochet, Amarillo, Blanco, Tendral and Hilo Carrete) are still cultivated nowadays, although mainly for self-consumption or for local markets, with the exception of Piel de Sapo). Due to social demands of traditional fruits grown using organic farming practices, Valencian government (CEICE, Generalitat Valenciana) funded a project (PROMETEO2017/078) to select traditional Spanish melon cultivars appropriated for organic cultivation. Fifty cultivars representing the six classes of sweet melons and the non-sweet alficós were selected among those maintained at the Gene bank of the Polytechnic University of Valencia. Two experimental fields in the peri-urban area of Valencia, where organic farming is being recovered, were established. A survey of the main pests and diseases was conducted during the growing cycle. Aphid-transmitted viruses, such as *Watermelon mosaic virus* (WMV) and *Cucumber mosaic virus* (CMV) were the most frequent. Powdery mildew differentially affected the cultivars; Rochet and alficós cultivars were more tolerant than other types. In one of the fields, where melon had been cultivated for years, many soilborne fungal pathogens were isolated from plants showing different degrees of vine decline symptoms (*Fusarium oxysporum*, *F. solani*, *Monosporascus cannonballus*, and *Macrophomina phaseolina*); cultivars of Blanco and Amarillo types were more susceptible. Traditional melon varieties were grafted onto both *Cucurbita* and melon rootstocks, the latter tolerated better the soil fungi since *F. solani* affected the roots of the *Cucurbita* hybrids rootstocks. These assays allowed us to select the most tolerant cultivars and to identify the biotic factors limiting organic farming of melon in the region. This information would be used to optimize traditional melon breeding for organic cultivation.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Ion Cultivars for Biosaline Agriculture

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Salinity is an important factor limiting the productivity of crop plants. Biosaline agriculture deals with salinity, using unconventional saline water resources and crop breeding to support saline stress. Spain is the main melon producer and exporter in Europe. Modern improved hybrid cultivars are grown under conventional agriculture, but market is demanding diverse traditional cultivars, grown under sustainable systems. Low availability of fresh water is limiting this production. We have cultivated under saline conditions 20 melon cultivars in a field experiment conducted in an agricultural Wetland area included in the Carrizales Agrarian Natural Park (Southeast of Spain) (PROMETEO2017/078 funded by the Generalitat Valenciana). The climate is arid to semiarid Mediterranean (average annual rainfall of 250 mm). Drip irrigation was applied, using water with a conductivity ranging from 4 to 6 dS/m. Melon cultivars were grown ungrafted and grafted onto two rootstocks, an F1 *Cucurbita* hybrid and an F1 melon hybrid. Yield and fruit quality were compared with an assay grown in a field irrigated with non-saline water (1.9 dS/m). Adverse effects of saline irrigation were observed on plant growth only in some cultivars, mainly in ungrafted plants. As expected, plants grafted onto *Cucurbita* rootstocks were more vigorous. Most varieties yielded acceptable levels of high quality fruits, but differences in yield and fruit quality allowed the selection of those more appropriated for salt stress cultivation. Salinity slightly reduced yield, but no effect on mean fruit weight was observed (1.42 kg vs 1.41, for non-saline vs saline irrigation). Total soluble solids contents (SSC) increased under salt stress (11.9 vs 13.4). Rootstock effect on fruit size and quality was significant in saline conditions. Most cultivars gave higher yields and bigger fruits when grafted onto the *Cucurbita* rootstock (1.55 kg), whereas fruit size was similar in plants grafted onto melon rootstock and non-grafted plants (1.46 and 1.41 kg). The highest SSC were found using melon rootstocks (13.6) compared to the *Cucurbita* and the non-grafted (13.3 and 13.1). The selection of the best traditional cultivars and best scion-rootstock combinations will enhance the implementation of biosaline agriculture in melon crop.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Selection of Traditional Spanish MeStudy of Hybridity in Cucumber (*Cucumis sativus* L.) Under Polyhouse Condition

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Present investigation was carried out at vegetable research centre and NAIP Lab, Department of vegetable science, G.B.P.U.A & T., Pantnagar, Uttarakhand during August 2015 to December 2016. The main objectives to study the hybridity among monoecious, gynoecious and parthenocarpic genetic background in cucumber (*Cucumis sativus* L.). Most of the genotypes were early to medium in flowering. Maximum values with respect to number of fruits per plant (19.00), fruit length (22.36 cm), average fruit weight (270.33 g) were found in PCUCP-4 x PCUC-8, PCUCP-3 and PCUCP-3 x PCUC-25, respectively under environment 2 (E2). Fruit yield (q/ha) varied from 105.24 to 1367 and E2 yield was higher than environment 1 (E1). The parthenocarpic lines and gynoecious lines exhibited superior performance in E2 as compared to E1 as environment differences influence the yield and yield contributing characters. Across the environments, PCUCP-4 x PCUC-8 and PCUCP-5 x PCUC-8 genotypes were found promising for heterobeltiosis and standard heterosis over check. PCUCP-4 x PCUC-8 and PGYG-3 x PCUC-25 showed significant specific combining ability for the maximum traits in both environments. Therefore, it is concluded that F1 hybrids PCUCP-4 x PCUC-8, PCUCP-3 x PCUC-25, PCUCP-3 x Pant Khira-1, PCUCP-5 x PCUC-8 and PGYG-3 x PCUC-25 can be exploited for commercial cultivation under protected cultivation. Genotypes PCUCP-4, PCUCP-3 and PCUCP-1 were found the best general combiner and tester PCUC-8 found the overall best general combiner in both the environments. Among genotypes PCUCP-5 and PGYC-2 were found the most diverse and PCUCP-4 and PCUCP-8 found most closely related with each other. Among markers, UBC-834 and UBC-840 can be used for further identification purpose as these have possessed highest PIC value and polymorphism. ISSR analysis amplified a total of 69 loci with 75-100% polymorphism. Use of ISSR markers will certainly help to identify genetic diversity, management and exploration of the genetic resources and assist in the genetic improvement of cucumber.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Genetic Characterization of DH-Kirkagac Melon Lines Revealed by SSR Markers

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Melon is an economically important vegetable crop in Turkey. The production amount is approximately 1.7 million tonnes and winter type melons (*Cucumis melo* L. var. *inodorus*) are widely grown in many regions using local genotypes. Haploidization technique is known to be a useful tool in plant breeding by reducing time for the production of 100% homozygous lines. We examined the genetic relationships of 96 DH-Kirkagac melon genotypes that we developed by irradiated pollen technique using SSR (Simple Sequence Repeat) markers. Twenty polymorphic SSR markers were used and based on SSR data, the genetic similarity coefficients were calculated and dendrograms were constructed using UPGMA (unweighted pair-group method with arithmetic average). According to cluster analysis DH-lines were separated into different groups. Our results provide us different heterotic groups for developing new breeding programmes in Kirkagac melons.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

QTL Mapping of Angular Leaf Spot Resistance in Cucumber

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Angular leaf spot (ALS) is an important disease of cucumber worldwide. The causal agent for ALS is the bacterial *Pseudomonas syringae* pv. *lachrymans*. Here, we reported quantitative trait locus (QTL) mapping and cloning of a resistance gene to a virulent strain of the ALS pathogen in two cucumber inbred lines Gy14 and WI 2757 with 129 recombinant inbred lines (RILs from the cross of Gy14 x 9930 (G9RIL) and 132 F_{2:3} families from the crosses between WI 2757 and True Lemon (WTF23). Phenotyping of angular leaf spot inoculation responses were conducted in five controlled environments (CA2011, WI2011, WI2015, WI2016, WI2017). QTL analysis suggested that the major-effect *ps/* locus for ALS resistance on Chromosome 5 was carried by Gy14 and WI2757. One additional minor-effect QTL *ps/1.1* was only detected in WI2011 environment and another minor-effect QTL *ps/3.1* was only detected in CA2011 environment in the WTF23 population. Additional 375 G9RILs were employed for fine mapping of the *ps/* locus, which allowed to delimit the resistance locus into a 93.7-kb region in which 12 genes were predicted including the cucumber staygreen (*CsSGR*) gene that is known to play a critical regulatory role in the chlorophyll degradation pathway. Local association analysis among 82 lines of the natural cucumber population with 623 SNPs provided further evidence that *CsSGR* is the candidate gene for ALS resistance QTL (*ps/*). A single nucleotide mutation in the coding region of *CsSGR* resulted in a nonsynonymous amino acid substitution in SGR protein, which is predicted to be responsible for the differential inoculation responses against the ALS pathogen infection between Gy14 and 9930. From qPCR, *CsSGR* was significantly upregulated upon pathogen inoculation in the susceptible 9930 as compared to the resistant Gy14. Genes involved in the chlorophyll degradation pathway exhibited differential expression between resistant and susceptible lines post pathogen inoculation. This work will help us further reveal host disease resistance mediated by a loss-of- susceptibility mutation of *CsSGR*. National Institute of Food and Agriculture, U.S. Department of Agriculture, under award numbers 2011-51181-30661 and 2015-51181-24285. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Physiological Responses of Melon Genotypes with Known Drought Tolerance Levels

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If available agricultural land in the world would be classified according to stress factors, then drought stress is one of the most common environmental stresses, affecting 26% of plant's growth and efficiency. Identification of plant species and genotypes that are resistant to drought stress, and development of varieties that are differentially tolerant to drought stress is one of the crucial priorities in agricultural sciences. The most important biochemical change in plants under drought stress is, formation of reactive oxygen compounds such as single oxygen, superoxide anion and hydrogen peroxide due to a decrease in photosynthesis rate. Reactive oxygen compounds accumulating under stress conditions are natural by-products of cell metabolism and play an important role in signal transduction mechanism. However, in case of excessive accumulation, they can lead to cell death by inducing lipid peroxidation, protein reduction and DNA fragmentation. Plants utilize enzymatic or non-enzymatic antioxidant molecules to deal with the oxidative stress caused by accumulation of reactive oxygen compounds. In this study, Kav-248 (drought tolerant) and Kav-20 (drought sensitive) genotypes were used, which were selected among 192 Turkish melon germplasms with two different screenings, were conducted in climate controlled greenhouses and climate chambers to determine drought tolerance levels. In the environment where melon genotypes were exposed to PEG 6000-induced drought stress, the defense mechanisms exhibited by the plants were compared with control groups. Antioxidant enzymes (SOD, POX, GR and CAT), known as internal defense systems of genotypes against drought stress and changes in reactive oxygen species were examined. While the SOD activity increased by 86% in Kav-248 genotype tolerant to dry conditions compared to control, it is increased by 75% in Kav-20 genotype sensitive to dry conditions compared to control. GR activity increased by 37% in Kav-248 genotype compared to control, while Kav-20 genotype increased by 10%. OH radical sweeping activities increased by 150% in the Kav-248 genotype, while increased by 181% in the Kav-20 genotype, and similarly, increased H₂O₂ radically due to drought application and the maximum increase in the Kav-20 genotype according to the control were determined.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Next-Generation Sequencing Bulk Segregant Analysis Reveals Multiple Loci Involved in Phytophthora Root and Crown Rot in Squash and Pumpkin

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Breeding for resistance to the various disease syndromes caused by the oomycete pathogen *Phytophthora capsici* has been challenging in Cucurbitaceae species. Few sources of resistance have been identified and evidence to date suggests that different genes play a role in resistance in distinct plant parts. Despite these difficulties, progress can be made in selection for quantitative resistance to specific disease syndromes. Our work is focused on squash and pumpkin (*Cucurbita pepo*), which are particularly susceptible to *Phytophthora* root and crown rot. Using white vegetable marrow landrace 'Austrian Bush' as the primary source of resistance, we have developed breeding lines that display reduced root and crown rot symptoms after inoculation with *P. capsici*. In order to elucidate the genetic basis of the trait and discover molecular markers for use in breeding, we performed QTL mapping using a next-generation sequencing bulk segregant analysis (NGS-BSA) approach. Over 13,000 F₂ individuals from a cross between susceptible zucchini cultivar 'Dunja' and a partially resistant breeding line were inoculated as seedlings in the greenhouse with a zoospore suspension of *P. capsici*. Pools consisting of the 15% most susceptible and resistant individuals, in addition to a control pool of random individuals, were visually selected in each of two biological replicates. DNA samples from each pool were then whole-genome resequenced. Allele counts at over 150,000 single-nucleotide polymorphisms were used to identify genomic regions where allele frequencies differed between control, susceptible, and resistant pools. Results suggest polygenic inheritance, and beneficial alleles originating from both the susceptible and partially resistant parents. We failed to observe any region with more than a 15% difference in allele frequency between susceptible and resistant pools, indicating small QTL effect sizes or poor accuracy in visual selection due to low single-plant heritability. Segments on chromosomes 4, 5, 8, and 16 displayed the most extreme allele frequency differences between pools. The effects of DNA polymorphisms located in these regions will be validated in additional populations in order to evaluate their usefulness in genomics-assisted selection.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

QTL Mapping of Resistance to Powdery Mildew in *Cucumis melo* MR-1 Using a Recombinant Inbred Line Population

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One hundred seventy-two lines of an MR-1 x AY RIL population (F₆ to F₁₁) were evaluated for resistance to cucurbit powdery mildew incited by *Podosphaera xanthii* race 1 (Px race 1) in greenhouse trials. The RIL population was evaluated in two independent greenhouse tests in April 2016 and February 2017 using a 0-10 scale of increasing disease severity. Disease severity scores across the population were heavily skewed towards resistance for all tissue types but were still continuous as the distributions all extended to a score of at least 5. Population disease severity means varied from 0.67 for the stem to 2.6 for rating of true leaves. There was no evidence for transgressive segregation. We identified two major QTL (*qPx1-5* and *qPx1-12*), two minor QTL (*qPx1-4* and *qPx1-10*), and one epistatic interaction associated with disease severity BLUPs of the true leaves. The same four QTL, or a subset thereof, defined by overlapping 1.5-LOD intervals were identified for all tissue types. The two major QTL alone (*qPx1-5* and *qPx1-12*) explained more than 74% of the variation in disease severity and had LOD scores of 46.1 and 36.6, respectively. MR-1 contributed the resistance alleles for all QTL. A significant epistatic interaction between *qPx1-5* and *qPx1-12* was detected, in which the resistance allele of *qPx1-5* masks the effect of the susceptible allele of *qPx1-12*. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Genomics-Assisted Molecular Breeding in Watermelon

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Watermelon is one of the most popular fruit crops worldwide. Accumulative genome and transcriptome resources have provided a solid foundation to help elucidate molecular mechanisms underlying horticultural traits and identify genes and molecular markers that can be used to facilitate watermelon breeding. To meet the increasing demands for watermelon varieties with improved flavor, nutrient composition and appearance, we have identified and characterized genes regulating fruit flesh sweetness and flesh color. We have also identified the genetic components that control watermelon sex determination. This knowledge has been applied to the generation of gynoecious varieties in order to increase the fruit yield and the efficiency of seed production. We have identified sources of resistance to Fusarium wilt, powdery mildew and *Zucchini yellow mosaic virus* (ZYMV) from the wild and semi-wild watermelon germplasms, which have been used in watermelon disease resistance breeding. In addition, we have performed genome-wide association studies (GWAS) on these fruit quality and disease resistance traits and identified a number of highly associated DNA markers. To increase the efficiency of watermelon improvement by taking advantage of genomics-assisted breeding, we have set up an ultra-high-throughput genotyping platform. Furthermore, we have successfully implemented the CRISPR/Cas9 gene editing technology in watermelon and used this technology to generate the world first tribenuron herbicide-resistant watermelon line.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

A High-Density Genetic Map Construction and Identifying QTLs Associated with Fruit Shape in Pumpkin (*Cucurbita moschata* Duchesne)

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Pumpkin (*Cucurbita moschata* Duch.) is an economically important crop belonging to the Cucurbitaceae family. However very few genomic and genetic resources are available for this species. A high-density genetic map can facilitate quantitative trait loci (QTL) mapping. A set of 186 F₂ progenies derived from the cross of pumpkin inbred lines Rifu and Honey jujube were genotyped using the genotyping-by-sequencing approach. Using the SNPs we identified, a high-density genetic map containing 656 bin-markers was constructed, spanning a total genetic distance of 3136.90 cM across the 20 linkage groups of *C. moschata* with a mean marker density of 4.78 cM. The high-density genetic map was used to identify genomic regions highly associated with an important agronomic trait, fruit shape index. Three QTLs on linkage groups (LGs) 2, 6 and 16, respectively, were recovered. One QTL, *qCoFs*, which was located in an interval of 0.15 Mb on LG 2 containing 36 putative genes, explained 55.7 phenotypic variations. The map provided a valuable resource for gene cloning and marker assisted breeding in pumpkin. The identified fruit shape index QTLs would help to further mapping the genes and dissect the underlying molecular basis regulating pumpkin fruit shape development.

Session Topic

Breeding and Genetics (BG)

Breeding and Genetics (BG)

Target SSR-seq: A New SSR Genotyping Technology and its Application In Genetic Analysis and Cultivar identification in *Cucumis sativus* L.

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Abstract: Simple sequence repeats (SSR) or microsatellites, as the second generation of molecular markers, have been extensively used in many research areas. However, the existing SSR exploitation and detection techniques cannot efficiently and accurately acquire hundreds of SSR genotypes. We designed a new technology called Target SSR-seq which combined the advantages of high throughput sequencing and multiplex amplification of SSR regions. It could automatically gain hundreds of SSR genotypes at once by sequencing the SSR motif at the coverage of 1000 times by HighSeq sequencing platform. The application of target SSR-seq in the present study was to establish 382 cucumber cultivar fingerprint database using 111 perfect SSR through analyzing 182 cucumber resources re-sequencing data. A total of 398 alleles within 111 perfect SSR loci were acquired and the average sequencing depths of all cultivars were 1289X. Population analysis showed that 382 cucumber cultivars were divided into four populations: “North China type,” “South China type,” “Europe fruit type” and “Xishuangbanna type.” The genetic relationship matrix among 382 cucumber cultivars was constructed based on the number of different SSR markers. We found “Jingyouyihao” has higher genetic similarity while fruit cucumber cultivars had further genetic distance with other cultivars. Consequently, 16 core SSR pairs were able to accurately distinguished 382 cucumber varieties, which demonstrated that target SSR-seq had high accurate and efficiency for SSR genotyping and especially suit for DNA fingerprinting research.

Session Topic

Breeding and Genetics (BG)

Breeding for Resistance (BR)

Breeding for Resistance (BR)

A Novel Putative Oligogalacturonan-Binding Receptor-Like Kinase is Involved in Quantitative Downy Mildew Resistance in Cucumber

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Cucurbit Downy Mildew (DM), caused by the obligate biotrophic oomycete *Pseudoperonospora cubensis* is a major foliar disease of cucumber. Cucumber accession PI 197088 was previously shown to be one of the most promising donors for DM resistance. The resistance in PI 197088 is controlled by multiple quantitative trait loci (QTLs), each with a relatively small effect. We recently fine-mapped one of the QTLs in order to identify potential causal genes. We combined fine mapping data with RNAseq and whole genome resequencing. In one of the mapped QTL regions, we identified a locus that contains several *Receptor Like Kinase* genes (*RLK*). Interestingly, we found evidence for the presence of a novel *RLK* gene at this locus in resistant genotypes. In susceptible genotypes, including the reference genotype 'Chinese Long 9930', this novel gene has a 551 base pair (frameshift) deletion, and was therefore not correctly predicted during the annotation of the cucumber genome. In order to functionally characterize the novel *RLK* gene, we cloned it from resistant and susceptible genotypes, and transiently expressed both alleles in leaves of *Nicotiana benthamiana* by infiltration with *Agrobacterium tumefaciens*. We found that whereas the functional allele of the gene triggered a strong defense reaction consisting of necrosis of infiltrated tissue, the loss-of-function allele had no effect. Comparing the *RLK* genes at this locus, our novel *RLK* gene is very similar (>90% identical) to the neighboring *RLK* genes concerning the predicted extracellular domain, whereas the predicted intracellular kinase domains are very different (<30%). We hypothesize, therefore, that the different *RLK* genes at the locus might be responsive to similar extracellular stimuli, but might trigger different intracellular signaling cascades. Interestingly, the kinase domain of the novel *RLK* gene is homologous to several *Arabidopsis thaliana* *RLK* genes with roles in disease resistance. The *RLK* genes in this locus contain predicted extracellular oligogalacturonan-binding domains. Oligogalacturonan, a breakdown product from pectin polymers in the plant cell wall, was previously shown to be a damage-associated-molecular-pattern (DAMP) which can elicit strong defence responses in plants. We are currently investigating whether genotypes with/without this novel oligogalacturonan-binding *RLK* gene react differently to oligogalacturonan.

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

Resequencing of Bottle Gourd Germplasm and Using QTL-seq to Fine-Map PRSV-W Resistance in Bottle Gourd (*Lagenaria sinceraria*)

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Bottle Gourd, a native of Africa is widely grown in the East Asian countries for food, medicine and decorative purposes. It is also used as an important rootstock to improve cold tolerance and disease resistance in other cucurbits, like watermelon and melon. Viral diseases are considered a major threat to cucurbit crop productions worldwide. The most prevalent viruses are aphid-transmitted *Papaya ringspot virus* watermelon strain (PRSV-W) and *Zucchini yellow mosaic virus* (ZYMV). Commercially available bottle gourd cultivars are susceptible to these viral diseases. Traditional breeding takes years to develop a disease-resistant cultivar. With the recent advancement in the sequencing technologies, we aim to accelerate the molecular breeding for trait improvement. We recently sequenced the bottle gourd genome and used genotyping-by-sequencing to map the PRSV-W resistance locus in an F2 population. A dominant monogenic locus *Prs* was mapped in a 317.8 Kb region on chromosome 1 still containing 39 annotated genes. In the current study, through QTLseq analysis on pooled libraries with the 10 most PRSV resistant or susceptible individuals from six F3 populations, we aim to fine-map the *Prs* locus and to identify the candidate resistance gene for PRSV-W. In addition, through screening the USDA collections of bottle gourd germplasm, significant variations in the level of resistance to PRSV-W were identified in 154 Plant Introductions (PI) evaluated. Through genome re-sequencing (GBS) of these PI accessions, we intend to use genome-wide association studies to confirm genes or SNPs linking to the PRSV-W resistance in bottle gourd. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

Advances in Molecular Breeding for Disease Resistance in Cucumber

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Cucumber is an economically important worldwide vegetable crop. However, serious losses in yield and quality are a frequent occurrence due to a range of diseases. Many of these are not able to be controlled by conventional means, so the development of resistant cultivars offers a way to increase both production and quality. This could be achieved by traditional breeding, but a more useful approach would be to use marker-assisted selection (MAS). In 2009, CAAS-IVF completed the cucumber whole genome sequence, thus providing numerous markers for MAS. In this paper two genetic maps were constructed: a high-density map made from 248 SSR loci, and an ultra-high-density map made from 116,710 SNPs. The inheritance of resistance to several important diseases was identified and mapped. Cucumber scab resistance was found to be controlled by a single dominant gene, *Ccu*, which was mapped to Chr2 with an accuracy rate of 100% for the flanking marker, Indel01. For resistance to Fusarium wilt, the peak marker SSR17631 had an accuracy rate of 87.88% for detecting the major QTL, *Foc2.1*. Four QTLs related to powdery mildew resistance (*pm5.1*, *pm5.2*, *pm5.3*, and *pm6.1*) and five QTLs related to downy mildew resistance (*dm1.1*, *dm5.1*, *dm5.2*, *dm5.3*, and *dm6.1*) were detected. Fine mapping of resistance to *Watermelon mosaic virus* indicated that it is controlled by the single recessive gene, Csa6G421660, and the accuracy of the SNP marker WMVSNP1 has an accuracy rate of 100%. Inheritance analysis of resistance to papaya ringspot virus indicated control by a single recessive gene flanked by two SSR markers, SSR11-177 and SSR11-1 on Chr6. Marker SSR11-1 has an accuracy of 94% in resistant lines. One major QTL, *cmv6.1*, which was delimited by SSR9-56 and SSR11-177, explained 31.7% of the phenotypic variation. Five QTLs related to resistance to Gummy stem blight (*gsb-s1.1*, *gsb-s2.1*, *gsb-s6.1*, *gsb-s6.2*, and *gsb-s6.3*) were identified. The major locus, *gsb-s6.2*, accounted for the highest phenotypic variation of 22.7% and was flanked by markers SSR04083 and SSR02940. Using these markers, more than 30 new cucumber cultivars with multiple resistance have been developed that are grown on more than 700,000 ha throughout China.

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

Relative Susceptibility of Commercial Watermelon Varieties to Powdery Mildew and Phytophthora Fruit Rot

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Powdery mildew (PM, *Podosphaera xanthii*) and Phytophthora fruit rot (*Phytophthora capsici*) have been occurring frequently in recent years in commercial watermelon fields and growers routinely apply fungicides to manage these two diseases. Both these diseases are known to cause significant yield reduction. The current study was conducted in 2014, 2015 and 2016 to determine the relative susceptibility of twenty six watermelon varieties (seeded and seedless) and three pollenizers to PM and fruit rot in South Carolina. USVL677-PMS, which is highly susceptible and USVL531-MDR, which is resistant to PM and fruit rot were included as controls. A randomized complete block design with three replications was used for planting each year. Naturally occurring PM infection on plants were rated on a 0-10 scale of increasing disease severity. Mature fruit were harvested from all the variety plots and inoculated with a 7-mm plug from an actively growing colony of *P. capsici*. Inoculated fruit were kept on wire shelves in a large chamber (Temperature 26±2 °C) with high relative humidity (≥90%) and free moisture to enhance disease development and prevent drying of agar plugs. Five days after inoculation the lesion diameter and sporulation intensity were recorded. During all three years USVL677-PMS was the most susceptible to PM (70%, 3 year mean disease severity) with highest area under disease progress curves. In comparison, USVL531-MDR (2%) was very resistant to PM. The commercial pollenizers, SP5, SP6 and Lion were all resistant to PM (4.2%). Among the red fleshed varieties, Suprema, (seedless variety) was relatively resistant (20%) compared to other seeded and seedless varieties. Most of the seeded varieties evaluated (e.g. Malali, Black Mama, Mickey Lee) were highly susceptible to PM, however, some were relatively less susceptible (e.g. Declaration) under field conditions. Except for the resistant control USVL531-MDR (0.9 cm lesions) and the pollenizer Lion (2.5 cm), all the other watermelon varieties were highly susceptible to Phytophthora fruit rot (7-13 cm). Although resistance sources exist in watermelons, the resistance has not been bred into commercial varieties. There is a critical need to develop varieties with high levels of resistance to PM and/or Phytophthora fruit rot. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

Novel Source of Resistance to Fusarium Wilt Race 1 Identified in Citron Melon

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The fungal pathogen *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *niveum* (*Fon*) causes Fusarium wilt of watermelon, which limits production world-wide through yield loss and plant death. Thus far, the only genetic source(s) of host-plant resistance to *Fon* race 1 have been identified on the distal end of chromosome 1 in both *Citrullus lanatus* (cultivated watermelon) and *Citrullus amarus* (citron melon). The breakdown of host-plant resistance can be inhibited through gene pyramiding of multiple sources of resistance into a common genetic background. Cultivated watermelon and citron melon readily cross so disease resistance alleles found in citron melon can be introgressed into cultivated watermelon. Here, we identified a novel source of resistance through QTL mapping using segregating F_{2:3} and recombinant inbred line *C. amarus* populations. The *Fon* race 1 resistance QTL (*qFon1-9*) may confer non-race specific resistance to Fusarium wilt as it collocates on chromosome 9 with a previously identified QTL (*qFon2-9*) for *Fon* race 2 resistance in citron melon. Thus, *qFon1-9* provides watermelon breeders with a valuable genetic resource for the improvement of Fusarium wilt resistance in watermelon.

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

Linkage Map Construction and QTL Analysis for *Cucurbit chlorotic yellows virus* Resistance in Melon

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Cucurbit chlorotic yellows virus (CCYV) infects many *Cucurbitaceae* species including important crops like melon, cucumber, watermelon and pumpkin. CCYV is transmitted by the sweet potato whitefly, *Bemisia tabaci*. CCYV infection induces chlorotic spots and yellowing symptoms on melon leaves and causes significant decrease in sugar contents of melon fruits, which reduces their market value. A melon accession with resistance to CCYV has been reported, but there is no genetic information on its CCYV resistance. In this study, we generated two F₂ populations using the resistant accession and two susceptible cultivars. Genetic linkage maps were constructed using SSR and In-Del markers, and each map contained 12 linkage groups. CCYV resistance of each F₂ plant was evaluated in a greenhouse using *B. tabaci* carrying CCYV. QTL analysis was performed using each F₂ population, and one locus for CCYV resistance was detected on chromosome 1. Our results will accelerate developing DNA markers for marker-assisted selection for CCYV resistance in melon breeding.

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

New Sources of Resistance to Phytophthora Crown and Root Rot in *Cucurbita moschata* **Chandrasekar Kousik¹, Jennifer Ikerd¹, Mihir Mandal^{1,2}**

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Winter and crook neck squash (*Cucurbita moschata*) are important vegetable crops grown and consumed in most states in the USA. Among *C. moschata*, butternut type squash are the most popular and widely used across USA. Many *C. moschata* lines are also used to develop interspecific hybrid rootstocks for grafting watermelon in parts of Asia. However, most commercially available *C. moschata* varieties are highly susceptible to crown and root rot caused by the oomycete pathogen *Phytophthora capsici* which is prevalent in southeastern USA. As part of an USDA, NIFA SCRI grant, we evaluated all the available plant introductions (PIs) of *C. moschata* (319 PIs) for resistance to *P. capsici*. Four-week-old plants growing in 6.3-cm square pots were inoculated with 10⁴ zoospores from a local South Carolina (SC) isolate of *P. capsici*. Plants were rated for disease severity two weeks after inoculation using a 0-5 rating scale. The experiments were conducted twice. Twelve potential new sources of resistance (e.g. Grif 1738, PI 438724, PI 438778, PI 442280) to crown rot caused by the local SC isolate of *P. capsici* were identified. Variability in resistance reaction among plants within a PI was also observed, and not all plants were resistant. Further evaluation of S₁ and S₂ generation from the most resistant plants indicated that highly resistant plants could be selected from the 12 PI to develop lines for use in breeding programs. In a previous study done in Florida (FL), five sources of resistance to *P. capsici* (e.g. PI 176531, PI 458740) were identified in *C. moschata* by evaluating 119 accessions (Chavez et al., 2011, HortScience 46(4):536-540). Interestingly, Grif 1738 which was resistant to the isolate from SC in the current evaluation was susceptible to a FL isolate (Chavez et al., 2011), indicating the potential for existence of host specific races of *P. capsici* based on *C. moschata*. This also suggests that new sources of resistance should be evaluated against isolates from other states. These new sources of resistance can be utilized for developing new crown and root rot resistant rootstocks for watermelon grafting and for developing resistant varieties for human consumption. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project), and by USDA, NIFA, SCRI Vegetable Grafting grant award 2016-1498-08.

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

Genetics of Resistance to Powdery Mildew in Watermelon Line USVL608-PMR

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Powdery mildew (PM) of watermelon (*Citrullus lanatus*) and other cucurbits caused by *Podosphaera xanthii* is a major factor limiting production in greenhouse and open field. In recent years, occurrence of PM has been increasing on watermelon across the United States, and commercial watermelon cultivars with resistance are rare. Four PM resistant germplasm lines with broad resistance to isolates from South Carolina, Georgia, Florida, California and New York were developed from plant introductions and released in 2018 by USDA ARS. All four lines have red-pink flesh and hybridize readily with commercial cultivars and inbred lines. One of these, USVL608-PMR (S₆), a red fleshed watermelon line with high levels of resistance to PM was used as the female parent (P₁) and crossed with USVL677-PMS which is highly susceptible (P₂). The parents, F₁, backcrosses to both parents (BC₁, BC₂) and a large F₂ population were inoculated with a local isolate of PM and assessed for disease severity on a 0-10 scale of increasing disease severity. All susceptible parent (USVL677-PMS) plants were rated >7 [mean disease severity (DS) = 94%], whereas most resistant parent (USVL608-PMR) plants were rated as 1 (DS=2.5%). Majority of the BC₁ plants were rated ≤2 and considered as resistant. Of the 466 F₂ plants, 221 were rated ≤2 (DS=3.1%). Of the 76 BC₂ plants, 23 were rated ≤2 (DS=2.9%). Chi-square analyses of the observed segregation of phenotypes for the F₂ plants indicated that two genes control PM resistance with a good fit for a 7:9 resistance to susceptibility ratio. The proposed model for this ratio is two genes with one recessive for high resistance and one dominant for high resistance. This is supported by a backcrossing segregation ratio of 1:3. We have observed some highly and moderately resistant plants in the F₂ indicating the cumulative effect of the two genes. QTL-seq analysis on the extremes from the F₂ populations and RNA-seq analysis of the parents during PM infection are being conducted to identify the chromosomal regions involved in resistance. USVL608-PMR will serve as a useful source to incorporate PM resistance into commercial cultivars. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

QTL-seq of Young Fruit Resistance to *Phytophthora capsici* in Cucumber

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Phytophthora fruit rot caused by *Phytophthora capsici*, a soil-borne oomycete pathogen, can be a devastating disease for cucumber production. As young fruit are especially susceptible, the objective of this work is to identify quantitative trait loci (QTL) associated with young fruit resistance using QTL-seq analysis. A cucumber accession, PI 109483, was previously identified as a source of young fruit resistance and a resistant breeding line, MSU109483-53, was developed. Crosses were made between the susceptible pickling type cucumber breeding line, Gy14, and two resistant MSU 109483-53-derived lines: an S₆ generation line, B5, and a doubled haploid line, DH A4-3. The *P. capsici* isolate, Barley's 1, was used in all experiments. In the summer of 2017, F₂ progeny of Gy14 x B5 (n=397) along with parental lines and F₁ were grown in the field. To facilitate accurate phenotyping, plants were trellised to reduce wounding due to removal of soil during the cleaning process and lessen the possibility of contamination from other pathogens. The second population, F₂ progeny of Gy14 x DH A4-3 (n=222), along with the parental lines and F₁ were screened in the greenhouse in the spring of 2018. Replicate harvests were performed for each experiment providing a total of 10-50 fruits for each plant, and allowing replicated scoring for each individual. The normal distribution of disease scores of the F₂ population in each experiment suggested that young fruit resistance is a quantitative trait. Individuals with extreme resistant and susceptible phenotypes were selected from each population for QTL-seq analysis. QTL-seq analysis was performed by QTLseqr using two statistical approaches: QTL-seq and G'. A major QTL was identified on chromosome 6 in the 2017 experiment, potential additional QTL were located on chromosomes 1 and 3. In the 2018 experiment, QTLs were found on chromosomes 1, 2, 3, and possibly 7. A second F₂ population of Gy14 x DH A4-3 (n=362) was grown in the field and phenotyped in 2018 summer. The third population will provide additional data to identify the genomic regions associated with young fruit resistance.

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

Breeding for Resistance to *Phytophthora* Crown Rot in Squash

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Breeding cultivars resistant to *Phytophthora* crown rot is an important goal for squash breeders worldwide. The disease is particularly severe in south Florida where heavy rainfall coupled with hurricane-driven flooding results in rapid establishment and distribution of *P. capsici* spores across grower fields, leading to significant crop losses. Over the last decade, the cucurbit-breeding program at the University of Florida has identified resistance to *Phytophthora* crown rot in *C. pepo*, *C. moschata* and *C. lundelliana*. This paper reports efforts to introgress resistance from breeding lines #181761-36P (*C. pepo*) and #394-1-27-12 (*C. moschata*) into various cultivar groups of summer and winter squash using conventional and molecular breeding methods. Crosses (R x S) were initiated to develop F₁, F₂ and backcross progeny of #181761-36P x Acorn/Crookneck and #394-1-27-12 x Butterbush. These populations were screened for resistance to crown rot and a sub-population of 50 lines each for *C. pepo* and *C. moschata* exhibiting high resistance to crown rot were identified. These lines are currently undergoing further selection for disease resistance and horticultural performance. To facilitate efficient discrimination of resistant and susceptible genotypes, a QTL Seq. approach was employed to identify SNP markers associated with crown rot resistance in *C. moschata* using an F₂ population (#394-1-27-12 x Butterbush). In total, 1,334,918 SNPs were found among the parents, F₁ and resistant and susceptible bulks. Of these SNPs, 10.24% were located in the coding regions. SNP-index for the resistant and susceptible bulks were determined across the genome. Delta-SNP values were determined by subtracting SNP-index values of susceptible bulk from those of the resistant bulk. Five candidate markers (delta SNP>0.6) located in coding regions were identified as potential causal SNPs for crown resistance in #394-1-27-12. Efforts to validate these SNPs in independent populations are currently underway.

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

Gummy Stem Blight Resistance in Melon: Screening, Inheritance Pattern and Development of Molecular Markers

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Gummy stem blight (GSB) is one of the most destructive and economically important, soil borne diseases of melon caused by the ascomycete fungus, *Didymella bryoniae* throughout the world. In Korea, however, no GSB resistant genotype has been reported yet. We aimed to identify GSB resistant melon germplasm and to develop molecular markers linked to GSB resistance. We identified six resistant melon genotypes including inbred lines (PI 482399, PI 140471, PI 136170 and PI 420145) and cultivars (Asia Papaya and Supra) against GSB based on bioassay and molecular screening. Further, we developed 168 F₂ plants from the F₁ of a cross between the susceptible Cornell ZPPM 339, and the resistant PI 482399 lines. A 3:1 ratio of susceptible and resistant genotypes was observed in the F₂ population, indicating control by a single recessive gene. Nucleotide-binding site leucine-rich repeat (NBS-LRR) genes confer resistance against insects and diseases in cucurbits including melon. We cloned and sequenced the TIR-NBS-LRR-type resistance gene MELO3C022157, located on melon chromosome 9, from resistant and susceptible lines. Sequence analysis revealed deletions in the first intron, a 2-bp frameshift deletion from the second exon, and a 7-bp insertion in the 4th exon of the resistant line. We developed two insertion/deletion (InDel) markers, GSB9-kh-1 and GSB9-kh-2, found in the first intron of MELO3C022157 linked to GSB resistance. We validated these markers with the F₂ population and inbred lines. These InDels may be used to facilitate marker-assisted selection of GSB resistance in melon. However, functional analysis of is needed to confirm the frameshift mutation. This research was supported by the Golden Seed Project under Grant no. 213007-05-2-CG100.

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

Identification of New Resistance Sources in Watermelon for Anthracnose Race 2

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Anthracnose (*Colletotrichum orbiculare*) is reappearing as a major problem on watermelon (*Citrullus lanatus*). Watermelon anthracnose has three races, 1, 2 and 3. Earlier studies screened small sets of PIs for resistance to these races, but not the whole USDA, NPGS germplasm collection. The objective of this study was to identify accessions resistant to *C. orbiculare* race 2. The available watermelon germplasm collection of 1408 PI accessions was screened for resistance to anthracnose race 2 using seedlings in a greenhouse. The study had 1408 accessions in 2 replications, with a spore concentration of 10^5 sp/ml. Seedlings were rated three times at 3, 5 and 7 days post-inoculation. Data were analyzed with a repeated measure analysis using mixed models. A retest was performed using the 30 most resistant and 20 most susceptible accessions to confirm the results. We have identified new resistance sources of *Citrullus* for anthracnose race 2. In the future, we will run a GWAS analysis to identify SNPs and candidate loci for resistance.

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

Improving Gummy Stem Blight Resistance and fruit Quality in Watermelon Germplasm

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Development of watermelon (*Citrullus lanatus*) cultivars with resistance to gummy stem blight (GSB), caused by *Stagonosporopsis cucurbitacearum* (syn. *Didymella bryoniae*), has been slow, indicating complex inheritance. We have worked to combine high resistance and high fruit quality in watermelon inbreds. We developed a panel of RILs, which carries resistance genes to GSB and segregates for fruit quality traits, by crossing and intercrossing resistant plant introduction (PI) accessions and elite cultivars. The 300 RILs were evaluated for disease severity and fruit quality traits under greenhouse and field conditions in a randomized complete block design with 10 replicates in 2017 and 2018. Disease was rated on a scale of 0 (no damage) to 9 (plant dead). Severity was based on weekly ratings, as well as mean and maximum rating for each plot in the field and greenhouse. Around 200 RILs had disease severity ratings below the mean value of the disease assessment scale (4.5), indicating that they may carry some resistance genes for GSB. All disease severity ratings were correlated with each other ($r=0.67$ to 0.98 , $P < 0.001$) but not correlated with fruit quality traits. The moderate heritability estimated for the RILs (69%) indicates that selection for GSB resistance in early generations would be effective. A group of resistant RILs showed good to excellent fruit quality for the market. Our results provide evidence of improved germplasm for cultivar development of GSB resistant watermelons with good fruit quality.

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

A Major QTL Located in Chromosome 8 of *Cucurbita moschata* is Responsible for Resistance to Tomato leaf curl New Delhi virus (ToLCNDV)

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Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite whitefly-transmitted begomovirus, responsible since 2012-2013 season of severe damages in cultivated cucurbits in Southeastern Spain, mainly squash (*Cucurbita pepo*) and melon (*Cucumis melo*). The use of genetic resistances is the most efficient way to reduce the virus incidence. In previous works (Sáez et al., 2016, Ann Appl Biol 169:91–105), a core collection of *Cucurbita* accessions was screened against ToLCNDV using mechanical inoculation and natural infection with whiteflies. All *C. pepo* accessions were very susceptible to the infection, but the screening provided two *C. moschata* sources of resistance. In this assay, we studied the genetic control of the resistance to ToLCNDV in both resistant *C. moschata* accessions, crossing both with a high susceptible *C. moschata* control and between themselves. F₁ generations were selfed and backcrossed to generate the F₂ and BC₃ segregating populations. After mechanical inoculation with ToLCNDV, the response of the hybrids and their respective progenies was evaluated by symptoms scoring and by measuring viral titers (qPCR and molecular hybridization). The F₁ and F₂ offsprings of *C. moschata* resistant accessions cross, were symptomless and with low viral titers. Instead, F₁ plants offspring of susceptible x resistant crosses were very susceptible and with high viral accumulation levels. F₂ and BC₃ populations derived from plants of these susceptible F₁ segregated, suggesting a recessive control of the resistance in both resistance sources. All plants of the different generations were genotyped with a SNPs collection covering the whole *C. moschata* genome. A mayor QTL was identified in chromosome 8, tightly linked to the resistance to ToLCDV. The region identify is synthetic with the region in chromosome 11 responsible of resistance to ToLCNDV in melon, described in Sáez et al., (2017, Plant Cell rep. 36:1571–1584). Since *C. moschata* and *C. pepo* are partially crossable, the SNPs linked to the resistance are facilitating the marker-assisted introgression of ToLCNDV resistance in commercial zucchini and pumpkin breeding programs. Acknowledgements to Spanish Instituto Nacional de Investigaciones Agrarias (INIA) and European Union (FEDER) for funding projects E_RTA2013-00020-C04-03 and RTA2017-00061-C03-03, and to Generalitat Valenciana for funding the project Prometeo 2017/078 and the predoctoral fellowship ACIF/2016/188.

Session Topic

Breeding for Resistance (BR)

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Effect of Two Potyviruses on Development and Yield of Tropical Pumpkin

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Tropical pumpkin (*Cucurbita moschata* Duchesne) was mechanically inoculated at the cotyledon stage with potyviruses *Papaya ringspot virus*, watermelon strain (PRSV-W), *Zucchini yellow mosaic virus* (ZYMV), and the combination of the two viruses (ZYMV+PRSV). Plants mock-inoculated with buffer were used as controls. 'Nigerian Local' and 'Menina', genotypes known to be resistant to PRSV and ZYMV, and three susceptible genotypes were used. At four weeks seedlings were transplanted from the greenhouse to the field in Puerto Rico. The 20 treatment combinations (5 genotypes x 4 inoculation treatments) were arranged in a CRD with 4 reps. Plots consisted of single plants spaced 3.7 m apart within and between rows on beds with silver plastic mulch and drip irrigation. Plants were evaluated for virus titer by ELISA (at 20 days post-inoculation in the greenhouse and at 59 and 103 days post-inoculation in the field), flowering date (male and female), number of fruits, total fruit weight, average fruit weight, fruit diameter, pulp width and color, °Brix and percentage dry matter. 'Nigerian Local' and 'Menina' tested negative for virus infection in both the greenhouse and field while the other genotypes tested positive. ELISA tests showed that some cross-infection occurred after plants were transplanted to the field potentially influencing results from control plots, but the impact was thought to be minimal. Virus-infected plants generally took more time to flower. Plants infected with PRSV produced an average of only 2.2 fruits while control plants produced an average of 3.4 fruits. Plants infected with ZYMV or PRSV+ZYMV had fewer fruits than the control but the difference was not significant. Total fruit yield was almost 50% less in infected compared to control plants of susceptible genotypes. Fruit quality traits were unaffected by virus infection. This is the first known study to document the effects of these two potyviruses in tropical pumpkin at the field level. Early infection of tropical pumpkin with PRSV or ZYMV can result in significant economic losses for growers, demonstrating the importance of developing cultivars with genetic resistance to these two potyviruses. (Supported by USDA-NIFA-SCRI no. 2015-51181-24285, sub-award no. RC105573UPRM and Hatch accession no.1000526)

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

North Carolina State Cucumber Lines Developed for Downy Mildew Resistance

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Downy mildew, caused by *Pseudoperonospora cubensis*, is a devastating foliar disease of cucumber. The use of disease resistant (low leaf damage) and tolerant cultivars (low yield loss) has proven an effective practice to develop useful lines. Since 2004, the highly resistant downy mildew accessions have become only moderately or slightly resistant, due to a shift in the pathogen population. New strains of *P. cubensis* that are present in midwestern and southeastern US can overcome the resistance of most cultivars. In an effort to assist the industry, we developed three populations of cucumber with moderately resistant (MR) to highly resistant (HR) families, combined with good fruit quality. The populations were NC-25 x PI 197088 (13 families), Gy 14 x PI 197088 (17 families), and Poinsett 76 x PI 197088 (15 families). The three populations were evaluated in Clinton, NC under natural disease incidence for several years using three replications in a randomized complete block design. Susceptible (S) and slightly resistant (SR), moderately resistant (MR), or highly resistant (HR) checks were included in the field trials: 'Poinsett 76' (MR), NC-25 (MR), Gy 14 (MR), PI 179088 (HR), 'Coolgreen' (S), and 'Ashley' (SR). Disease damage was rated weekly using a 0-9 scale based on 0-100% disease incidence. Yield was measured (number of fruit per plot) in two harvests, and fruit quality was rated on a 1-9 scale (1-3 = low, 4-6 = moderate, 7-9 = high). Data were analyzed using PROC GLM (SAS 9.4). Selections of the best families obtained from self-pollination in the greenhouse were made from the three populations based on resistance and quality. Those included three lines from NC-25 x PI 197088, four from Gy 14 x PI 197088, and two from Poinsett 76 x PI 197088. The lines were again self-pollinated in the greenhouse to produce nine lines. The nine lines will be self-pollinated again in the greenhouse, and resistant germplasm will be released after a repeat of the process in the summer of 2019.

Session Topic

Breeding for Resistance (BR)

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A Loss-of-Susceptibility Mutation in the *STAYGREEN* Gene (*CsSGR*) Provides Durable, Broad-spectrum Disease Resistances for U.S. Cucumber Production

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The Gy14 and WI2757 cucumber inbred lines are resistant to multiple diseases including the oomyceteous downy mildew (pre-2004 strains), the bacterial angular leaf spot (ALS) and the fungal anthracnose (AR) pathogens. These resistances have been widely deployed in commercial cucumber varieties and provided effective protection to cucumber production in the U.S. since the 1960s. However, the causal genes and underlying molecular mechanisms are unknown. We conducted QTL mapping for DM, AR and ALS resistances in the two lines and further map-based cloning to identify the candidate genes for the resistant loci. We show that the triple-disease resistances in Gy14 were controlled by the *STAYGREEN* (*CsSGR*) gene that encodes the magnesium dechelatase that plays important regulatory roles in senescence-inducible chlorophyll degradation. Its candidacy was validated with evidence from spatial-temporal gene expression profiling, allelic diversity and phylogenetic analysis, as well as local association studies. We found that the triple-resistance was due to a SNP in the coding region of *CsSGR* that resulted in a nonsynonymous amino acid substitution of Q to R amino acid in the *CsSGR* protein. Genes in the chlorophyll degradation pathway showed differential expression between resistant and susceptible lines in response to pathogen inoculation. The region harboring the causal SNP was significantly associated with disease resistances in natural and breeding populations, which has undergone selection in cucumber breeding. We conclude that the resistance is due to loss-of-susceptibility of the *CsSGR* gene. A working model to explain the molecular mechanisms of *CsSGR*-mediated multiple disease resistance is proposed. Supported by the Agriculture and Food Research Initiative Competitive Grants under award numbers 2013-67013-21105 and 2015-51181-24285 from the U.S. Department of Agriculture National Institute of Food and Agriculture. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

Disease Assessment in Seedlings of Tropical Pumpkin Infected with PRSV and ZYMV

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Papaya ringspot virus (PRSV) and *Zucchini yellow mosaic virus* (ZYMV) frequently infect tropical pumpkin (*Cucurbita moschata*) in Puerto Rico. Breeding programs depend on efficient and reliable methods of assessing resistance. A series of experiments were conducted to determine the best method to differentiate the level of resistance among genotypes of tropical pumpkin for both PRSV and ZYMV, to determine if there are differences in seedling development of resistant vs. susceptible genotypes when infected with these viruses, and to determine if resistant genotypes inoculated with these viruses can infect susceptible genotypes. In Experiment 1, cotyledons of lines known to be either resistant or susceptible were inoculated with PRSV or ZYMV, and ELISA readings were taken from the first four leaves, sampling as each leaf expanded or sampling after all four leaves were expanded. In Experiment 2, cotyledons were inoculated with PRSV, ZYMV or PRSV+ZYMV, and then harvested and weighed at 18 days post-inoculation. In Experiment 3, susceptible genotypes were inoculated with sap from resistant genotypes 'Nigerian Local' and 'Menina' that had been previously inoculated with PRSV or ZYMV. The susceptible genotypes were tested with ELISA. ELISA readings <0.400 were considered negative for presence of virus. Small leaves, interveinal chlorosis, leaf deformation, curled leaves, and mosaic were some of the symptoms observed in susceptible genotypes inoculated with PRSV and ZYMV. Smaller leaves and chlorosis were observed in 'Menina' inoculated with PRSV and ZYMV and no symptoms were observed in 'Nigerian Local'. For PRSV, sampling of the fourth leaf was required to clearly differentiate between resistant vs susceptible genotypes. For ZYMV, leaves 2, 3, and 4 can be used to differentiate resistant from susceptible genotypes. No differences were found in ELISA readings from plants with single versus double inoculation. No differences in fresh and dry weight were found between uninoculated and inoculated plants. Negative ELISA readings were obtained when susceptible genotypes were inoculated with sap of previously inoculated resistant genotypes. Both 'Nigerian Local' and 'Menina' were observed to be useful sources of resistance to ZYMV and PRSV in tropical pumpkin. (Supported by USDA-NIFA-SCRI no. 2015-51181-24285, sub-award no. RC105573UPRM and Hatch accession no.1000526)

Session Topic

Breeding for Resistance (BR)

Breeding for Resistance (BR)

Innovation Research on Germplasm Resources of Watermelon with Resistance to *Fusarium Wilt*

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Watermelon *Fusarium* wilt disease caused by *Fusarium oxysporum* f. sp. *niveum* is a destructive soil-borne disease in watermelon production. Breeding for resistance is the most advocated strategy to circumvent this disease. Marker assisted backcross breeding was employed to incorporate the *Fusarium oxysporum* f. sp. *niveum* race 1 resistance gene *Fon-1* from donor parents 'Calhoun Gray', 'F211' and 'BW85' into nine cultivated susceptible watermelon varieties (No.1 to No.9) used as recurrent parents. The molecular markers tightly linked to gene *Fon-1*, CAPS marker 7716_fon was published in 2013 by Beijing Vegetable Research Centre and Indel marker InDel1_fon1 was published in 2017 by Zhengzhou Fruit Research Institute. 'Calhoun Gray', 'F211' and 'BW85' were used as male parent and the cultivated susceptible watermelon varieties (No.1 to No.9) were used as female parent, 25 combinations (F_1) were assembled, then F_1 plants were backcrossed with respective recurrent parents (No.1 to No.9). Molecular identification of *Fusarium* wilt resistance for the recurrent parents and F_1 plants using CAPS marker (7716_fon) was carried out. The identification results of molecular detection were associated with *Fusarium* wilt resistance except in No.4 variety. The result indicated that 8 recurrent parents were with susceptible genotype for *Fusarium* wilt race, and 22 combinations expressed as heterozygous resistant genotype, No.4 variety and 3 combinations assembled using No.4 variety as female parent were *Fusarium* wilt resistant genotype. The marker is co-dominant marker, which can be used for the distinguishing of homozygous and heterozygous genotypes. The backcross generation BC_1F_1 foreground selection was carried out by the indoor seedling artificial inoculation, molecular marker detection, and screening in natural disease nursery (population levels of *Fusarium* in soil samples were 2.52×10^4 CFU·g⁻¹ of soil), BC_1F_1 plants are at fruiting stage currently. The gene positive BC_1F_1 plants which were heterozygous genotypes chosen through molecular identification planted in natural disease nursery show different *Fusarium* wilt resistance, eight combinations were expressed as highly resistance with the diseased plant rate under 20 %, seven combinations were susceptible with the diseased plant rate over 60 %, it may have related to the recurrent parent genetic background. The survival BC_1F_1 plants will be backcrossed with respective recurrent parents, the best BC_3F_1 plants from each of the combinations will self to generate BC_3F_2 populations and plants homozygous for the target gene will be isolated. Further, selected plants will advance till BC_3F_4 generation and best families with resistance to *Fusarium* wilt from each of the combinations will be identified. At present, our team has bred 3 high quality watermelon varieties with high resistance to *Fusarium* wilt.

Session Topic

Breeding for Resistance (BR)

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Development of a Real-Time Fluorescence-Based Microplate Assay for Pathogen Growth on Plant Tissue: *Phytophthora capsici* Infection of Cucumber Fruit

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Cucumber fruit rot, caused by the oomycete pathogen *Phytophthora capsici*, can cause significant losses for cucumber production in the Midwestern U.S. An age-related resistance (ARR) to this pathogen was observed previously in fruit of cultivar 'Poinsett76'. Young rapidly growing fruits are highly susceptible, but become resistant as they approach full size. ARR is increasingly recognized as an important defense against pathogens, however, the basis for resistance is still unclear. Our objectives were to develop a high-throughput, reliable, and sensitive, real-time detection method for *P. capsici* in cucumber fruits to allow for quantitative measurement of pathogen growth in different genotypes, ages or treatments. A fluorescence-based microplate assay was developed using the *P. capsici* isolate NY0664-1 expressing red fluorescent protein gene *tdTomato* and the Spark[®] multimode microplate reader. Cucumber peel sections (5-6 mm thick) were sampled with a 6 mm biopsy punch placed in individual 6.5 mm microtiter plate wells, and inoculated with 5 ul of 1.0×10^6 *P. capsici* zoospores/ml. By applying this method, pathogen on the tissue could be detected immediately after inoculation. Thereafter, the increase in signal was rapid in susceptible fruit and slower in resistant fruit. The amount of pathogen quantified was highly correlated with susceptibility to *P. capsici*. The method developed in this study provides rapid, accurate and sensitive quantification of pathogen before symptoms were observed, allowing real-time monitoring of pathogen growth on tissue. The quantitative nature of the bioassay described in this study may be useful both in cucumber breeding and basic research.

Session Topic

Breeding for Resistance (BR)

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Molecular Mechanism of *CsWIN1* in regulating Cuticular Wax Biosynthesis in Grafted Cucumber

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Fruit appearance quality is one of the important commercial quality characters for cucumber. Previous studies showed that cucumber bloom on the fruit surface was not only affected by environmental factors but also regulated genes including those related to wax synthesis related genes and silicon transport. Grafting to suit pumpkin was considered as an effective method to decrease bloom on cucumber fruit. To understand the molecular regulation mechanism of de-blooming by grafting, a cucumber 'Jingyan 116' was grafted on pumpkin 'Jingxinzhen 6' in the present study. RNA-seq and DNA methylation sequencing analysis were conducted on grafted and self-rooted cucumber. A total of 69 different expression genes were identified between grafted and self-rooted cucumber, including five up-regulated wax synthesis related genes (*Csa3G127750*, *Csa6G079750*, *Csa6G151790*, *Csa6G151810*, *Csa6G302180*) and four down-regulated aquaporin transporters (*Csa3G743400*, *Csa5G162580*, *Csa5G199270*, *Csa5G623360*). We also found 20 genes with DNA methylation in grafted cucumber. Consequently, we isolated an important candidate gene *CsWIN1*(*Csa3G017320*), which encodes an AP2/ERF-type transcription factor. *CsWIN1* is a homologous gene in *Arabidopsis thaliana* and positively regulates the biosynthesis of wax. Our results demonstrated that grafted cucumber fruit surface contained more wax and lower silicon than self-rooted cucumber. This study also provided valuable information for breeding glossy cucumber varieties by MAS and to facilitate decrease bloom research in other cucurbit crops.

Session Topic

Breeding for Resistance (BR)

Floral and Fruit Development (FD)

Floral and Fruit Development (FD)

Flowering, Yield and Fruit Quality of Three Organically Grown Zucchini Cultivars Evaluated in two Different Cropping Seasons

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In Germany the demand for organically produced zucchini is increasing. The usual cultivation period is from mid-May to mid/end-September. Temperature and growing-degree-days (GDD) influence plant development, they differ within this cultivation period. In this research it was hypothesized that the temperature affects the time from planting to flowering and the production of the first fruit in zucchini. Further, the needed temperature sum to build up the first flower is genetically driven and differs between cultivars. Hence, the objectives of this study were to evaluate the impact of temperature in different cultivation periods within a year on days to flowering and first fruit production for various zucchini cultivars and on overall marketable yield. For this purpose, three open pollinating cultivars were grown in 2017 in an organic field trial in Kleinhohenheim (Stuttgart, Baden-Württemberg, Germany). One green (cv. Leila), one yellow (KSZ-KB-Gelb.1) and one striped cultivar (cv. Cocozelle) were planted on 23.05.2017 and on 27.07.2017. The results showed that a minimum of 314 GDD in the first set and 291 GDD in the last set was necessary to induce the first flower. In both cultivation-sets, cv. Leila was the earliest cultivar showing the first female flower 16 and 19 days after planting with a GDD of 265 and 224, followed by the flowering of cv. Cocozelle 22 and 28 days after planting and a GDD of 327 and 318. The last cultivar KSZ-KB-Gelb.1 flowered 24 and 29 after planting with a GDD of 351 and 332. To produce the first fruit within the first planting date a higher GDD of 328 on average was needed when compared to the last planting date with 312. Cv. Leila had the first fruits 20 days after planting in comparison to 23 days at the later planting date, followed by cv. Cocozelle and KSZ-KB-Gelb.1. Overall the study indicated that a lower requirement of GDD results in earlier flowering, thus earlier fruiting and finally higher yields. In addition, cultivars with a lower GDD requirement might be more suitable also for planting dates in the later season of a year, when temperatures start to decrease.

Session Topic

Floral and Fruit Development (FD)

Floral and Fruit Development (FD)

Genetic Analysis of Trimonoecy in Watermelon

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Sex expression and sex determination are both regulated by the plant hormone ethylene in watermelon. The arrest of stamen development in female flowers of monoecious cultivars depend on the ethylene biosynthesis gene *CitACS4*. A single missense mutation in the coding region of this gene (*m*) promotes the conversion of female into hermaphrodite flowers, and therefore of monoecy (*MM*) into trimonoecy (*Mm*) or andromonoecy (*mm*). Andromonoecy and trimonoecy are undesirable traits in watermelon, since hermaphrodite flowers need to be emasculated when acting as female parents in hybrid seed production, and also because the traits are usually associated with a reduction in fruit set and fruit quality under greenhouse conditions. Trimonoecy occurs in heterozygous plants for the mutant *m* allele (*Mm*), but also in certain cultivars that are homozygous for the *CitACS4* WT allele (*MM*). 48 Spanish traditional cultivars and 4 commercial inbred lines (P84, P85, P86 and P87) were phenotyped for sex associated traits, and then genotyped for *M* and *m* alleles of *CitACS4*. Most of the *MM* cultivars were monoecious, but two *MM* cultivars (BG24 and BG31) and two *MM* lines (P84 and P85) were phenotyped as trimonoecious, which suggests the existence of additional mutant *CitACS4* alleles or genes regulating sex determination in watermelon. The sequencing of *CitACS4* in *MM* lines and cultivars demonstrated that the trimonoecious phenotype was not caused by a new *CitACS4* allele. By contrast, the segregation ratio between monoecious and trimonoecious plants in the F2 derived from the cross between P86 (monoecious) and P84 (trimonoecious) indicated that trimonoecy is regulated by a recessive gene other than *CitACS4*.

Session Topic

Floral and Fruit Development (FD)

Floral and Fruit Development (FD)

Sex Determinism Genes in Cucurbit Crops: Evolutionary History and Domestication

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Whereas most angiosperms plants are hermaphrodite, a diversity of type of flowers (male, female, or bisexual) on separate or on the same plants are present in Cucurbitaceae. Our previous works have shown that the genes *CmWIP1*, *CmACS7* and *CmACS11*, associated with three loci (*G*, *M*, *A*), control sex expression in melon. The ethylene biosynthesis enzyme ACC synthase-7 is responsible for the sex transition from monoecious (female and male flowers) to andromonoecious (hermaphrodite and male flowers). We demonstrated that syntenic orthologous genes encode for the ethylene rate limiting enzyme in *C. melo*, *C. sativus*, and *Citrullus lanatus* and that the inactivation of the enzyme leads to the development of stamens in female flowers in these plant species. These results clearly indicate that the ACS7 gene and the ethylene pathway was recruited for sex determinism across Cucurbitaceae before *Cucumis* and *Citrullus* divergence, which was estimated to 20 Myago. Interestingly, in cucurbit crops, functional alleles were lost in some landraces (in andromonoecious and androecious plants) but were maintained in some others (monoecious landraces). This made it possible to use genetic approaches, positional mapping and TILLING, to elucidate key components of the mechanisms that contribute to sexual type in cucurbit crops. This also highlights that andromonoecy was selected several times during the domestication processes of cucurbit crops.

Session Topic

Floral and Fruit Development (FD)

Floral and Fruit Development (FD)

Quantitative Trait Loci for Parthenocarpic Fruit Set in Cucumber Identified from Biparental and Natural populations

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Parthenocarpy is a horticulturally important trait in cucumber that is required for production in protected environments. It is also a desirable trait with potential for increasing yield and quality in processing cucumber production. Although many successful parthenocarpic cucumber varieties have been developed, the genetic and molecular mechanisms behind parthenocarpic fruit set (PFS) in cucumber are still not well understood. In our previous study, we identified several PFS QTL using an F_{2:3} population derived from a cross between highly parthenocarpic inbred line 2A and low parthenocarpic line Gy8. In this study, we conducted QTL mapping for PFS in cucumber using a double haploid segregating population derived from 2A×Gy8, and validated the QTL, *parth7.1* for early PFS. We also conducted a genome-wide association analysis (GWA) of this trait using a diverse panel of 129 cucumber lines. Genotyping of lines was performed through genotyped by sequencing (GBS) and resequencing data resulting in ~6,700 high quality SNPs. Phenotypic data for PFS were collected in multiple pollen exclusion environments. Six regions in cucumber chromosomes 1, 2, 3, 6, and 7 of the cucumber genome were detected with significant association of PFS between the environments. These regions included the *parth7.1* identified in the 2A with biparental populations. This study will provide meaningful insight for future genetic investigations of loci associated with PFS from this natural and biparental populations. National Institute of Food and Agriculture, U.S. Department of Agriculture, under award numbers 2015-51181-24285 and 2017-67013-26195. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project).

Session Topic

Floral and Fruit Development (FD)

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Genetic Interactions between *EIN1*, *EIN2* and *EIN3* in the Regulation of Sex Expression and sex Determination in Squash

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The plant hormone ethylene regulates different developmental processes, including sex determination and sex expression in the species of the *Cucurbitaceae* family. We have developed an EMS collection consisting on 3,751 mutant families in zucchini squash (*Cucurbita pepo* L.). The high throughput screening of the collection for ethylene triple response resulted in the identification of three ethylene insensitive mutants: *ein1*, *ein2*, *ein3*. Mutant were backcrossed with the background line MUC16, and sex expression and sex determination traits compared in WT and mutant plants of BC2S1 or BC3S1 segregating populations. The mutations *ein1*, *ein2* and *ein3* promoted the conversion of female into bisexual or hermaphrodite flowers, and therefore of monoecy into andromonoecy, but they also delayed the transition to female flowering and reduced the number of pistillate flowers per plant. The *ein2* and *ein3* mutations segregated as semi-dominant for ethylene triple response and sex expression, but andromonoecy only occurred in homozygous mutant plants. Homozygous plants for *ein1* were completely blocked in female flowering transition, but heterozygous *Ein/ein1* plants produced some hermaphrodite flowers. Since the three mutations were female sterile in homozygous condition, the genetic interactions between these three loci were studied in double heterozygous plants for each two mutant alleles. The loci did not complement each other, but they were found to be dose-dependent under certain mutant combinations. The additive or synergistic effects *ein* mutations on female flowering transition and sex determination suggests functional redundancy of genes regulating ethylene sensitivity, and sex expression and sex determination in *C. pepo*. The molecular homology between *EIN2* and *EIN3* genes also supports this conclusion.

Session Topic

Floral and Fruit Development (FD)

Floral and Fruit Development (FD)

Fine-mapping of a Major Quantitative Trait Locus *Qdff3-1* Controlling Flowering Time in Watermelon

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Flowering time is crucial in watermelon (*Citrullus lanatus*) production as it determines time of fruit set. Early flowering is desirable because it enables crops to escape biotic and abiotic stresses that are intensified by long production cycles. Production of seedless watermelon is also reliant on synchronized flowering of diploid pollenizers and the triploid watermelon cultivars. Incorporation of single nucleotide polymorphisms (SNPs) for marker assisted selection (MAS) of flowering time in watermelon breeding would potentially aid in selection for the early flowering trait, which would shorten the production time. Moreover, seedless watermelon breeding would be enhanced through appropriate triploid-diploid pairings. A major quantitative trait locus (*Qdff3-1*: 12Mbp-17Mbp) associated with days to female flower was previously identified on chromosome 3 of watermelon. This QTL contributes approximately 50% of the phenotypic variance. The objective of this study was to determine more precisely the interval of *Qdff3-1* and the gene controlling flowering time in watermelon. A combination of QTL-seq and candidate gene sequencing was used to identify SNP markers in the region. Validation and fine mapping through QTL-seq identified three candidate genes underlying *Qdff3-1*: *FT*, *TEMPRANILLO* and *PIP-kinase*. Kompetitive Allele Specific PCR (KASP™) assays were developed for the SNP markers identified. Potential markers for selection were tested on the recombinant inbred line (RIL) mapping population and a panel of cultivars to establish marker-trait association and determine their applicability in MAS for flowering time in watermelon. SNPs that represent potential tools for the refinement of the QTL have been identified and may be applicable in MAS of this trait in watermelon. To further delineate the QTL, recombinants were identified in the RIL and an F₂ population of 372 lines. Six recombinants were selected following genotyping, and F₃ populations developed from them for flowering time evaluation in summer 2018. Fine-mapping is currently ongoing and preliminary data indicates *FT* as the most likely candidate gene. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Floral and Fruit Development (FD)

Floral and Fruit Development (FD)

Genetic Characterization of a Key Regulator of Pigment Accumulation in Melon and Watermelon

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Color and pigment content are important aspects of fruit quality and consumer acceptance of cucurbits. Here, we describe the independent mapping and cloning of a common causative gene regulating pigment accumulation in melon and watermelon. We initially show that this gene is causative for the qualitative difference between dark and light green rind in both crops. Further analyses demonstrate the link between sequence or expression level variations at this gene and pigment content in rind and flesh of mature melon fruits. GWAS of young fruit rind color in a panel composed of 177 diverse melon accessions did not result in any significant association, leading to an earlier assumption that multiple genes are involved in shaping phenotypic variation of this trait. Through sequencing of 25 representative accessions and allelism tests between light rind accessions, we show that multiple independent SNPs in the gene are causative for the light rind phenotype. The multi-haplotypic nature of this gene explains the lack of detection power obtained through GBS-based GWAS and confirms the pivotal role of this gene in shaping fruit color variation in melon. This study demonstrates the power of combining bi- and multi-allelic designs with deep sequencing to resolve lack of power due to high haplotypic diversity and low allele frequencies. Due to its central role and broad effect on pigment accumulation, this gene is an attractive target for bio-fortification of cucurbit crops.

Session Topic

Floral and Fruit Development (FD)

Floral and Fruit Development (FD)

Towards Understanding and Predicting Fruit Quality in Winter Squash

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Fruit quality in winter squash is a complex trait made up of many components. To better understand fruit quality, we used RNA-Seq to obtain gene expression profiles in four squash cultivars from *C. moschata* and *C. maxima* that cover the spectrum of fruit quality. Through comparison of gene expression across key fruit developmental time points within and between cultivars, we are identifying gene expression differences that may underlie variation in important fruit quality components such as Brix, dry matter, starch, sugar, and carotenoid content. Results from this study along with the application of genomic prediction to improve fruit quality in squash will be discussed.

Session Topic

Floral and Fruit Development (FD)

Floral and Fruit Development (FD)

Chemical Characterization of *Cucurbita ficifolia* Bouché During its Development and its Hypoglycemic Effect

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Cucurbita ficifolia is known in Mexico as chilacayote and is consumed mainly in the Valley of Mexico. Recent studies have shown that the extract of this fruit has important hypoglycemic properties similar to the drugs used for the treatment of diabetes mellitus type 2 (DM 2) such as glibenclamide and tolbutamide. However, it is still unknown which compounds attribute this property to the chilacayote as well as its state of development in which this effect is greater. The objectives of this study were: 1) to characterize the main chemical compounds of chilacayote during its development and 2) to determine its hypoglycemic effect during its development. In order to achieve these objectives, seeds of *C. ficifolia* were sown and the flowers were marked with the date of anthesis, from this date samples were taken at 10, 15, 25, 30, 40 and 45 days for further analysis. The chemical characterization was by means of high performance liquid chromatography (HPLC) Agilent. For the determination of the hypoglycemic effect, male mice of strain CD-1 were used, groups were formed to which the different extracts were administered, and a positive control was used administered with glibenclamide and a control with isotonic saline solution. The compounds found were gallic acid, quercetin, catechin, kaempferol and myristicin in different concentrations throughout their development. The hypoglycemic effect showed significant differences in its early stages ($P < 0.05$) of development, however this effect is in all stages of the development. So it can be concluded that this fruit is a good alternative for the treatment of DM 2.

Session Topic

Floral and Fruit Development (FD)

Floral and Fruit Development (FD)

Use of Grafting to Promote Flowering in Late and Short-day Flowering Cultigens of Squash

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Wild species and landraces of *Cucurbita* are important resources for plant breeders as they often contain novel genes for economically important traits. Utilizing such germplasm presents challenges to breeders in temperate climates because wild species and landraces from tropical and subtropical regions may flower late in development or only under short days. Although previous researchers have induced flowering in recalcitrant flowering cucurbit cultigens by grafting them to early flowering rootstocks, details of methodology and floral development are scant, and there has been no confirmation of fruit set and seed production from these efforts. We sought to develop a grafting method that would reliably induce flowering and fruit set in a short-day flowering landrace of *C. moschata* and the short-day flowering species, *C. ficifolia*. Initial experiments revealed leaf removal from the scion is necessary, presumably to prevent synthesis of an inhibitor molecule. Grafts performed at the one and two leaf stage of rootstocks failed to induce flowering of scions. Scion insertion into rootstock by a cleft graft at or above the 4th node together with leaf removal from the main stem of the scion for 20 nodes was effective in causing floral induction. Vigorous shoot development in a lateral branch of the rootstock between nodes 0-4 also proved to be necessary as a source of photosynthate for the scion. In a greenhouse study, once floral induction occurred, flowering continued even with renewed leaf growth along the scion main stem; however, flowers aborted prior to fruit set. Without leaf removal, flower buds were initiated at early nodes on scions, but aborted early in development. In field studies conducted in 2018, flowering was again induced with grafting and leaf removal for 20 nodes, and in the *C. moschata* accession, fruit set and growth was obtained. Self-grafted plants with and without leaf removal, un-grafted plants with and without leaf removal, and grafted plants without leaf removal all failed to flower. This method should be a useful tool for plant breeders and curators of *Cucurbita* germplasm.

Session Topic

Floral and Fruit Development (FD)

Floral and Fruit Development (FD)

CsERF31 and CsERF39 Play Key Roles in Cucumber Female Flower Differentiation by Activating M (CsACS2)

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In cucumber (*Cucumis sativus* L.), the differentiation and development of female flowers are important processes that directly affect the fruit yield and quality. Sex differentiation is mainly controlled by three ethylene synthase genes, F (CsACS1G), M (CsACS2), and A (CsACS11). Thus, ethylene plays a key role in the sex differentiation in cucumber. The “one-hormone hypothesis” posits that *F* and *M* regulate the ethylene levels and initiate female flower development in cucumber. Nonetheless, the precise molecular mechanism of this process remains elusive. The functional analysis of the *M* gene suggested that the activation of *M* was essential to accumulation of ethylene signal. To investigate the ethylene-mediated sex differentiation process and the interplay mechanism between *F* and *M*, three cucumber chromosome segment substitution lines with different *F* and *M* loci were generated. According to the transcriptome analysis of the three lines, we identified two *ERF1*-like genes, *CsERF31* and *CsERF39*, as the key players in female flower initiation. *CsERF31* and *CsERF39* had especially high expression levels in *FFMMAA* lines. Both of genes showed high expression in 1-2 mm female bud, declining with the development of female flower, which is similar to *M*. In addition, the expression of *CsERF31* and *CsERF39* were significantly induced by ethephon and suppressed by AgNO₃ and Aminoethoxyvinyl Glycine (AVG). These results indicated that *CsERF31* and *CsERF39* were involved in female flower differentiation through response to the ethylene signal. The biochemical experiments further demonstrated that *CsERF31* and *CsERF39* bind directly the ERE-box in the promoter of *M* and activate its expression. Thus, we suggest in female flower initiation, *CsERF31* and *CsERF39* responded to the ethylene signal derived from *F* and mediated the positive feedback regulation of ethylene by activating *M*; *M* amplifies the ethylene signal via *CsERF31* and *CsERF39*, which form a “Ethylene-*CsERF31/39*-*M*-Ethylene” positive feedback regulation. Then, the genes, activated by the high-level ethylene signal promote female flower initiation. In conclusion, our research offers an extended “one-hormone hypothesis” of sex differentiation in cucumber.

Session Topic

Floral and Fruit Development (FD)

Genetic Resources (GR)

Genetic Resources (GR)

Where in The New Melon Classification Schemes Does *Cucumis melo* ssp. *agrestis* var. *texanus* Belong?

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Melon, *Cucumis melo* L., is one of the most important fruit crops worldwide, and is highly diverse for many vegetative characters, fruit color, size, shape, soluble solids, flavor, aroma, and ripening. Thirteen schemes for melon classification have been proposed since 1859, the most recent of which recognized 19 horticultural groups. Wild North American melon populations were recognized as *C. melo* ssp. *agrestis* var. *texanus* in 2002, but were overlooked in 3 subsequent revisions to melon classification. This wild melon is found in southeastern U.S. and Mexico, is weedy and bears many small, non-sweet, often bitter, yellow fruit, and is a potential source of resistances to powdery mildew and nematodes. It is similar for some traits to groups Chito (syn. ssp. *melo* var. *chito*), which is found in Central America and Caribbean countries, and Dudaim (syn. ssp. *melo* var. *dudaim*), which is cultivated in Central Asia, but were considered to be distinctively different from *texanus* based on expression of 43 morphological and 2 phenological quantitative traits, and a total of 40 RAPD and SSR markers. We analyzed genetic variation among more than 2,000 *C. melo* accessions in the U.S. National Plant Germplasm System, including 44 *texanus*, 4 putative Chito and 2 putative Dudaim accessions, as well as heirloom varieties and important cultivars using genome-wide SNP markers that were derived via the Genotyping-by-Sequencing (GBS) method. The *texanus* accessions clustered together, isolated from other Groups in phylogenetic and principal component analyses (PCA). The Chito accessions fell in 2 different groups distinct from *texanus*. Dudaim clustered with 3 of the Chito accessions. Nine additional Chito accessions were, therefore, obtained from M. Pitrat, Institut National de la Recherche Agronomique (INRA), Avignon-Montfavet, France for an ongoing comparative GBS and phenotypic analyses with the *texanus* accessions in order to more clearly characterize Chito and determine whether *texanus* constitutes a distinct Group or is a sub-group of Chito. Several of the Chito reference accessions used to delimit *texanus* were also included in this analysis. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Genetic Resources (GR)

Genetic Resources (GR)

Diversity of Lesser Known Cultivated *Cucurbita* from Latin America: Landraces to Locally Grown Cultivars

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There are perhaps over a thousand named varieties, or cultivars, of *Cucurbita*, which represent an astounding diversity. The exact number is difficult to ascertain since names change over time and from region to region, and there are many synonyms, as well as new varieties released by breeders every year. Taxonomically, these fall into multiple species from the Americas, including three primary species, i.e., *Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata*; two species of lesser worldwide economic importance, i.e., *Cucurbita argyrosperma* and *Cucurbita ficifolia*; and landraces from an additional species, *Cucurbita ecuadorensis*. Seed catalogs today generally list far more cultivars than in the past. But whether this represents an increase in genetic diversity is doubtful. For example, what was once a landrace, may have been selected for a number of named cultivars, but with an overall erosion in total genetic diversity. While there are fewer major seed houses than in the past, this is not a major issue as far as a loss in squash diversity, since many alternative online sources of seeds have arisen, from start-up private seed companies to seed saver organizations to e-commerce, such as eBay and Etsy. In addition to these seed sources, there are noteworthy locally grown cultivars. These are not more widely distributed for a number of reasons. Perhaps the main reason is they are locally adapted to certain growing conditions, such as short-day photoperiodism in the tropics, and thus will not set fruit in temperate regions. Most of the cultivars of *C. ficifolia*, as well as many *C. moschata*, are short-day sensitive, to the extent that there is far more morphological variation within both of these species than is generally recognized. Some cultivars are selected for uses not popular elsewhere, such as their edible leaves and shoot tips, sprouts, flowers, or seeds. The *C. moschata* 'loche' is grown only on the northern coast of Peru in a special way that helps concentrate its flavor, so that it becomes more of a seasoning than a vegetable.

Session Topic

Genetic Resources (GR)

Genetic Resources (GR)

Development of Novel Sets of Reciprocal Introgression-line Collections in Melon

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Introgression line (IL) collections are composed of individuals sharing a high proportion of genetic background from a recurrent parent, differing only in a specific region of a genomic introgression inherited from the donor parent. Two sets of reciprocal ILs were generated from the intraspecific cross between a non-climacteric, inodorous 'Piel de Sapo' (PS) type (*C. melo* ssp. *melo* group *inodorus*) and the highly climacteric Charentais-type 'Vedrantais' (VED) (*C. melo* ssp. *melo* group *cantalupensis*). Marker assisted backcrossing and selection followed by selfing was employed for up to six generations to develop the core IL sets. The VED IL collection is composed of 38 lines with a mean introgression size of 15.6 Mb covering 91% of the VED genome in the PS genetic background. The PS IL collection was also comprised of 38 lines with introgression lengths ranging from 2.4 to 33 Mb and covering 95% of the PS genome in the VED genetic background. A preliminary phenotypic analysis of the IL collections in autumn of 2017 showed a high level of segregation for different traits related to plant architecture and vigor, flowering time, fruit set rate, and especially for fruit quality and ripening behavior. Several QTL that were mapped in a VED x PS RIL population were successfully validated in the ILs. In summer of 2018 exhaustive phenotypic analyses for n=5 fruits of each line of both populations will be performed. These collections should serve as an outstanding resource for trait diversification in melon, and fine-mapping and cloning of QTL for traits related to fruit quality.

Session Topic

Genetic Resources (GR)

Genetic Resources (GR)

Exploring Spanish Watermelon Diversity for Resistance to Fungal Diseases

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Watermelon [*Citrullus lanatus* (Thumb.) Matsum. and Nakai] crops are affected by several soil-borne fungi as *Fusarium oxysporum* f. sp. *niveum* (*Fon*), *Monosporascus cannonballus* and *Macrophomina phaseolina* and more recently, by powdery mildew caused by *Podosphaera xanthii* which has become a concern among growers. In Spain, little research has been done until now searching for resistance sources, in spite of the occurrence and spread of these diseases and the existing and unexplored Spanish watermelon diversity. One hundred and twenty one Spanish watermelon accessions were artificially inoculated with the isolate RZ1 belonging to *P. xanthii* race 1W by using a conidia suspension ($4,2 \times 10^4$ cel/mL). No immune watermelon accession to powdery mildew was found and most of them were highly susceptible, with profuse sporulation of the fungus. However two accessions of *C. lanatus citroides* (NC079249 and NC0100745) and two of *C. lanatus lanatus* (NC054847 and NC082460) showed very low level of sporulation. Sixty-four of these accessions were also inoculated with an isolate of *Fon* race 2 by using a conidial suspension (3×10^6 cel/mL). Most of the accessions were susceptible and only four accessions of *C. lanatus lanatus*, NC042492, NC026156, NC054866 y NC047502 showed a high level of resistance. This subset was also evaluated against *M. cannonballus* and *M. phaseolina*, inoculating the roots with an aggressive isolate of each pathogen, grown in wheat seeds (200 gr of infected wheat seeds/kg of peat) and with the toothpick/stem method, respectively. *M. phaseolina* was less aggressive in our conditions, and six highly resistant accessions were found. Interestingly, one of them was NC079249, also resistant to powdery mildew, and a second *citroides* accession NC100274. Two of the accessions resistant to *Fon*, NC042492 and NC026156, were also moderately resistant to *M. phaseolina*. *M. cannonballus* was very aggressive and severe root and hypocotyl damage was found in most accessions; the *citroides* accession NC100274 was one of the few highly resistant to *M. cannonballus*. Results allowed the selection of accessions multi-resistant to the main fungi affecting watermelon that will be used in breeding programs. This work was partially funded by Spanish grants AGL2017-85563-C2-1-R and AGL2017-85563-C2-2-R.

Session Topic

Genetic Resources (GR)

Genetic Resources (GR)

Oleaginous Potential of Seeds of *Cucurbita moschata* and *C. argyrosperma*

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Cucurbita species are New World cucurbits that have been grown since pre-Columbian times for their fruit pulp and seeds. Of the five cultivated species of *Cucurbita*, the two best adapted for growing in the warmest parts of the Americas are *C. moschata* Duchesne (tropical pumpkin) and *C. argyrosperma* Huber (silverseed gourd). The purpose of the present investigation was to evaluate the seeds of these two species as energy sources, focusing on ethereal extract (EE) content. We evaluated 394 accessions, 295 of *C. moschata* and 99 of *C. argyrosperma* subsp. *sororia*, from the *Cucurbita* collection maintained at the Vegetable Program of the Universidad Nacional de Colombia, Palmira. Five plants of each accession were grown out in a completely randomized design, within- and between-row spacing 3 m in a 1.6-hectare plot. A weighted selection index (WSI) was used for each variable: EE ($34.53 \pm 15.0\%$), Seed production per fruit (SPPF) (63.29 ± 20.0 g) and Number of fruits per plant (NFP) (5.3 ± 2.0). All three variables expressed significant differences ($P < 0.05$) between and within species and a highly significant ($P < 0.01$) positive correlation was observed between Seed weight per plant (PSP) and Total ethereal extract per plant (TEEPP) ($r^2 = 0.95$). *C. moschata* accessions that were identified by WSI as having the highest oleaginous potential were numbers 308, 129, 142, 144, 136, and 160. The most promising *C. argyrosperma* subsp. *sororia* accessions were numbers 256, 140, 260, 132, 107, and 68. The composition of the oil from the EE was predominantly polyunsaturated fatty acids, 70.1% and 68.3% from *C. moschata* and *C. argyrosperma*, respectively.

Session Topic

Genetic Resources (GR)

Genetic Resources (GR)

Reactions of Cornell Melon Breeding Lines to *Cucumber mosaic virus* and Their Horticultural Qualities

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Cucumber mosaic virus (CMV) has been classified into major subgroups I and II. CMV subgroup I has reemerged as a concern for melon (*Cucumis melo* L.) production in California and Arizona. Twenty-five melon breeding lines developed by Cornell University for resistance to CMV were, therefore, evaluated for their reactions to CMV in a greenhouse test, and for horticultural quality and adaption to the Central Valley of California in a field test. Cotyledons of melon seedlings were mechanically inoculated with a CMV subgroup I isolate collected from infected melon plants in California. Plants were individually evaluated for their reaction to CMV as evidenced by mosaic symptoms, and by ELISA using commercial antiserum against CMV for quantification of virus titer. Eleven lines were susceptible, with rates of infection ranging from 78% to 100% (n ranged from 8 to 10). Eight lines may be considered to be segregating for resistance, with 1 to 6 infected plants (n ranged from 5 to 9). Six lines were uniformly resistant, with zero infected among 5 to 9 plants. The lines were planted 25 June 2018 in a field at University of California, Westside Research and Extension Center, Five Points, CA for evaluation of adaptation (plant size and condition) and 11 fruit quality traits in late August and early September. Fruit of one of the lines resembled Group Chandalak subgroup zami, while those of three other lines were characteristic of Group Inodorus subgroup honeydew. Fruit of most of the other lines could be considered members of Group Cantalupensis but could not readily be categorized as members of a particular subgroup. One of the honeydew lines segregated for resistance; the other two were susceptible. Fruit of the segregating honeydew line were smaller than desired for commercial production. Fruit of the six CMV-resistant Group Cantalupensis-type lines resembled subgroup American eastern, and were undersized compared with standard western shipping type cantaloupe (Group Cantalupensis subgroup American western). These tests identified the best CMV subgroup I-resistant melon breeding lines from the 25 Cornell CMV-resistant lines for continued introgression of CMV resistance into western U.S. shipping type cantaloupe and honeydew. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Genetic Resources (GR)

Genetic Resources (GR)

The Mis-Understood History of the Cucumber, *Cucumis sativus*

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Cucumbers, *Cucumis sativus* L., are among the most cosmopolitan and widely consumed vegetables, and are eaten fresh, pickled, or cooked. Cucumbers are native to the Indian sub-continent, but the history of their westward diffusion from that region is widely misunderstood. On-line sites and literature are riddled with misinformation, in which there is inane repetition of claims that the ancient Egyptians, Greeks, and Romans were fond of cucumbers. Much of this misunderstanding is derived from a combination of two factors: confusing cucumbers with long-fruited melons, *Cucumis melo* L., and lack of familiarity of the scientific community with the latter because most of this community, historically, hailed from cooler climates where these melons are ill-adapted for producing good crops. Young, long-fruited melons do closely resemble cucumbers, but have two outstanding traits not possessed by cucumbers: their surfaces are hairy and they are usually longitudinally furrowed. The *sikyos* of Greek writers, *cucumis* (or *cucumeres*) of Latin writers, and *qishu'im* of Hebrew writers were described as hairy and ancient Egyptian depictions of elongate cucurbits show them to be furrowed. Hence, the ancient Egyptians, Greeks, Romans, and Jews were growing long-fruited melons, not cucumbers. There is no substantiated evidence for the presence of cucumbers, *Cucumis sativus*, in Europe prior to the medieval period. Also, in contrast to what is stated in English dictionaries, the word "gherkin," for a small or pickled cucumber, can be traced all the way back to the Indian sub-continent, through late medieval German *kychern* and Latin *chache* and *circea*, to medieval Arabic and Persian *khiyar*, to Urdu *khira* and Hindi *k(h)ira*.

Session Topic

Genetic Resources (GR)

Genetic Resources (GR)

Genome-Wide Diversity for Worldwide Watermelon Collections: Analysis of Population Structure, Haplotype Networks, Selective Sweeps and LD Decay to Characterize Domestication Signals

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Genotyping by sequencing (GBS) technology was employed to differentiate among 1259 watermelon (*C. lanatus*) and egusi (*C. mucospermus*) accessions. Among these accessions, 1052 United States Plant Introductions (PIs) maintained at the USDA, ARS, Plant Genetic Resources Conservation Unit, Griffin, GA (<https://npgsweb.ars-grin.gov/gringlobal/site.aspx?id=22>) were sampled to represent the geographical diversity of these taxa. The GBS analysis produced 64,205 SNPs. A minor allele frequency cutoff of >0.01 and a call rate of >70% retained 17,558 SNPs for use in genetic diversity and population structure analyses. Neighbor joining analysis produced a phylogenetic tree that resolved all genotypes into distinct clusters. Population structure analysis confirmed the clustering patterns produced by the neighbor joining and the principal component (PCA) analyses. A core collection containing 384 accessions was identified and selected. Tajima's D windows for various chromosomes were used to identify selective sweeps. Linkage disequilibrium (LD) decay was estimated across chromosomes and haplotypes were deduced for various LD blocks and selective sweeps. Haplotype networks were generated for various LD blocks characterized across chromosomes. These networks are analogous to genealogical histories and will be useful in efforts to resolve reticulation histories for the materials analyzed. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Genetic Resources (GR)

Genetic Resources (GR)

Characterization of Bitter Gourd Breeding Lines Developed at World Vegetable Center

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The bitter gourd breeding program of the World Vegetable Center (WorldVeg) is globally recognized for development of unique products which are shared with its stakeholders in the private and public sectors. Understanding of different market segments (based on fruit length, shape, color, skin pattern) of WorldVeg's bitter gourd breeding lines helps seed requesters to select appropriate lines for their breeding program. We evaluated the fruit traits of 100 WorldVeg bitter gourd breeding lines at the World Vegetable Center, East and Southeast Asia/Oceania, Research and Training Station, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand. Entries were planted in a single block with row spacing of 1.6 m, within-row spacing of 1 m and five plants per plot. Five marketable fruit of each entry were harvested for evaluation of five fruit traits: 1) shape = cylindrical, spindle, elongated; 2) color = green, light green, medium green, dark green; 3) skin pattern = smooth, spiny; 4) length = short (20 cm); 5) bitterness = low, medium, high. Three types of fruit shape were identified among the lines. The majority of lines produced spindle-shaped fruit (69%); cylindrical and elongated fruit were produced by 25% and 6% of the lines. Four distinct fruit skin colors were observed among lines: green (46%), light green (26%), medium green (11%) and dark green (17%). Two fruit skin patterns were observed among lines: spiny (90%) and smooth (10%). Three classes of bitterness among the lines were noted based on taste panels: low (40%), medium (35%) and high (25%). Lines were categorized into three market segments based on the fruit length: short (10%), medium (53%) and long (37%). Fruit pictures of lines were also recorded. The bitter gourd fruit database is available to seed requesters to facilitate ordering lines from the WorldVeg genebank.

Session Topic

Genetic Resources (GR)

Genomics (G1, G2)

Genomics (G1, G2)

Exploring Genetic Diversity in the U.S. National Melon Collection

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It is essential to maintain, as well as to incorporate genetic diversity into breeding programs for the improvement of quality, nutrients, yield, and biotic and abiotic stress tolerances. The U.S. National Plant Germplasm System (NPGS) maintains more than 2K *Cucumis melo* L. accessions from >70 countries. The NPGS melon collection has a paucity of systematically collected phenotypic data, and few of them have been classified to the horticultural Group level. These accessions and additional heirloom cultivars not in the NPGS collection were genotyped by the Genotyping by Sequence (GBS) method to understand the genetic diversity, population structure, and phylogenetic relationships within the U.S. melon collection. Over 27K single nucleotide polymorphism (SNP) markers with missing rate less than 0.5 and minimum allele frequency greater than 0.01 were obtained. Eleven sub-populations were identified via population structure analysis. We then developed a core panel subset (n=384) based on genome-wide SNPs, origin and known phenotypic properties, which captured 98.96% of the allelic diversity of the base population. The core panel was evaluated in a non-replicated field test in Imperial Valley, CA for 10 phenotypic traits (leaf shape, sex expression, and fruit traits including exocarp, flesh and cavity color, shape, weight, and soluble solids). Each member of the core panel was classified for horticultural Group based on phenotypic data, and we examined the relationship between genotypic data and horticultural Group classification. The core panel data were subjected to genome-wide association study in order to identify marker-trait associations for the key phenotypic traits. Overall, characterization of the genetic diversity and structure of the entire U.S. melon collection based on the high-density SNP markers derived from GBS and establishment of a melon core panel provide a valuable resource for melon genetic improvement. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Genomics (G1, G2)

Genomics (G1, G2)

Transcriptome Methods Identified Mobile mRNAs from Pumpkin Rootstock in Watermelon Scions that Respond to Chilling Stress

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Grafting of scion onto resistant pumpkin rootstock is an effective method to enhance chilling tolerance of watermelon seedlings. Previous studies showed that grafting watermelon scion onto rootstock led to differential expression of watermelon genes. However, the regulatory mechanisms remain to be investigated. In this study, the RNA-sequence technique was used to analyze the mechanism of mobile mRNAs from pumpkin rootstock to regulate chilling tolerance in watermelon. The leaves of self-grafted and rootstock-grafted watermelon were respectively collected at 12 h and 72 h from control (28/18 °C) and chilling group (10/10 °C), and were used for building library and sequencing. The results showed the detection of 91, 73, 97, and 100 mobile mRNAs in the treatment groups of control-12 h, control-72 h, chilling-12 h, and chilling-72 h from the pumpkin rootstock, respectively. Gene Ontology (GO) analysis of these mobile mRNAs indicated that certain biological processes, such as metabolic process (64.6 %), cellular (63.5 %), single-organism process (49.0 %), were overrepresented. Within the cellular component category, cell (66.1 %), cell part (66.1 %), and organelle (60.6 %) were overrepresented. Moreover, binding (65.5 %) and catalytic activity (49.0 %) were overrepresented in the molecular function category. After 12 hours of chilling stress, total 46 specific mRNAs were detected to be involved in long-distance transportation, and the number was increased to 83 at 72 hours of chilling stress. These results provide further insights into the molecular mechanisms of pumpkin rootstock grafting to increase chilling tolerance of watermelon seedlings, and can be helpful to identify some suitable candidate genes that conveying valuable information for improving chilling tolerance in watermelon through genetic engineering.

Session Topic

Genomics (G1, G2)

Cucurbitaceae 2018
Conference abstracts

Genomics (G1, G2)

Cucurbit Genomics Database

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The Cucurbitaceae family (cucurbit) includes several economically important crops, such as melon, cucumber, watermelon, pumpkin, squash, and gourd. During the past several years, genomic and genetic data have been rapidly accumulated for cucurbits. We have been developing the Cucurbit Genomics Database (<http://cucurbitgenomics.org/>) to store, mine, analyze, and disseminate the large-scale cucurbit genomic and genetic datasets in an efficient way and to provide a center portal for the cucurbit research and breeding community. The database currently contains all available genome and EST sequences, genetic maps, and transcriptome profiling for cucurbit species, as well as sequence annotations and biochemical pathways. A set of analysis and visualization tools and user-friendly query interfaces have been implemented in the database. Future development of the database will be discussed.

Session Topic

Genomics (G1, G2)

Genomics (G1, G2)

Mapping of QTLs Controlling Seed Size by Whole-Genome Sequencing and Bulk Segregation Analysis in Watermelon

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Seed size controlled by QTLs dispersed in watermelon genome is a quantitative trait that influences both the quality and yield of watermelon. QTL-seq a rapid high-resolution genome-wide strategy, combining bulked segregation analysis (BSA) with whole genome sequencing, is the first steps for further research, such as cloning and function analysis. In this study, an F₂ population derived from a cross between an inbred line K₁ which the seed length was 5.8 mm and an inbred line L₁ which the seed length was 9.8 mm, was used for excavation of controlling seed size traits genes by BSA-seq. The “L-Bulk” and “S-Bulk” DNA bulks were constructed using 20 plants selected from the F₂ population. Next-generation sequencing (NGS) was applied for the resequencing of the parental bulks and two bulks. The result of the BSA showed a major region on chromosome 6 was identified about 2.82 Mb. Biparental QTL was conducted in the candidate region. An F₂ genetic linkage map which included 21 CAPS markers and 1 dCAPS markers was constructed. The map contained 1 linkage groups which corresponded with the chromosome and spanned 60.03 cM with a mean marker interval of 2.73 cM, a major-effect QTL named SS6.1 was detected between the markers WSS-18 and CAPS-29 which was tightly linked to the seed length and 100 seed weight. The QTL analysis indicated the presence of one quantitative trait loci, SS6.1 was found in about 460 kb region WSS-18 and CAPS-29, which mapped on chromosome 6 of watermelon genome. There were 39 candidate genes were predicted in this region. Based on the watermelon genome and function analysis, five genes including Cla009263, Cla009266, Cla009289, Cla009292, Cla009303 were predicted as candidate genes related to the seed size. The results of candidate gene analysis provided some potential target for further cloning and functional identification of the seed size for breeding.

Session Topic

Genomics (G1, G2)

Genomics (G1, G2)

The CucCAP Project: Genomic Tools and Resources to Facilitate Breeding for Disease Resistance in Cucurbits

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The USDA-SCRI 'CucCAP' project is an effort by the U.S. cucurbit community to develop genomic and bioinformatic tools for the Cucurbitaceae to facilitate introgression and stacking of disease resistance loci. Tool development includes upgrading of the Cucurbit Genomics Database (<http://cucurbitgenomics.org/>) which provides sequence data, search capacity, and bioinformatics tools, and genetic characterization of the National Plant Germplasm System (NPGS) plant introduction (PI) collections of watermelon (*Citrullus lanatus*), melon (*Cucumis melo*), cucumber (*Cucumis sativus*), and squash (*Cucurbita pepo*). Genotyping-by-sequencing (GBS) was performed on the full collections (1000-2000 accessions/crop) providing 0.9-1.7 billion GBS reads and 20,000-30,000 SNPs per species that are well distributed across the genomes (average density: one SNP per 10.6, 14.6, and 15.7 kb for cucumber, melon and watermelon, respectively). The SNPs were used to characterize genetic diversity, population structure, phylogenetic relationships, linkage disequilibrium, and population differentiation of the collections and perform GWAS of horticulturally important traits. This information is being used to establish publicly available, re-sequenced functional panels of 300-400 accessions per crop representing >95% of the diversity present in the collections along with key disease resistance, fruit quality, horticultural and agronomic traits. The CucCAP disease priorities, which were identified by the cucurbit industries and vary among crops, include downy mildew, *Fusarium*, gummy stem blight, *Phytophthora capsici*, powdery mildew and several viruses. The participating research groups are characterizing resistances, identifying QTL, developing markers, and introgressing resistances for the different crop-disease combinations. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic
Genomics (G1, G2)

Genomics (G1, G2)

Differential Gene Expression in Fruits Across Reciprocal Grafts and Effects of Grafting on Fruit Quality and Weight

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To assess the impact of grafting on gene expression in fruits, we compared transcriptome generated using RNAseq for fruit tissues collected in scions and ungrafted plants of watermelon and *Lagenaria* (bottle gourd). When watermelon is used as scion (WM/LAG), 57 genes were shown differentially expressed (DEGs), when compared to non-grafted watermelon of which, 11 were upregulated and 46 were downregulated. Four genes were exclusively absent in WM/LAG while they highly expressed in nongrafted watermelon. Upregulated genes were of plant cell wall modification, carbohydrate metabolism, synthesis of volatile compounds, hormone metabolism and stress response. Pectinesterase (Cla004251), 5-dehydro-2-deoxygluconokinase (Cla007008) germacrene D synthase (Cla004416), protein forked1 (Cla021595) and phloem filament protein (Cla001440) were examples for upregulated DEGs. On the other hand, downregulated genes were associated to transport, response to stimulus, transcription factors, signaling and metabolic process including peptide transporter (Cla018169), calcium-dependent lipid binding protein (Cla006159), ethylene responsive transcription factor (Cla014156), elicitor-responsive protein (Cla016038) and biosynthetic arginine decarboxylase (Cla016612). 478 genes were significantly differentially expressed in fruits of LAG/WM when compared with nongrafted bottle gourd. Of these genes, 263 were upregulated and 215 were downregulated. Hormone metabolism, transporter activity, response to stimulus and oxidative stress were found among the DEGs. ATP-dependent 6-phosphofructokinase (Lsi07G005330), auxin efflux carrier family protein (Lsi01G016410), ethylene-responsive transcription factor (Lsi06G008160), high-affinity glucose transporter (Lsi09G013320), potassium transporter (Lsi02G015540), calcium-binding EF hand protein (Lsi02G010860) and amine oxidase (Lsi09G013240) were some of the notable DEGs in the fruits of LAG/WM. In this study, we also field evaluated reciprocal grafts to understand the effects of grafting on fruits.

Session Topic

Genomics (G1, G2)

Genomics (G1, G2)

The Whole-Genome Resequencing Reveals the Evidence of Selective Sweeps during Melon Domestication

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As an important member of cucurbitaceae family, melon exhibits the most diversity in phenotype. However, the genomic evidence of melon domestication remains poorly understood. The goal of this study is to investigate the genomic signatures in the history of melon domestication. We resequenced 294 melon accessions to get an ultra-genomic variation map with 2,045,412 SNPs. Genomic F_{ST} analysis of wild versus domesticated populations suggests that genes which affect aroma, sweetness, flower time and stress resistance have undergone strong positive selection during domestication. Based on the results of the domesticated signal selection, we speculated that the evolution in melon from wildtype to modern cultivated species underwent two stages (the domestication stage and the improvement stage). The first one is the domestication from the wildtype to two kinds of landraces closely to the *C. melo ssp. melo* and *C. melo ssp. agrestis* with different selective sweeps (203 and 178 candidate genes in the selective regions with the 1% top F_{ST} values, respectively). For the landrace closely to the *C. melo ssp. agrestis*, the genes related to sugar accumulation and disease-resistance were selective, while in the *C. melo ssp. melo*, the stress tolerance and plant development genes performed high selective signals. The aroma related genes were selected in both landraces. The two kinds of landraces might have undergone independent evolution to the varieties of *C. melo ssp. melo* and *C. melo ssp. agrestis*, respectively in the improved stages. In the second stages, the *C. melo ssp. agrestis* underwent much more genome variation than the *C. melo ssp. melo* to perform a high degree domestication events. The *C. melo ssp. melo* and *C. melo ssp. agrestis* groups did not have any domesticated relationships in the evolutionary history. This study advances the understanding of melon domestication and also gives the genome signals to enhance the melon breeding.

Session Topic

Genomics (G1, G2)

Genomics (G1, G2)

Metagenomic and Metatranscriptomic Analyses of Diverse Watermelon Cultivars Reveal the Role of Fruit-Associated Microbiome in Carbohydrate Metabolism and Ripening of Mature Fruits

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The plant microbiome is a key determinant of plant health and productivity, and changes in the plant microbiome can alter the tolerance to biotic and abiotic stress and the quality of end produce. In this study, we aimed to understand the diversity and function of microorganisms in relation to ripening and carbohydrate metabolism in ripe watermelon fruits. We used 16S metagenomics and RNAseq metatranscriptomics for analysis of red (PI 459074, 'Congo' and SDRose) and yellow (PI 227202, PI 435990 and 'JBush') flesh watermelon germplasm of geographically and metabolically diverse origin. Metagenomics data showed that proteobacteria were abundant in Sweet Dakota Rose (SDRose) and PI227202, whereas cyanobacteria were most abundant in Congo and PI4559074. In the case of metatranscriptome data, Proteobacteria was the most abundant in all cultivars. High expression of genes linked to infectious diseases and the expression of peptidoglycan hydrolases associated to pathogenicity of eukaryotic hosts was observed in SDRose, which could have resulted in low microbial diversity in this cultivar. Production of carotenoids and other volatile aroma compounds due to carotenoid degradation could be correlated with presence of active microbial groups Cyanobacteria and Deinococcus-Thermus. Moreover, Basidiomycota and Ascomycota also could contribute to volatile aroma compounds and their activity is observed in the metatranscriptomic results. Further, ethylene production activity leading to fruit ripening could be associated with Proteobacteria. The presence of GH28, associated with polygalacturonase activity in JBush and SDRose could be related to cell wall modifications including de-esterification and depolymerization, and consequent loss of galacturonic acid and neutral sugars. Moreover, based on the KEGG annotation of the expressed genes, nine α -galactosidase genes involved in key processes of galactosyl oligosaccharide metabolism, such as raffinose family oligosaccharide were identified and galactose metabolism pathway was reconstructed. Results of this study underline the links between the host and fruit-associated microbiome in carbohydrate metabolism of the host and fruit ripening. The cultivar difference in watermelon reflects the quantum and diversity of the microbiome, which would benefit watermelon and other plant breeders aiming at the holobiont concept to incorporate associated microbiomes in breeding plan.

Session Topic

Genomics (G1, G2)

Genomics (G1, G2)

A Multispecies SNP Array for High-Resolution Genotyping of Melon, Cucumber and Watermelon

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With the availability of a set of sequenced cucumber, watermelon and melon genomes in public databases, a large source of SNPs is now available for these important Cucurbitaceae species. We have used these resources of molecular markers for the development of a new multispecies SNP array using the Affymetrix Axiom genotyping platform. The array has been set up with 29,961 melon, 47,537 watermelon, and 49,497 cucumber SNP markers. For each species, the markers have been selected based on (i) general allele frequency in all sequenced lines; (ii) chromosomal distribution along the physical length of the chromosomes with a higher marker number towards the end of the chromosomes reflecting the distribution of crossing overs; and (iii) expected marker functionality over a wide range of material. This new Cucurbitaceae SNP genotyping array has been used for the characterization of sets of cucumber, melon and watermelon breeding lines and varieties for defining the individual marker functionality and quality as well as the general level of polymorphism. With many functional markers, this SNP genotyping array provides a significant improvement for large-scale genotyping in these Cucurbitaceae species.

Session Topic

Genomics (G1, G2)

Genomics (G1, G2)

New Genetic and Genomic Resources in Melon and their Application for Fruit Quality Improvement

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During the last years, a big effort has been done in order to develop genetic and genomic tools in melon (*Cucumis melo* L.) that are useful to identify the genes or QTLs underlying traits of high agronomical importance. In this sense, we have improved the assembly of the melon reference genome (v3.6.1, <http://melonomics.net>) by using an optical mapping approach, obtaining the correct order and orientation of 21 scaffolds, and defining the gap-size in the 12 pseudomolecules. A new annotation v4.0 integrating RNA-seq data has been released, with more than 8,000 new genes identified. These resources are very useful to identify candidate genes obtained from mapping experiments. With the aim to improve melon fruit quality, we developed a RIL population between two commercial cultivars: the highly climacteric “Védreantais” from the *cantalupensis* group, and the non-climacteric “Piel de Sapo” from the *inodorus* group. This population segregates for many fruit quality and morphology traits, and we used a GBS strategy to map QTLs for sugar and carotenoid content, fruit and seed morphology, and the external appearance of the fruit. The high mapping resolution allowed the identification of five major loci, and 33 QTLs in intervals of 1 Mb containing 100 genes on average.

Session Topic

Genomics (G1, G2)

Genomics (G1, G2)

Genomics-Aided Development and Characterization of *Cucumis hystrix* Introgression Lines in Cucumber

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Cucumis hystrix ($2n = 2x = 24$, HH) is the only known species in the genus *Cucumis* that is cross-compatible with cucumber (*C. sativus*, $2n = 2x = 14$, CC) and has a great potential for cucumber improvement. To facilitate introgression of *C. hystrix* chromatins into cucumber genetic background, we developed a draft genome for the *C. hystrix* genome (accession TH1) which contained 16,865 scaffolds (~78× coverage) for a total of 226.0 Mb, representing ~51% of the estimated 447 Mb *C. hystrix* genome. The largest scaffold was 342 kb with the N50 scaffold size of 23.3 kb. Through genotyping-by-sequencing (GBS), a linkage map for *C. hystrix* was developed with 1,692 SNP loci, which was then integrated with a previously developed genetic map with 410 SSR markers. The resulting consensus map consisted of 12 linkage groups spanning 1119 cM, which was used to anchor 1,069 scaffolds accounting for 49.4 Mb or ~22% of *C. hystrix* draft genome assembly. A karyotype for the *C. hystrix* genome was developed with molecular cytogenetic landmarks. These new genomic resources allowed refinement of the syntenic relationships among *C. hystrix*, cucumber and melon (*C. melo*, $2n = 2x = 24$) chromosomes. An improved model was proposed to elucidate the evolutionary history in which the seven cucumber chromosomes were evolved from an $x=12$ ancestor through dysploid chromosome reduction that involved in four translocations, four chromosomal fusions, and 53 inversions. A synthetic tetraploid, *C. x hytivus* ($2n = 4x = 38$, HHCC) was developed through induction of chromosome doubling of the interspecific hybrid between *C. sativus* and *C. hystrix*, which was used as the bridge to develop an introgression library through marker-assisted backcrossing. The molecularly characterized introgression lines provide insights into homoeologous pairing between cucumber and *C. hystrix* chromosomes. Supported by Agriculture and Food Research Initiative Competitive Grant nos. 2013-67013-21105 and 2015-51181-24285. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic
Genomics (G1, G2)

Genomics (G1, G2)

The USDA Cucumber (*Cucumis sativus* L.) Collection: Genetic Diversity, Population Structure, Genome-Wide Association Studies and Core Collection Development

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Germplasm collections are a crucial resource to conserve natural genetic diversity and provide a source of novel traits essential for sustained crop improvement. Optimal collection, preservation and utilization of these materials depends upon knowledge of the genetic variation present within the collection. Here we use the high throughput genotyping-by-sequencing (GBS) technology to characterize the United States National Plant Germplasm System (NPGS) collection of cucumber (*Cucumis sativus* L.). The GBS data, derived from 1,234 cucumber accessions, provided more than 23K high quality single nucleotide polymorphisms (SNPs) that are well distributed at high density in the genome (~1 SNP/10.6 kb). The SNP markers were used to characterize genetic diversity, population structure, phylogenetic relationships, linkage disequilibrium, and population differentiation of the NPGS cucumber collection. These results, providing detailed genetic analysis of the U.S. cucumber collection, complement NPGS descriptive information regarding geographic origin and phenotypic characterization. We also identified genome regions significantly associated with thirteen horticulturally important traits through genome-wide association studies (GWAS). Finally, we developed a molecularly-informed, publicly accessible core collection of 395 accessions that represents at least 96% of the genetic variation present in the NPGS. Collectively, the information obtained from the GBS data enabled deep insight into the diversity present and genetic relationships among accessions within the collection, and will provide a valuable resource for genetic analysis, gene discovery, crop improvement and germplasm preservation. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Genomics (G1, G2)

Genomics (G1, G2)

Genome-Wide Identification of Calcium-Dependent Protein Kinase and its Related Kinase Gene Families in Cucurbitaceae Species

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Both the calcium-dependent protein kinase (CDPK) and CDPK-related kinases (CRKs) play numerous roles related to plant growth, development, and stress response. Despite genome-wide identification of both families in *Cucumis* species, comparative evolutionary and functional analysis of both *CDPKs* and *CRKs* in Cucurbitaceae remain unclear. Here, we totally identified 128 *CDPK* and 56 *CRK* genes in six Cucurbitaceae species (*C. lannatus*, *C. sativus*, *C. moschata*, *C. maxima*, *C. pepo*, and *L. siceraria*). Structural variation analysis indicated that two *CDPKs* (*CpCDPK19* and *CpCDPK27*) in *C. pepo* may have undergone subfunctionalization via self-duplication of conserved domains. Using the watermelon genome as reference, an integrated map containing 23 loci (16 *CDPK* and nine *CRK* loci) were obtained, 16 of which (12 *CDPK* and four *CRK*) were shared by all seven Cucurbitaceae species. Phylogenetic analysis obtained four *CDPK* groups and one *CRK* group with one or two major intron phase patterns. Moreover, the topologies of most loci are consistent with a recently published evolutionary scenario of seven modern Cucurbitaceae species. Comparative syntenic analysis detected few segmental duplication events in Benincaseae tribe species, but many in Cucurbita tribe species. In addition, expression patterns of *CICDPKs* and *CICRKs* were studied under different abiotic stresses, as well as subcellular localizations of several *CICDPKs* and *CICRKs*.

Session Topic

Genomics (G1, G2)

Genomics (G1, G2)

Transcriptome Dynamics of the Whitefly *Bemisia tabaci* in Response to Feeding on Melon Plants Infected with *Cucurbit yellow stunting disorder virus*

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Whiteflies are a serious threat to crop production. They can cause direct damage through feeding, as well as indirect damage through production of honeydew leading to sooty molds, and particularly through transmission of plant viruses. Whiteflies are known to transmit economically important viruses in the genera *Begomovirus*, *Crinivirus*, *Ipomovirus*, *Carlavirus*, and *Torradovirus*, and all are known to cause economic loss to the production of cucurbit crops. Recent studies have demonstrated common patterns of gene regulation between whiteflies fed on tomato infected with either a crinivirus or begomovirus (Kaur et al. 2017; BMC Genomics, 18: 370; Hasegawa et al., 2018, Virology 513: 52). In order to gain a better understanding of the whitefly response to virus-infected cucurbits and cucurbit viruses, RNA-Seq was performed on whiteflies following acquisition feeding on melon leaves infected with the semipersistently transmitted crinivirus, *Cucurbit yellow stunting disorder virus* (CYSDV) for three different time periods, 24 h, 72 h, and 7 days. CYSDV is transmitted exclusively by the whitefly, *Bemisia tabaci*, and can be retained for seven to nine days in the vector. A total of 275 differentially expressed genes (DEGs) were identified in response to feeding on CYSDV-infected melon plants. Interestingly, only 3 DEGs (all down-regulated) were observed at 24 h, followed by 221 DEGs at 72 h, and 51 DEGs at 7 days. Several distinct gene categories were represented among the DEGs in the whiteflies. As was found in previous studies involving another crinivirus, *Tomato chlorosis virus* (ToCV), a large percentage of the DEGs were orphan genes that are unique to the whitefly and do not show any homology to known genes in other species. Further, we found 59 DEGs common between whiteflies fed on CYSDV-infected melon and ToCV-infected tomato plants, and 14 in common between those fed on CYSDV, ToCV and the begomovirus, *Tomato yellow leaf curl virus*, suggesting certain common responses by whiteflies to feeding on crinivirus-infected host plants and perhaps virus-infected plants in general, that can be harnessed to interfere with transmission to melon and other crops.

Session Topic

Genomics (G1, G2)

Genomics (G1, G2)

Pan-Genomes of *Citrullus* Species

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Watermelon (*Citrullus lanatus*) is among the most important vegetable crops in the world. It belongs to the *Citrullus* ($2n=2x=22$) genus, which includes six other species, namely egusi watermelon (*C. mucospermus*), citron watermelon (*C. amarus*), colocynth (*C. colocynthis*), *C. ecirrhosus*, *C. rehmii* and *C. naudinianus*. To maximize the capture of genome variations within and among these *Citrullus* species and to identify novel agronomically important alleles for facilitating watermelon breeding, we are constructing pan-genomes of the *Citrullus* species. We have been generating high-quality reference genomes from selected individuals of four *Citrullus* species, including two *C. lanatus* accessions, two *C. amarus* and one *C. colocynthis* whose genome assemblies and annotations are finished, and one in the process for *C. mucospermus*. We also generated whole genome resequencing data from more than 400 additional accessions of these four species, which are being *de novo* assembled for each accession. A pan-genome for each of the four species will be constructed by combining the reference genome(s) and *de novo* assembled novel non-redundant sequences. Presence-absence variations (PAVs) of protein-coding genes will be analyzed, and collections of core and distributed gene sets for each species will be identified. Comparative analysis of the four pan-genomes will be performed to highlight syntenic regions and species-specific variations. The *Citrullus* pan-genomes and the identified PAVs will enable us to trace the distribution of functionally important alleles and gain insights into watermelon evolution, domestication and introgression, and provide an important resource to facilitate the mining of natural variation in *Citrullus* for scientific studies and the improvement of watermelon. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Genomics (G1, G2)

Genomics (G1, G2)

Developing Genome and transcriptome Information Database for functional Genomics Research of Japanese Muskmelon

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Melon (*Cucumis melo* L.) is an important Cucurbitaceae crop that is widely produced in the world. It exhibits a wide range of natural variation especially in fruit phenotypes (i.e., climacteric and non-climacteric ripening types). The semi-climacteric type inbred cultivar 'Earl's favorite Harukei-3' (Harukei-3) is known for its sweetness and rich aroma, and hence popular as a breeding material for development of high-grade muskmelon in Japan. To promote functional genomics research and breeding of Japanese muskmelon, we have developed gene information database 'Melonet-DB' (<http://gene.melonet-db.jp>). In the database, a user is able to visually compare tissue-specific gene expression patterns as well as gene coexpression across multiple input queries. To update Melonet-DB, we recently conducted additional RNA-seq studies, and the updated dataset will contain new RNA-seq datasets such as imbibed seeds, seedlings, and post-harvest ethylene-emitting fruit tissues. In addition, by combining a non-destructive fruit phenotype analysis method, leaf RNA-seq datasets have been also collected in a weekly manner in a greenhouse to analyze sink-source tissue interaction. In addition to the transcriptome study, the whole genome information of the Harukei-3 melon has been also developed using the 2nd and 3rd next-generation DNA sequencers. By combining 43-fold Pacbio long reads, 190-fold Bionano single molecule data, 82-fold Illumina Hiseq short reads, and 52-fold mate pair reads, we assembled Harukei-3 genomic scaffolds. Some scaffolds span chromosome arm, indicating that combining these technologies was effective to construct long scaffolds. In this presentation, we'd like to introduce the results of our recent research activities.

Session Topic

Genomics (G1, G2)

Production and Quality (PQ)

Production and Quality (PQ)

Green Nanotechnology: An Effective Approach for Watermelon Growth while Maintaining Quality

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Nano-priming is an innovative technique for improving seed germination and growth. The present study demonstrated a synthetic process that involves the production of spherical silver nanoparticles (AgNPs) by treating aqueous onion juice with silver nitrate. No “man-made” chemicals other than the silver salt was used in the green nanotechnological process and characterized using ultraviolet-visible (UV-Vis) spectroscopy, dynamic light scattering (DLS) techniques, X-ray diffraction (XRD) and transmission electron microscopy (TEM). Similarly, turmeric oil nanoemulsion (TurNE) was prepared by dropwise reduction method using curcumin removed turmeric oleoresin (CRTO). AgNPs and TurNE were used for priming two varieties of watermelon (Riverside and Maxima) for 12 hr and compared with the unprimed control seeds. Internalization of silver (Ag) in watermelon seeds after treatment with AgNPs was determined by Neutron Activation Analysis (NAA). Germination and emergence tests were conducted in an incubator and greenhouse, respectively, where significant enhancement was observed in treated seeds compared with the unprimed seeds. Seedlings grown in the greenhouse were transplanted at four different locations across Texas: Edinburg, Pecos, Grapeland, and Snook. At 40-days after transplanting (DAT), vine length and stem thickness were found to be significantly higher in AgNPs treated watermelons for both varieties at all locations. Results show that AgNPs priming of seed has potential to decrease production costs of watermelon by enhancing seed germination and plant growth. However, no consistent changes in nutritional levels were observed in watermelon fruits. Lycopene level in the treated fruits of both varieties was observed to be similar to the control watermelons in most of the locations. Our results demonstrate that naturally occurring plant extracts can be used in the production of biocompatible nanoparticles thus paving the way for future application in germination and growth of watermelon without deteriorating the nutritional quality. This study was supported by Research award # SC-1607-013 from Texas Department of Agriculture.

Session Topic

Production and Quality (PQ)

Production and Quality (PQ)

Recent Progress of Cucurbit Grafting in China

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The earliest literature about vegetable grafting in China was recorded in an ancient book “Fan-Sheng-Zhi-Shu” in the first century, BC. However, commercial grafted vegetable production began in the 1970s. The main purposes of grafted cucurbit production in China are to overcome soil-borne diseases and increase resistance to abiotic stress. In recent years, commercial grafted cucurbit seedling production developed rapidly in China, which is now the largest country that produces grafted cucurbit seedlings in the world. There are about 3000 companies producing grafted seedlings in China and distribute in different provinces. The facilities and equipment related to grafted seedling production also developed very quickly, including greenhouse, plug trays, automatic seedling machine, substrate production, and environmental controlling systems. There are about 30 cultivars of rootstock that have been bred and released, especially for the rootstocks of cucumber and watermelon. Recent research on vegetable grafting method, grafting and fruit quality, grafting and abiotic stress, rootstock-scion interaction will be presented and discussed.

Session Topic

Production and Quality (PQ)

Production and Quality (PQ)

Maximizing Internal Quality of Butternut Squash (*Cucurbita moschata*) through Harvest Timing and Storage

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While yield is a critical factor when producing a crop, quality is needed to market the crop. The primary goal of this study was to examine soluble solid and nutrient content in butternut squash at stages of fruit development and after subsequent storage. The test was conducted in Salisbury, in the Piedmont region of North Carolina. A randomized complete block design with four replications utilizing 'Butterfly' was direct seeded on 26 May 2016. Plants were spaced 0.61 m in-row with 3.04 m between rows on black plastic mulch with drip irrigation. Fruit set was coordinated for 48, 55, 62, and 69 days after seeding (DAS). Fruit were harvested at maturity intervals of 14, 21, 28, 35, and 42 days after anthesis (DAA) and stored for 0, 5, and 9 week periods at 13° C and 70% RH. At the end of the storage period, fruits were sampled for soluble solids (SSC), carotenoids, flesh color, and weight loss. SSC increased significantly between 21 and 28 DAA and slightly between 28 and 35 DAA. Alpha carotene reached a maximum content on the vine at 35 DAA. Carotenoids and SSC did not increase significantly from 35 to 42 DAA and did not decrease during the 5 to 9 week storage period. Maximum values were obtained in squash that were set at 55 DAS, harvested at 35 DAA and stored for 5 weeks with SSC of 11.6, alpha carotene of 1034.09 mcg/100g fwt and beta carotene of 1937.58 mcg/100g fwt. At 0 to 5 weeks storage, β -carotene increased significantly. The above results indicated that in order to maximize both soluble solid content and provitamin A relatives, butternut squash should be harvested 35 DAA and stored for a minimum of 5 weeks when grown in the warm climates of central North Carolina. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

Session Topic

Production and Quality (PQ)

Production and Quality (PQ)

Phloem Unloading and Intracellular Transport of Carbohydrate in Developing Melon Fruit: A Global Transcriptional View of Sugar Transporters Responsible for Sucrose Accumulation

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Sugar accumulation in melon fruit has a metabolic transition during its development and leads to high sucrose concentration in the mature fruit. However, it remains unclear whether the phloem unloading pathway alters to adapt to the transition and which sugar transporters are involved in this process. The transport of the phloem-mobile symplasmic tracer carboxyfluorescein (CFDA), a global transcriptional view of four sugar transport relative gene families and functional identification of one tonoplast sugar transporter (CmTST2) were studied during the development of melon fruits. CFDA experiment result indicated that carboxy fluorescein was released from the functional phloem strands during the early and middle stages of fruit development, whereas the symplasmic tracer was confined to the phloem strand during the late stage. This reveals a shift of phloem unloading from symplasmic to apoplasmic pathway during fruit development. In addition, the expression of two sucrose transporter (SUT/SUC) genes, three sugar will eventually be exported transporter (SWEET) genes and one tonoplast sugar transporter gene (CmTST2) are increased around the onset of ripening and sucrose accumulation, while the expression of two cell wall invertase genes, two SWEET genes, three hexose transporter genes, and one tonoplast sugar transporter gene (CmTST1) are decreased. These results provide further evidence for an operation of the apoplasmic unloading pathway after onset of ripening. Furthermore, overexpression of the CmTST2 gene in strawberry and cucumber fruits can increase sucrose, fructose, and glucose accumulation in fruit. These results indicated that sucrose accumulation to a high level in ripening melon fruit need a more strong unloading pathway (apoplasmic) to unload and transport the sucrose to the flesh cells and then to the vacuoles for storage.

Session Topic

Production and Quality (PQ)

Production and Quality (PQ)

Melon Texture Diversity: Sensory and Physical Assessments

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Melon (*Cucumis melo* L.) is a commercially important horticultural crop worldwide that presents extensive phenotypic and genetic variation. Texture is one of the key attributes defining melon fruit quality and overall consumer acceptance. The aim of this research was to develop rapid phenotyping tools to characterize differences in texture among a diverse panel of melon varieties during ripening throughout postharvest storage. Texture-related physical and sensory measurements were compared to assess their correlations. We compared eight melon varieties with diverse textural characteristics using sensory (taste panel) and physical (texture analyzer) evaluations and correlated the obtained results. Fruits from each variety were harvested at optimal commercial harvest and stored for six days at 5°C plus one day at room temperature, during two production seasons. Our results show that both methodologies detected significant and reproducible differences in texture among the eight assayed melon varieties. Furthermore, texture-related sensory attributes of firmness, crunchiness, and juiciness significantly correlated with several parameters obtained through the physical assessment of texture by using different probes of the texture analyzer instrument. These results indicate that selection of texture-related attributes in breeding programs targeting overall fruit quality improvement could be accelerated significantly with the application of high-throughput physical measurements to select for phenotypes that highly associate with consumer perception.

Session Topic

Production and Quality (PQ)

Production and Quality (PQ)

Effect of Ethrel and Gibberellic Acid on Sex Expression and Seed Production of Snake Gourd, *Trichanunium anguna* L.

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Snake gourd (*Trichanunium anguna* L.) belongs to Cucurbitaceae family and it is cultivated all over the world. It is consumed as fresh vegetable which due to its high nutritional value. Cucurbit plants bear more male flowers than female flowers. If the temperature rises high, pistillate flowers decreases and male flowers increase, and as a result yield is decreased. The yield of the plant is increased by increasing the number of pistillate flowers. The plant growth regulators ethrel and gibberellic acid increased pistillate flowers with different concentrations, resulting in increased fruit yield and seed production of cucurbits. GA₃ at the rate of 50 ppm significantly increased the plant height, maximum seedling fresh weight, number of male flowers per plant, male-to-female flowering ratio, fruit size, germination percentage, seedling dry weight, plumule length of seedling, radical length of seedling, weight of seeds, and days to first female flower. GA₃ at the rate 10ppm of maximized number of fruit per plant and number of seeds per plant. GA₃ at the rate 30 ppm maximized the length of fruit. Ethrel at the rate of 250ppm recorded maximized fruit diameter and number of female flowers per plant.

Session Topic

Production and Quality (PQ)

Production and Quality (PQ)

Challenges and Opportunities for Grafted Cucurbit Plants in the United States

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Grafting cucurbits vegetables has been going on in the world for over 60 years. However, grafting in the United States is just starting to happen. The need has never been greater due to the increase in soil borne diseases. Unfortunately, the main reason for this delay in the United States is associated mainly with cost. Grafting is both labor intensive and with the lack of skilled labor force inadequate. Currently the cost of cucurbit grafted transplants is four to five times the cost of a normal transplant. Our research has been focused on bringing these costs down. Things that will be discussed are: 1. The availability of rootstock material; focusing on the pros and cons of each type and what regions in the world they are grown. 2. The release of a new USDA-ARS rootstock, 'Carolina Strongback', and effects it may have on the world market. 3. The treatment of the rootstock to prevent re-growth both in the greenhouse, as well as the field. I will discuss the effects on the rootstock and ways to totally eliminate the need for at least one cotyledon on the rootstock (a standard practice with cucurbit grafting) and the ease of making grafting automated. 4. Growing grafted plants in an artificial environment to reduce the overall cost of growing both scion and rootstock material. We are looking at developing a cookbook type system, so that we are not dependent on the constant changing outside environment. We have converted two shipping containers into grow rooms and a healing chamber for this purpose. 5. Finally the effects grafting has on melon fruit; quality and quantity which can be both beneficial and determinable if one does not understand these effects.

Session Topic

Production and Quality (PQ)

Production and Quality (PQ)

Comparative Transcriptome Analysis Reveals Key Genes Potentially Related to Soluble Sugar and Organic Acid Accumulation in Watermelon

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Soluble sugars and organic acids are important components of fruit flavor and have a strong impact on the overall organoleptic quality of watermelon (*Citrullus lanatus*) fruit. Several studies have analyzed the expression levels of the genes related to soluble sugar accumulation and the dynamic changes in their content during watermelon fruit development and ripening. Nevertheless, to date, there have been no reports on the organic acid content in watermelon or the genes regulating their synthesis. In this study, the watermelon cultivar 203Z was used as recurrent parent, has spherical fruits with a green rind, dark green pencil stripes at the mature fruit stage, pink red flesh, a high total soluble sugar content, and a low total organic acid content. The wild watermelon PI 271769, which was the donor parent, has spherical fruit with pale green, a low total soluble sugar content and a high total organic acid content with white flesh at the mature fruit stage. Near-isogenic line (NIL) SW (in the '203Z' background) with a sweet and sour flavor was developed by backcrossing 7 times and self-pollinating 4 times. Then '203Z' and SW were used as experimental materials for measuring the soluble sugars and organic acids and performing comparative transcriptome analysis. The results suggested that soluble sugar consist of fructose, glucose and sucrose while malic-, citric-, and oxalic acids are the primary organic acids in watermelon fruit. Several differentially expressed genes (DEGs) related to soluble sugar- and organic acid accumulation and metabolism were identified. These include the DEGs encoding raffinose synthase, sucrose synthase (SuSy), sucrose-phosphate synthase (SPSs), insoluble acid invertases (IAI), NAD-dependent malate dehydrogenase (*NAD-cyt MDH*), aluminum-activated malate transporter (*ALMT*), and citrate synthase (*CS*). This is the first report addressing comparative transcriptome analysis via NILs materials in watermelon fruit. These findings provide an important basis for understanding the molecular mechanism that leads to soluble sugar and organic acid accumulation and metabolism during watermelon fruit development and ripening.

Session Topic

Production and Quality (PQ)

Production and Quality (PQ)

Fungicide Resistance Occurrence Determined for Cucurbit Powdery Mildew (*Podosphaera xanthii*) and Downy Mildew (*Pseudoperonospora cubensis*) using a Seedling Bioassay

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Effectively managing downy and powdery mildew in cucurbit crops in the US necessitates applying fungicides with high risk for resistance development. A seedling bioassay was used to examine fungicide sensitivity in Maryland, New Jersey, New York, and Pennsylvania in 2016-18. Plants at about the 2-leaf stage were sprayed with various fungicides at high and low or half label rates, exposed to natural pathogen populations by placing them next to field-grown plants with one of these diseases for up to 2 days, then kept in a greenhouse until symptoms developed and severity was compared to nontreated plants. In 2017 bioassays for *Podosphaera xanthii*, thiophate-methyl (FRAC code 1) and trifloxystrobin (11) were ineffective likely due to resistance at all locations where the bioassay was conducted. Boscalid (7) was ineffective in NY and moderately effective in NJ and PA. Cyflufenamid (U6) was moderately effective in NJ and effective in NY and PA. Myclobutanil (3) was effective in all assays but in NJ it was only moderately effective at low label rate. Quinoxifen (13), metrafenone (U8), and fluopyram (7) were highly effective at all locations. Results were more variable with bioassays for *Pseudoperonospora cubensis*. Azoxystrobin (11) was ineffective likely due to resistance at all locations. Fluopicolide (43) and mandipropamid (40) were also ineffective in NY whereas fluopicolide was effective in PA and MD. Dimethomorph (40) exhibited moderate efficacy in NY and PA. Cymoxanil (27) also exhibited moderate efficacy in NY and in PA at one location; it was highly effective in the other location in PA and in MD. Propamocarb hydrochloride (28) was ineffective at low rate but moderately effective at high rate in MD and PA whereas it was highly effective at both doses in NY. Ametoctradin (45) formulated with dimethomorph was also ineffective at the low rate in PA but only at one location whereas it was moderately effective in MD and highly effective at both doses in NY. The most effective fungicides were zoxamide (22) formulated with chlorothalonil (M5), chlorothalonil, cyazofamid (21), and oxathiapiprolin (U15). Knowing when resistance impacts fungicide efficacy is critical for determining which fungicides to use.

Session Topic

Production and Quality (PQ)

Production and Quality (PQ)

Performance of Pumpkin Lines Bred by World Vegetable Center in Different Highland Areas of Thailand During the Winter Season

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Pumpkin is one of the most widely grown crops worldwide with immense economic potential and nutritional importance. Performance of cultivars is influenced by environments. The objective of this study was to evaluate the performance of three pumpkin lines bred by World Vegetable Center (AVPU1502, AVPU1504, AVPU1505), along with the local commercial check cultivar (Deliga), in different highland areas of Thailand in the winter season: Location 1- Royal Park Rajapruek, Muang Chiang Mai (200 meters above sea level, Location 2 - Royal Agricultural Station, Pangda (500 meters above sea level; Location 3 - Royal Agricultural Station, Anghang (1,000 meters above sea level). Plot size was a 5 m-wide bed with one 16 m-long row per bed (15 plants). Entries were replicated 3 times and plots were arranged in a randomized complete block design. In Location 1, AVPU1502 and AVPU1504 produced significantly ($P=0.05$) greater fruit weight per plant (2.02 and 2.71 kg/plant) compared with the commercial check (1.31 kg/plant). In the location 2, AVPU1504 and AVPU1505 yielded better (10.66 and 11.41 kg/plant) compared with the commercial check (2.3 kg/plant). Beta-carotene content of AVPU1504 and AVPU1505 in location 1 were highest (6017 and 4759 $\mu\text{g}/100$ g fresh weight, respectively) compared with the commercial check (1213 $\mu\text{g}/100$ g fresh weight). Similarly in location 2, beta-carotene content of AVPU1504 and AVPU1505 were nearly four times higher (5692 and 4283 $\mu\text{g}/100$ g fresh weight) than recorded in the commercial check (1807 $\mu\text{g}/100$ g fresh weight). In location 1, total sugars of lines ranged from 12.7 to 15.9 °Brix, while in location 2, it ranged from 11.7 to 15.6 °Brix) and it was comparable to the sugar content of the commercial check (14.3 and 14.0 °Brix, in locations 1 and 2, respectively). The pumpkin trial failed in Location 3. In conclusion, pumpkin lines developed by World Vegetable Center recorded better yield and beta-carotene content in trials in highland areas of Thailand compared with the current commercial cultivar grown by local farmers.

Session Topic

Production and Quality (PQ)

Production and Quality (PQ)

**Agronomic Performance of Some New Kirkagac Melon (*Cucumis melo* L. var. *inodorus*)
Hybrids Developed by DH Technique**

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Kirkagac melons are the primary melon groups grown widely and economically in Turkey. These melons are odourless winter melons belong to *Cucumis melo* var. *inodorus*. Their fruits are thick rinded, dark dotted and spotted on yellow rind. In this study irradiated pollen technique was used to obtain 100 % homozygous pure lines in 18 months. A total of 49 experimental hybrids were developed by the cross breeding programme of distant relatives in pure lines.

The plant growth, total yield and some fruit characteristics performances of hybrids were examined in open field conditions for two years. As a result; some of the hybrids were found promising in terms of investigated parameters and will be registered to be used as new Kirkagaç hybrid cultivars.

Session Topic

Production and Quality (PQ)

Production and Quality (PQ)

Sucrose Concentration and Watermelon Flavor Quality

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Watermelon flavor is mainly associated with sweetness. Brix is used as indicator for watermelon flavor quality. Our previous studies indicated selecting flavor quality using Brix alone will not result in varieties with high flavor acceptance. Ripe watermelons have varying levels of glucose, fructose, and sucrose. Fructose is considered the most important saccharide for watermelon flavor because of its sweetness intensity. Our previous studies indicated small variation in fructose concentrations among watermelon hybrids and breeding lines. Therefore, variation in watermelon sweetness intensity is unlikely attributed to fructose. Conversely, the role of sucrose on the watermelon flavor quality is rarely revealed. The current study was aimed at exploring sucrose contribution to watermelon flavor quality. Understanding the role of sugar components on watermelon flavor acceptance will assist breeding program to setup screening criteria for flavor quality. Seventeen F₁-crosses were made using selected parental lines (P_n). High sucrose line (A) was one of the parents in nine F₁-crosses (P_n×A). Low sucrose line (B) was one of the parents in eight F₁-crosses (P_n×B). Sensory and sugar analysis were performed on parental lines and F₁ populations. Fructose concentrations of parent A and all F₁-crosses were 3.5%-4.0%. Fructose concentration of parent B was 2.2%. Sucrose concentrations of parents A and B were 6.0% and 0.7%, respectively. Sweetness intensities and flavor acceptance of A were significantly different from B. Sucrose concentrations of P_n×A were 4.7%-6.2%. Sweetness intensities and flavor acceptance scores of P_n×A were similar to A. Likewise, sweetness intensity and flavor acceptance scores of the highest sucrose genotype among P_n×A were similar to the lowest sucrose genotype. Conversely, P_n×B had sucrose concentrations 2.2%-4.3%. Sweetness intensities and flavor acceptance scores of P_n×B were significantly different from parent B. Moreover, sweetness intensity and flavor acceptance scores of the highest sucrose line among P_n×B were higher than the lowest sucrose line. Sensory and sugar data indicate the importance of sucrose in determining watermelon flavor quality. Sucrose level of 4.5%-5.0% seems to be optimal for good flavor quality. Increasing sucrose level >5.0% does not seem to improve flavor quality of watermelons.

Session Topic

Production and Quality (PQ)

Production and Quality (PQ)

Tissue Firmness and Hollow Heart Development in 2012, 2013 and 2014 Triploid Watermelon Variety Trials

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Hollow heart (HH), a serious internal defect most commonly found in triploid (seedless) watermelon, causes a crack in the center of the fruit. Watermelon cultivars with lower tissue firmness are thought to be more prone to HH. Using data from three triploid watermelon cultivar studies conducted from 2012-2014 at the Central Crops Research Station, Clayton, NC correlations were made between tissue firmness and HH incidence within 5 different HH categories. Transplants were planted in May; plot size was one row, 10 plants/plot at 1 m in-row-spacing. Plots were 7.5 m long with 3 m alleys. Pollinizers 'Ace' and/or 'SP-6' were planted within plots after plants 1, 4, 7 and 10. Trickle irrigation was utilized and fertigation was applied weekly. Fruit were harvested and rated (0-4, none-severe) for HH after cutting fruit from the stem to blossom end. Flesh firmness was taken using a Penetrometer FT 011 with a 7/16" plunger tip, (QA Supplies LLC, Norfolk, VA). Pressure was taken in five areas of the fruit; stem end, top side, ground spot side, blossom end, and center. Averaging across all cultivars and reps, a negative correlation (<0.32) was indicated between tissue firmness and incidence of moderate to severe HH (2-4) in 2012 and 2013. A positive correlation (>0.15) was found between increased tissue firmness and fruit with little or no HH (0-1) and a negative correlation (<0.15) between tissue firmness and fruit with moderate to severe HH (2-4) in the 2014 study. Tissue firmness correlations were highest in all three studies with pressure readings taken at the center of the fruit. The correlations suggest that cultivars with lower tissue firmness may have higher incidences of HH. In all three studies, cultivars that appear to be linked to HH include Bold Ruler, Liberty and Affirmed, having the highest percent of HH. With further analysis, we plan to determine if tissue firmness and cultivar interact, and which factor most contributes to the incidence and severity of HH. Partial Funding provided by the Specialty Crop Block Grant Program at the U.S. Department of Agriculture (USDA) through Grant 14-SCBGP-CA-0006 (The CucCAP Project)

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Research Aspects of Cucurbits in India

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Cucurbits are vegetable crops belonging to family Cucurbitaceae, which is mainly comprised of species that are consumed worldwide. The family consists of about 118 genera and 825 species. There is tremendous genetic diversity within the family, and the range of adaptation includes tropical, temperate and subtropical regions, arid deserts. The genetic diversity in cucurbits extends to both vegetative and reproductive characteristics and considerable range in the monoploid (x) chromosome number including 7 *Cucumis sativus*, 11 *Citrullus* spp., *Momordica* spp., *Lagenaria* spp., *Sechium* spp., and *Trichosanthes* spp., 12 *Benincasa hispida*, *Coccinia cordifolia*, *Cucumis* spp. other than *C. sativus*, and *Praecitrullus fistulosus*, 13 *Luffa* spp., and 20 (*Cucurbita* spp.). Cucurbits are consumed in various forms, *i.e.*, salad (cucumber, gherkins, long melon), sweet (ash gourd, pointed gourd), pickled (gherkins), desert (melons), culinary purposes (bitter gourd), and are well known for their unique medicinal properties. Plant genetic resources management, varietal development, hybrid development, breeding for resistance, clonal selection and off-season vegetable production are some areas that require special concern. Cucurbits are affected by a number of diseases and pests that are of national importance and cause of important economic losses in cucurbits. Breeding objectives of cucurbits can broadly defined by five categories, *viz.* earliness, sex ratio, growth habit and sensitivity to photoperiod, fruit yield, and fruit quality. The main goal of research on Cucurbitaceae in India is to improve productivity on sustainable basis. Cucurbits, comparatively, have small genomes, which is very useful for gene identification and marker development. Productivity is a major criteria to get maximum return, but like other vegetable crops, quality and availability of the product during lean periods are also equally important to fetch better price in the markets. There is a need to introduce diverse germplasm of cucurbits with an emphasis on biotic and abiotic stresses, desirable yield and quality characters. The mapping of gynoecious and parthenocarpic genes needs much more attention, as it may be commercialized for economic seed production and off-season protected cultivation. Cucurbit germplasm including wild relatives assembled at NBPGR and other centers across the country should be characterized and evaluated in different agro-climatic zones.

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