Pineapple Mealybug Wilt Associated Viruses

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Report:

Mealybug wilt of pineapple (MWP) is a devastating disease of pineapple worldwide. The disease is characterized by severe leaf tip dieback, downward curling of the leaf margins, and reddening and wilting of the leaves that can lead to total collapse of the plant. *Ampeloviruses*, *Pineapple mealybug wilt associated virus-1* (PMWaV-1), PMWaV-2, and PMWaV-3 have been previously identified in pineapple grown in Hawaii. *Pineapple mealybug wilt associated virus-2* (PMWaV-2) infection and mealybug exposure are both involved in the etiology of MWP whereas PMWaV-1 is not a necessary component in MWP. Both viruses correlate with reduced yields.

Molecular diversity of viruses

Pineapple mealybug wilt associated virus-1 (PMWaV-1) and PMWaV-2 are mealybug transmitted *Ampeloviruses* that infect pineapple plants. The complete genome of PMWaV-1 was sequenced recently and found to contain seven open reading frames (ORFs). The genome organization of PMWaV-1 is distinct from PMWaV-2 and other ampeloviruses with the lack of an intergenic region between the RdRp and p6, a small coat protein (CP), and the apparent absence of a minor CP. Two additional putative viruses were identified in pineapple using degenerate primers targeting the closterovirus heat shock protein-70 (HSP-70) homolog gene. PMWaV-3 shares 63.9%, 72.5%, and 79.2% amino acid identity with PMWaV-1 for the RNA dependent RNA polymerase, small hydrophobic protein, and HSP-70-homolog ORFs, respectively. A virus tentatively designated PMWaV-4 shared less than 40% sequence homology with PMWaV-2 in the HSP-70 homolog region. Based on sequence available for PMWaV-4, it is a distinct virus from PMWaV 1, 2, and 3. Phylogenetic analyses placed PMWaV-1, PMWaV-3, PMWaV-4, PBNSPaV, and some GLRaVs in a distinct clade within the genus *Ampelovirus*.

Bacilliform-shaped particles with morphologies similar to *Badnaviruses*, (*Caulimoviridae*) have been identified in pineapple. Oligonucleotides degenerate for the conserved sequences in the reverse transcriptase/RNase H and protease regions of badnavirus open reading frame III were used in PCR assays to identify badnavirus-like sequences from commercially-grown pineapple plants in Hawaii. Cloning and sequencing of resulting amplicons of the expected size (400 to 650 bp) revealed the presence of at least four different clades, designated A, B, C, and D. Amino acid (aa) sequence

similarity of the 200 aa spanning the RT/RNase H region within each clade is above 98% and between clades ranges from 46% to 86%. Sequences within the B clade contain a stop codon within this region, suggesting a nonfunctional sequence, and possible integrated remnants of a pararetrovirus or retroelement. Specific PCR assays and DIG-labeled probes have been developed to detect and distinguish the variants amplified from field pineapple samples. The phylogenetic relatedness of clades A, B, C, and D to other badnaviruses, other Caulimoviridae members, and retro-like elements, and the distribution of the clades were examined.

Virus transmission by mealybug vectors

Several vector transmission characteristics of PMWaV-2 such as acquisition access period (AAP), persistence and retention of infectivity of the virus in grey pineapple mealybug vectors were evaluated. PMWaV-2 was detected in viruliferous grey pineapple mealybugs and in infected plants using a 2-step RT-PCR assay that involves the use of ImmoMix (Bioline), a hot-start Taq polymerase. Grey pineapple mealybug, a known vector of PMWaV-2, obtained maximum transmission efficiency (100%) after a 3 day- acquisition access period. The minimum acquisition access period for grey pineapple mealybug was 1.5 days. PMWaV-2 persists in viruliferous grey pineapple mealybugs up to 3 days after daily sequential transfer to healthy pineapple plants within a 7-day period. The maximum retention time that viruliferous grey pineapple mealybugs can retain PMWaV-2 while feeding on a non-host plant (*Cucurbita pepo*) and still remain infective when allowed to feed to the host plant (pineapple) was 24 hours. Transmission experiments provided evidence that PMWaV-2 is transmitted by the grey pineapple mealybug in a semi-persistent manner.

A real-time PCR assay has been developed to monitor virus concentrations in mealybugs and pineapple plants. Comparisons between virus concentrations of infected plants in the presence and absence of mealybug feeding may provide insight into the mechanisms underlying the development of mealybug wilt of pineapple (MWP), and may lead to novel prevention strategies.

Screening for viruses in newly-imported pineapple and germplasm accessions.

Reverse transcription-polymerase chain reaction (RT-PCR) and PCR assays for PMWaVs and badnaviruses were used to determine the distribution and incidence of these viruses in two new commercially important pineapple hybrids (one of which was imported from two different countries), and the germplasm accessions maintained at the ARS-USDA National Clonal Germplasm Repository in Hilo, HI. PMWaV-3, a virus previously identified at low incidence in the traditionally-grown hybrids and cultivars in Hawaii, was present in about 70% of one new hybrid. Incidences of PMWaV-2 were nearly 85% in the USDA-ARS accessions in Hilo, HI. Such high incidences indicate the need for screening of plant material prior to mass propagation and further planting expansions. Assays for the incidences of badnavirus-like sequences with members belonging to four different clades (designated A, B, C, and D) showed that badnavirus

clade B types were present in all plants tested, whereas incidences of clades A, C, and D varied from hybrid to hybrid.

Development of transgenic pineapple plants with virus resistance genes

Transformation with *Agrobacterium tumefaciens* was used to introduce gene constructs within a pCAMBIA backbone that contained either 1) the coat protein gene (CP) of *Pineapple mealybug wilt associated virus-2* (PMWaV-2), or 2) a sequence composed of a portion of the CP gene in sense orientation, a portion of the HSP70h gene in sense orientation, and a portion of the CP gene in sense and antisense orintations aligned in tandem to form a hairpin-like structure of complementary sequences (Rerez 2006; Perez et al. 2006). PCR analyses using oligonucleotide primer-pairs specific for the CP region or CP antisense sequence and the NOS-terminator region of 105 putatively transformed plants in the greenhouse showed that 8 plants, representing up to 7 distinct lines are positive for the transgene. Dig-labeled probes of the gene construct confirmed the specificity of the PCR assays. These plants will be further evaluated with genomic Southern hybridization experiments.

Impacts:

Our approach to control *Pineapple mealybug wilt associated viruses* and badnaviruses utilizes non-transgenic and transgenic methods. The high incidences of some PMWaVs and badnaviruses in newly-imported pineapple hybrids verify the importance of using virus screening assays to select virus-free plant material prior to mass propagation. Our results of virus persistence suggest that trap crops may have potential to reduce the spread of PMWaVs in commercial fields. Our development of a highly efficient tissue culture procedure for inducing adventitious bud formation from very young leaf bases will be used to develop efficient transformation protocols that may lead to the genetic improvement of pineapple with enhanced virus resistance.

Our efforts will continue to utilize non-transgenic and transgenic approaches for virus control in pineapple. We are currently investigating virus transmission characteristics and evaluating the virus titers in pineapple plants with and without mealybug exposures to better understand the mechanism behind PMWaV spread and MWP. This information could provide insight into novel genetic manipulations of pineapple that could lead to virus or mealybug resistance. We will also continue characterization of the pineapple plants that have tested PCR-positive for our anti-viral transgenes. This may help us better understand the mechanisms and possible modifications to future transgene constructs or siRNA pathways.

Publications:

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