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Molecular markers are ideal to investigate genetic effects on the resistance/susceptibility to disease. Simple Sequence Repeats (SSRs), repetitions of nucleotide motifs of 1-5 bases, are currently the markers of choice due to their abundant distribution in the genomes, and suitability for high-throughput analysis. Yam, (*Dioscorea alata* L), the main food source for over 100 million people in humid and sub-humid tropics, is vulnerable to anthracnose (*Colletotrichum gloeosporioides*) disease. This is one of the major limiting factors in the production of yam worldwide. A collaborative project between the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, Africa, and Virginia State University, Petersburg, VA, was developed for genetic improvement and germplasm characterization of yams using molecular tools. Very limited sequence information is available from public genome databases. Total RNA was isolated from young leaves of resistant and susceptible genotypes and cDNA libraries corresponding to these two lines were constructed using Clontech's Creator SMART cDNA library construction kit. The libraries from the resistant and susceptible genotypes now have a total of 85,000 and 80,000 cDNA clones, respectively. These cDNA clones are currently being sequenced and nearly 80,000 EST sequences generated from this project are presented.

5:00–5:15 pm

Development of SSR Markers for *Chionanthus retusus* (Oleaceae) and Cross Amplification of Closely Related Taxa

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Chionanthus is a genus of the Oleaceae family along with *Olea*, *Fraxinus*, and *Forsythia*. Albeit being used as ornamental and commercialized as a natural product, the genetic information of *Chionanthus* spp. is negligible. We have created microsatellite-enriched libraries of *C. retusus*, assembled 1072 contigs, and detected 1010 repeats. The frequency of the repeats decreased exponentially with the increase in repeat length, and the most abundant motives were: AG, AC, AAG, ACC, AT and ACTC. We have screened 384 markers on 12 *Chionanthus* related taxa, characterized 57 microsatellite loci across four species of Oleaceae and characterized 195 within the species *C. retusus*, most of these being polymorphic. Polymorphic information content (PIC) values varied from zero to 0.85, and the percentage of heterozygous loci was in a

range from 24.6% to 68.4%. The SSR markers developed here could assist in the botanical characterization for breeding programs and in the industry for the quality control and authentication of varieties of these medicinal plants.

Specified Source(s) of Funding: USDA-ARS

5:15–5:30 pm

Fine Mapping Ty-3, a Major Begomovirus Resistance Gene in Tomato

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Fla. 8680, a *Tomato Yellow Leaf Curl Virus* (TYLCV) resistant breeding line, was used to develop an F₂ population for fine mapping Ty-3. Fla. 8680 has a *Solanum chilense* introgression of approximately 27cM on chromosome 6, spanning markers C2_At2g39690 (5.3cM) to T0834 (32cM). Seven-hundred-nineteen F₂ plants were screened with molecular markers to identify 30 recombinants within this region. F₄ and F₅ recombinant inbred lines (RILs) were tested for resistance to TYLCV in fall 2007 and spring 2008, respectively. Results indicate that Ty-3 maps to the 7 cM interval between markers T0774 (18cM) and P6-25 (25cM). Two recombinants, each containing this region within a shortened introgression, were used to develop a large segregating population to further fine map Ty-3. In spring 2009, over 9000 seedlings were screened with molecular markers at the distal ends of the introgressions, and over 200 recombinants were selected and transplanted to the field. Multiple cuttings taken from each recombinant were inoculated with whiteflies viruliferous for TYLCV and tested for resistance in a replicated field experiment. These results will be presented.

Specified Source(s) of Funding: USDA NRI

5:30–5:45 pm

Cloning and Characterizing Two PpSFBB-Alpha Genes in Chinese Sand Pear (*Pyrus pyrifolia* Nakai.)

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Gametophytic self-incompatibility (GSI) is genetically controlled mechanism to prevent inbreeding and promote out-crossing. It is controlled by a single multi-allelic S-locus, which contains two separate genes (a stylar S-RNase gene and a pollen S-gene). In gametophytic self-incompatible Japanese pear (*Pyrus pyrifolia* Nakai.) and apple (*Malus domestica*), three types of SFB genes (SFBB-alpha, SFBB-

beta, and SFBB-gamma) have been isolated of which the SFBB-alpha genes were identified as the best candidates for pollen S-genes. To investigate sequence characteristics of the possible pollen S-gene and elucidate possible GSI mechanism of Chinese sand pear (*P. pyrifolia*), SFBB-alpha homologies in Chinese sand pear cultivars were cloned and characterized by degenerate primers, PSFBA-F and PSFBA-R, that were designed based on Japanese pear and apple SFBB-alpha sequence information. Results showed that the PSFBA-F and PSFBA-R primers successfully amplified a fragment of approximately 1,300 bp from 'Maogong' ($S_{12}S_{13}$), 'Hongsucui' (S_4S_{12}), 'Tianchengzi' (S_7S_{12}), and 'Mantianhong' (S_4S_{12}), corresponding to pear SFBB-alpha gene. A total of two distinct sequences derived from the 1,300 bp product were identified that were named PpSFBB12-alpha (*P. pyrifolia* SFBB12-alpha) and PpSFBB13-alpha, respectively. RT-PCR revealed that both PpSFBB-alpha genes were expressed specifically in the pollen grains. The coding region of PpSFBB12-alpha was 1,194 bp in length encoding 397 amino acids with predicted molecular weight of 45.8 kDa and isoelectric point of 5.03. The coding region of PpSFBB13-alpha was 1,179 bp in length encoding 392 amino acids with predicted molecular weight of 45.4 kDa and isoelectric point of 4.92. Both genes displayed normal structural characteristics of SFB/SLF genes, i.e. an F-box motif and four variable regions. At the deduced amino acid level, they shared 18.6% to 97.7% similarities with other SFB/SLFs of rosaceous plants. These characteristics of the two PpSFBB-alpha genes fully demonstrated that they are good candidates of pollen S-gene. Phylogenetic analysis revealed that 34 rosaceous SFB/SLFs were divided into two subfamily groups, but did not further form species subgroup. The evolutionary pattern of SFB/SLFs concurred with that of rosaceous S-RNases, suggesting that SFB/SLFs occurred after divergence of subfamily, but before the divergence of species as S-RNases in Rosaceae. The two PpSFBB-alpha sequence data should be useful for studying the interaction between SFBs and S-RNases and clarifying the mechanism of GSI at the molecular level in Chinese sand pear.

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5:45–6:00 pm

Multiple Approaches for Genetic Engineering of Citrus for Disease and Pest Resistance

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Citrus in Florida is threatened by non-indigenous but now endemic bacterial pathogens causing the diseases Huanglongbing (HLB, caused by *Candidatus Liberibacter asiaticus*) and citrus canker (caused by *Xanthomonas citri* ssp. *citri*). These diseases have the potential to wipe out the entire industry due to unavailability of resistance in commercially cultivated Florida cultivars. HLB is vectored by the Asian citrus psyllid (*Diaphorina citri* Kuwayama), while canker is dispersed by windblown rain, contaminated equipment or human activity. In efforts to control the diseases, several transgenic strategies have been adopted. Citrus scion and rootstock cultivars were transformed with gene(s) encoding antimicrobial peptides, systemic acquired resistance (SAR) proteins or insecticidal proteins. Each gene was under control of an enhanced CaMV 35S promoter. Several genes were also under control of a phloem specific *Arabidopsis* SUC2 (*AtSUC2*) promoter as

C. Liberibacter asiaticus is a phloem limited bacterium and targeting gene expression in the phloem potentially resolves issues of presence of transprotein in the fruit. Molecular analysis confirmed the presence of the gene(s) in the citrus genome. We employed a rapid micrografting technique on tender rootstocks to quickly propagate our transgenic lines. Results from preliminary disease resistance tests suggested that some of the antimicrobial peptides could provide varying levels of resistance against both HLB and canker. Several studies are currently underway to evaluate resistance to either of the pathogens and also to the insect vector.

Oral Session 4:

Jefferson A

Horticultural Crops Culture and Management: Pest Management

Saturday, 25 July 2009, 4:00–6:00 pm

Moderator: Beiquan Mou, beiquan.mou@ars.usda.gov

4:00–4:15 pm

Effectiveness of Host Resistance Inducers and Humic Acid for Fire Blight Control and Shoot Growth on Apple Cultivars with M9 Rootstock

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Fire blight, caused by *Erwinia amylovora*, is the most devastating bacterial disease of apples with M9 rootstocks and often results in the death of the tree. The resistance-inducing compounds prohexadione-Ca (PC), harpin protein (Hp) and benzothiadiazole (acibenzolar-S-methyl) (BTH), the fertilizer humic acid (HA), the bactericides streptomycin and copper, and combinations of copper with chemicals were evaluated for their ability to control fire blight and shoot growth on apple cultivars, Gala, Red Elstar, Jonagold and Pinova, with M9 rootstock in two years. PC was applied at a rate of 125 mg·L⁻¹ at two shoot lengths (6–12 cm and 15–20 cm), while BTH (135 mg·L⁻¹) and harpin (50 mg·L⁻¹) were applied when the shoots measured between 15–20 cm, and again at 30–35 cm. HA (200 mg·L⁻¹), copper and streptomycin were applied at three phase of shoot lengths. In bioassay tests, bacterial populations were determined in plant tissues at 5th, 10th and 15th days. Disease severity and effectiveness of the applications were calculated using the following formulas: Disease severity (%) = (a/b) × 100; where a is the length of the blighted part of the shoot (cm), and b is the whole length of the shoot (cm) and Efficacy (%) = [(DI_{ck} – DI_{tr}) / DI_{ck}] × 100 where DI_{ck} is mean disease severity in water sprayed control plants and DI_{tr} is mean disease severity in treated plants. When the apple varieties and years were taken into consideration, PC alone (44% to 70%) and in combination with copper compound (48% to 63%) were the most effective treatment during both years on disease control, following by the streptomycin (76% to 95%) ($P \leq 0.05$). PC reduced both shoot length and shoot blight on the apple cultivars. The cultivar Red Elstar showed the lowest disease severity than the other cultivars and followed Jonagold, Pinova and Gala, respectively. HA applications were unsuccessful in controlling fire blight on all of the apple cultivars. Streptomycin treatment yielded the lowest rate of bacterial population, and it was followed by PC, Hp, BTH, copper and HA respectively. The use of resistance-inducing substances during the early phase of shoot growth may offer a means of managing the shoot blight phase of fire blight disease on apple cultivars with M9 rootstock.

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