Title: Virus elimination from Arkansas blackberry breeding lines

Final Report

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Research Proposal

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Objectives

1. To introduce a group of advanced blackberry breeding selections for virus testing based on potential variety release from the Arkansas breeding program.
2. To conduct a range of virus tests on the genotypes using ELISA, PCR and double-stranded RNA methodologies as appropriate.
3. To move selections to tissue culture and provide foundation stock for cultivar release.

Justification

There are more than 5,000 acres of blackberries grown in the Southeast US with the acreage increasing each year to meet fresh market demands (Strik et al., 2008). An important factor for the success of blackberry in the southeast are the varieties developed at the University of Arkansas from Dr. James Moore and the leading PI of this proposal. The potential of greater expansion and growth of the industry is threatened by Blackberry yellow vein, a disease that has emerged in the last eight years in the region. The problem with Blackberry yellow vein disease is such that is has become one of the top priorities for the industry.

There has been little work on blackberry viruses in the last three decades until a coordinated effort led by the co-PIs of this proposal. As a result there have been 14 new viruses - to *Rubus* or science - identified in the last five years (Halgren et al., 2007; Martin and Tzanetakis, 2008; Susaimuthu et al., 2008; Tzanetakis et al., 2007a;b; Tzanetakis et al., 2008; Tzanetakis and Martin, unpublished data).

All blackberry cultivars developed at the University of Arkansas have at least a five year field growth cycle before selection and release. The U of A advanced selections have been tested for the standard *Rubus* viruses before release. In the past, those tests have been sufficient for minimizing the effects of virus diseases in new blackberry fields. The virus-caused problems observed today and the new viruses discovered (the majority of which do not have commercially available tests) make evident the need for a virus clean-up procedure that will guarantee that the released material is free of all the viruses known to infect *Rubus*. We expect that this work will have a great effect in the viability of the plants in the field and the life span of the blackberry plantations in the southeast and elsewhere.

Methodologies

Multiple plants of a group select clones (the genotypes determined by JR Clark based on commercial cultivar potential) were or will be tested by ELISA for the presence of *Raspberry bushy dwarf, Tobacco ringspot, Strawberry necrotic shock* and *Tomato ringspot, Cherry leaf roll, Cherry rasp leaf, Strawberry latent ringspot, Arabis mosaic* and *Cucumber mosaic viruses* using ELISA. Extract virus enriched nucleic acids were also used or will be used as a template for testing for the following viruses using RT-PCR: Blackberry yellow vein associated virus, Blackberry virus Y, *Beet pseudo yellows virus*, Raspberry mottle virus, Blackberry virus X, Blackberry virus E, Blackberry yellow mottle virus, Raspberry mottle virus, Raspberry leaf spot virus - North America, Blackberry chlorotic ringspot virus, and *Impatiens necrotic spot virus*. Plants that tested negative in these tests were or will be graft-inoculated onto *Rubus occidentalis* 'Munger' which is an indicator for most of the uncharacterized virus-like agents reported in *Rubus* species. In cases where none of the selected plants of a clone test negative for viruses in each of the above tests, two individual plants will be subjected to heat therapy. In cases where a plant is identified that tests negative in each of the virus assays, an individual plant that tested negative will be subjected to heat therapy. The latter is to reduce the chance of infection of a released cultivar by an unknown virus. Since 2002 at least 14 new viruses of *Rubus* have been identified, thus the plan to go through the virus elimination program even when a plant tests negative for all known viruses.

The resulting clean plant material will be tissue cultured, and if the genotype is released for public use then the culture will be used by commercial propagators for the initial

multiplication of foundation stock. This system should ensure clean stock is used for all new blackberry cultivar releases.

Results

Advanced selections were provided by JR Clark for virus testing. These included:
APF-40
APF-41
APF-45
APF-77
APF-132
APF-136
APF-138
A-2252
A-2271
A-2339
A-2361
A-2362

The selection APF-45 had all virus testing completed in June 2009 (no viruses found), was placed in tissue culture and was moved to commercial tissue laboratories in late summer 2009 to begin propagation for release. This selection was released as Prime-Ark®45 in late summer. Initial stock is being produced for conventional propagators along with tissue culture plants to be available in 2010 for commercial growers.

Additionally, selections APF-41, APF-77, APF-136, APF-138, A-2252, A-2271, A-2339 and A-2361 had graft indexing conducted along with a partial testing of viruses as proposed. Virus testing continues on these selections at present. One, APF-136, was found initially to be virus infected, and is currently in heat treatment.

Selections APF-40, APF-132, A-2339 and A-2362 are currently being established in pots from root cuttings, and virus testing will begin on these in late winter 2010.

Conclusions

The activities proposed have been vigorously pursued in the past year. The release of Prime-Ark®45 with extensive virus tested foundation stock should ensure clean plant stock is introduced into the commercial industry. This is likely the most virus-tested of any blackberry in history, at least prior to release.

The many other selections continue to be worked with in various stages of establishment for testing, the conducting of testing, and as needed, heat treatment.

Impact Statement

The clear impact is that initial stock of Prime-Ark®45 was tested extensively for viruses, and this will allow thoroughly tested initial stock to enter into the marketplace at the time of first introduction of the cultivar. Other selections will later be introduced, and the extensive testing will provide for further clean stock for nurseries and commercial growers. This should have a positive impact on reducing virus infections in commercial plantings.

Citation(s)

None resulting from this work.

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