



Short communication

Development of reliable detection assays for blueberry mosaic- and blackberry vein banding- associated viruses based on their population structures



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ABSTRACT

Blueberry mosaic associated virus (BIMaV), the presumed causal agent of the homonymous disease and blackberry vein banding associated virus (BVbAV), a component of the blackberry yellow vein disease complex, are recently characterized RNA viruses. There is a need for efficient and sensitive detection protocols for the two viruses, not only for screening during the nursery propagation process but also in commercial fields to better understand virus epidemiology and minimize disease spread. RNA viruses display significant nucleotide variation forming quasi-species. Therefore, sequence-based detection methodologies, even though sensitive, may lead to false negative results. For this reason, information on the genetic diversity of virus populations is essential to develop diagnostic assays that have the potential to detect all variants. Detection assays for BIMaV and BVbAV were developed based on existing genetic diversity data and were validated by screening samples from different geographical areas in the United States. These detection tests provide sensitivity and specificity and will serve as the protocols of choice for virus screening in *Vaccinium* and *Rubus* certification programs in the United States and elsewhere. Given the increasing global trade of both blueberry and blackberry these tests will be valuable in avoiding virus introductions to new areas.

1. Type of research

- i The genetic information of the putative disease agent of blueberry mosaic, blueberry mosaic associated virus (BIMaV), was recently unveiled (Thekke-Veetil et al., 2014). BIMaV is a tripartite negative-sense RNA virus in the *Ophioviridae* family encoding viral replicase and a 24 kDa protein of unknown function in RNA 1, the movement protein in RNA 2 and the nucleoprotein in RNA 3.
- ii Blackberry vein banding associated virus (BVbAV), a member of subgroup I of the genus *Ampelovirus* in the *Closteroviridae* family, is a component of blackberry yellow vein disease and found widespread in wild and cultivated blackberries (Thekke-Veetil et al., 2013).
- iii RNA viruses exhibit higher rates of sequence variation due to their error prone replication mechanisms (Lauring and Andino, 2010). The genetic variation in BIMaV and BVbAV populations were studied in depth (Thekke-Veetil et al., 2013; Thekke-Veetil et al., 2015) and sequence information obtained from these studies were utilized in designing PCR-based detection assays for both viruses.

2. Time required

Whole protocol: 7 h

- a Total nucleic acid extraction: 2 h
- b Reverse transcription: 1.5 h
- c PCR: 1.5 h
- d Gel electrophoresis and imaging: 2 h

3. Materials

3.1. Special equipment

No special equipment is required. Any microcentrifuge, vortexer, conventional thermocycler, gel electrophoresis apparatus and gel imaging system could be used for performing the steps mentioned in the protocols.

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Table 1

Details of the isolates used for designing detection primers for blueberry mosaic associated virus. Isolates AR1 through OR22 are U.S. isolates.

Isolate	State/Country	GenBank accession Number
AR1	Arkansas	KJ849098
AR2	Arkansas	KJ849099
AR3	Arkansas	KJ849100
AR4	Arkansas	KJ849101
AR5	Arkansas	KJ849102
KY1	Kentucky	KJ849127
KY2	Kentucky	KJ849128
KY3	Kentucky	KJ849129
KY4	Kentucky	KJ849130
MI1	Michigan	KJ849092
MI2	Michigan	KJ849093
MI3	Michigan	KJ849094
MI4	Michigan	KJ849095
MI5	Michigan	KJ849096
MI6	Michigan	KJ849097
NJ1	New Jersey	KJ849131
NJ2	New Jersey	KJ849132
NJ3	New Jersey	KJ849133
NJ4	New Jersey	KJ849134
NJ5	New Jersey	KJ849135
NJ6	New Jersey	KJ849136
NJ7	New Jersey	KJ849137
NJ8	New Jersey	KJ849138
NJ9	New Jersey	KJ849139
NJ10	New Jersey	KJ849140
NJ11	New Jersey	KJ849141
OR1	Oregon	KJ849103
OR2	Oregon	KJ849104
OR3	Oregon	KJ849105
OR4	Oregon	KJ849106
OR5	Oregon	KJ849107
OR6	Oregon	KJ849108
OR7	Oregon	KJ849115
OR8	Oregon	KJ849121
OR9	Oregon	KJ849113
OR10	Oregon	KJ849109
OR11	Oregon	KJ849112
OR12	Oregon	KJ849116
OR13	Oregon	KJ849125
OR14	Oregon	KJ849117
OR15	Oregon	KJ849118
OR16	Oregon	KJ849119
OR17	Oregon	KJ849124
OR18	Oregon	KJ849111
OR19	Oregon	KJ849110
OR20	Oregon	KJ849126
OR21	Oregon	KJ849122
OR22	Oregon	KJ849114
BC1	Canada	KJ849120
BC2	Canada	KJ849123
Slo1	Slovenia	KJ849142
Slo2	Slovenia	KJ849143
Slo3	Slovenia	KJ849144
Slo4	Slovenia	KJ849145
Slo5	Slovenia	KJ849146
Slo6	Slovenia	KJ849147
Slo7	Slovenia	KJ849148
Slo8	Slovenia	KJ849149
Slo9	Slovenia	KJ849150

NA- Not available

3.2. Chemicals and reagents

a Total nucleic acid extraction: RNA extraction buffer (200 mM Tris-HCl [pH 8.5], 300 mM lithium chloride, 1.5% sodium dodecylsulfate, 10 mM ethylene diamine tetra-acetic acid [EDTA], 1% sodium deoxycholate, 1% NP-40, and 1% 14 M β -mercaptoethanol solution [vol/vol] added just before use), 5.8 M potassium acetate (pH 6.5), 100% isopropanol, RNA wash buffer (10 mM Tris-HCl [pH 7.5], 0.5 mM EDTA, 50 mM NaCl, and 50% ethanol), silica, and Tris-

Table 2

Details of the isolates used for designing detection primers for blackberry vein banding associated virus.

Isolate	Location	GenBank accession number
AR1	Arkansas	KC912179
AR2	Arkansas	KC912188
GA1	Georgia	KC912181
GA2	Georgia	KC912185
GA3	Georgia	KC912186
GA4	Georgia	KC912182
GA5	Georgia	KC912183
GA6	Georgia	KC912187
GA7	Georgia	KC912184
MS1	Mississippi	KC912165
MS2	Mississippi	KC912189
NC1	North Carolina	KC912171
NC2	North Carolina	KC912170
NC3	North Carolina	KC912166
NC4	North Carolina	KC912169
NC5	North Carolina	KC912168
NC6	North Carolina	KC912172
NC7	North Carolina	KC912173
NC8	North Carolina	KC912167
NC9	North Carolina	KC912178
NC10	North Carolina	KC912175
NC11	North Carolina	KC912176
NC12	North Carolina	KC912177
SC1	South Carolina	KC912180
SC2	South Carolina	KC912174

EDTA (10 mM Tris-HCl and 1 mM EDTA, pH 8.0).

b Reverse transcription: Maxima[®] reverse transcriptase, RiboLock[™] RNase inhibitor and random hexamer primer, UltraPure[™] DNase/RNase-Free distilled water (Thermo Fisher Scientific, USA).

c PCR: *Taq* DNA polymerase and dNTP Mix (Genscript, USA), detection primers synthesized by Integrated DNA Technologies (USA).

d Gel electrophoresis and imaging: sodium borate (SB) agarose gel buffer, 1X (10 mM NaOH, 66 mM boric acid, pH8.2) and GelRed (Biotium, USA).

4. Detailed procedure

4.1. Assay design and standardization

Sequence information obtained from GenBank (61 BlMaV and 25 BVBaV isolates; Tables 1 and 2) were aligned using MUSCLE (Edgar, 2004) and conserved regions were identified. Degenerate primer sets and PCR assays were designed for each virus. After preliminary experiments primers that provided sensitive and reliable results were selected and PCR conditions were optimized. For BlMaV, the detection primers BlMaVMPdegF (5'-CCWGTATCAAGCATAGTYACAAG-3') and BlMaVMPdegR (5'-AAGAAGGTRGTGATTGAGA-3') target a 254- base region of the MP gene, and for BVBaV, primers BVBaVdetF (5'-CTGC-TRTAYAGCACRGTAAACA-3') and BVBaVdetR (5'-TACGTGCGGACT-TTGTTAGTG-3') amplify a 157- base region of the minor coat protein gene. Reaction mixtures and conditions were optimized to also amplify a 721-base region of the NADH dehydrogenase ND-2 subunit transcript (Thekke-Veetil et al., 2016; Tzanetakis et al., 2007), to ensure the quality of the extracted nucleic acids.

4.2. Detailed steps

4.2.1. Total nucleic acid extraction

Total nucleic acids were extracted from 50 mg of leaf tissue following the steps essentially as described by Poudel et al. (Poudel et al., 2013).

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