2012 SRSFC report

Title: Understanding blueberry mosaic disease

Progress Report, SRSFC Project # 2012-11

Research Proposal

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Objectives

Our efforts will focus on **A.** understanding the causal agent of the disorder, including its identification and characterization, **B.** develop detection protocols that will be used to survey fields and determine the areas affected by the agent.

Justification

Blueberry cultivation has expanded dramatically in the last twenty years and fruit consumption has reached historic highs primarily because of the publicity on the beneficial effects of the antioxidants found in the fruit. Arkansas has the potential to become a major growing state in the Midsouth, alas the industry is lingering due to cultivation problems, a combination of cultural, abiotic and biotic stresses, caused primarily by pathogens. The industry may be facing another problem in Mosaic or Variegation. The disease has been reported in major blueberry producing areas (West coast, Michigan, New Jersey) and the affected area is expanding as there are reports from Europe (Plesko, personal communication). During a blueberry virus survey in Arkansas in 2009, we observed several plants showing typical mosaic disease symptoms. In 2010, the disease was observed in additional plants indicating movement of the causal agent. Mosaic was never reported in Arkansas before and its discovery in the state adds to an already stressed industry.

Methodologies

Contigs obtained from blueberry mosaic samples were compared to those found in sequence databases. Virus-like sequences were aligned and PCR primers were designed to amplify the viral genome. PCR products were cloned into the TOPO® TA 2.1 vector and transformed into *Escherichia coli* cells. Plasmids were extracted from

recombinant *E. coli* cells and sequences were assembled to contigs by CAP3 to obtain the three genomic RNAs of Blueberry mosaic virus. Confirmation of the 5' and 3' termini of the RNAs, RACE-PCRs were conducted and the resultant products were cloned and sequenced as described above. Further characterization of the virus was done by subjecting the RNA dependent RNA polymerase (RdRP) to phylogenetic analysis using MEGA5.

Results

Sequence and phylogenetic analysis of Blueberry mosaic indicated that the virus belongs to the *Ophioviridae*, a family of multipartite negative sense RNA viruses. We have sequenced three RNAs of the viral genome which are 7.9 kb, 1.9 kb and 1.5 kb in length. The sequence information obtained has been used for the development of a molecular diagnostic test (RT-PCR).

Conclusions

We identified the virus closely associated with blueberry mosaic disease as an ophiovirus with three genomic RNAs. Our future efforts will include the development of universal detection protocols, based on a large number of isolates so as to minimize the possibility of escapes due to sequence variability. We will also focus on the identification of the virus vector(s). The majority of the ophioviruses are reported to be transmitted by soilborne chytrid fungi. Information obtained from our work provides valuable knowledge that can be used to identify vectors and implement effective control measures.

Impact Statement

Identification of Blueberry mosaic virus as an ophiovirus is of concern since the majority of viruses in the family are transmitted by the soilborne fungi in the genus *Olpidium*. Members of the family can be found on zoospores as well as in resting spores of the fungus. It is common that ophioviruses persist in infected soil for years without a living host making eradication a difficult undertaking. This project will minimize the movement of infected material and spread of the disease to new areas. This will have a major effect not only in the farm level but also in nurseries, as non-meristemed clonal propagation of blueberry could lead to the dissemination of the virus to all produced propagules.