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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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ARTICLE INFO	ABSTRACT
Keywords: Detection Sensitivity Specificity Reliability Certification	Blueberry mosaic associated virus (BlMaV), the presumed causal agent of the homonymous disease and black- berry 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 varia- tion 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 BlMaV 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 <i>Vaccinium</i> and <i>Rubus</i> 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 (BlMaV), was recently unveiled (Thekke-Veetil et al., 2014). BlMaV is a tripartite negativesense 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 BlMaV 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 apparati and gel imaging system could be used for performing the steps mentioned in the protocols.

Methods

CrossMark

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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.

AR1ArkansasKJ849098AR2ArkansasKJ849100AR4ArkansasKJ849100AR4ArkansasKJ849101AR5ArkansasKJ849102KY1KentuckyKJ849127KY2KentuckyKJ849127KY2KentuckyKJ849128KY3KentuckyKJ849130MI1MichiganKJ849092MI2MichiganKJ849093MI3MichiganKJ849094MI4MichiganKJ849096MI5MichiganKJ849097NJ1New JerseyKJ849131NJ2New JerseyKJ849132NJ3New JerseyKJ849133NJ4New JerseyKJ849135NJ5New JerseyKJ849135NJ6New JerseyKJ849135NJ7New JerseyKJ849136NJ7New JerseyKJ849138NJ9New JerseyKJ849138NJ9New JerseyKJ849130NJ10New JerseyKJ849136NJ11New JerseyKJ849136NJ3New JerseyKJ849136NJ4OregonKJ849106OR1OregonKJ849106OR2OregonKJ849106OR4OregonKJ849106OR5OregonKJ849107OR6OregonKJ849112OR9OregonKJ849112OR10OregonKJ849112OR10OregonKJ849113OR11OregonKJ849114 </th <th>Isolate</th> <th>State/Country</th> <th>GenBank accession Number</th>	Isolate	State/Country	GenBank accession Number
AR3ArkansasKJ849100AR4ArkansasKJ849102KY1KentuckyKJ849102KY1KentuckyKJ849128KY2KentuckyKJ849129KY4KentuckyKJ849129KY4KentuckyKJ849129KY4KentuckyKJ849023M11MichiganKJ849093M13MichiganKJ849093M13MichiganKJ849096M16MichiganKJ849096M16MichiganKJ849096M16MichiganKJ849096M16MichiganKJ849097NJ1New JerseyKJ849132NJ3New JerseyKJ849133NJ4New JerseyKJ849135NJ5New JerseyKJ849136NJ7New JerseyKJ849136NJ7New JerseyKJ849138NJ8New JerseyKJ849138NJ9New JerseyKJ849139NJ10New JerseyKJ849139NJ11New JerseyKJ849136NJ11New JerseyKJ849136NJ3OregonKJ849106OR1OregonKJ849107OR2OregonKJ849108OR2OregonKJ849103OR4OregonKJ849106OR5OregonKJ849113OR6OregonKJ849113OR10OregonKJ849113OR11OregonKJ849113OR12OregonKJ849113OR13OregonKJ849116<	AR1	Arkansas	KJ849098
AR4ArkansasKJ849101AR5ArkansasKJ849102KY1KentuckyKJ849128KY2KentuckyKJ849128KY3KentuckyKJ849129KY4KentuckyKJ84902M11MichiganKJ849092M12MichiganKJ849093M13MichiganKJ849096M14MichiganKJ849096M15MichiganKJ849097NJ1New JerseyKJ849131NJ2New JerseyKJ849133NJ3New JerseyKJ849134NJ4New JerseyKJ849135NJ5New JerseyKJ849136NJ7New JerseyKJ849137NJ8New JerseyKJ849140N111New JerseyKJ849140N111New JerseyKJ849140N111New JerseyKJ849137N111New JerseyKJ849137N111New JerseyKJ849140OR1OregonKJ849107	AR2	Arkansas	KJ849099
ARSArkansasKJ849102KY1KentuckyKJ849127KY2KentuckyKJ849128KY3KentuckyKJ849129KY4KentuckyKJ849092MI1MichiganKJ849092MI2MichiganKJ849093MI3MichiganKJ849095MI5MichiganKJ849096MI6MichiganKJ849097NJ1New JerseyKJ849132NJ2New JerseyKJ849133NJ4New JerseyKJ849133NJ5New JerseyKJ849135NJ6New JerseyKJ849135NJ7New JerseyKJ849137NJ8New JerseyKJ849138NJ7New JerseyKJ849138NJ8New JerseyKJ849138NJ9New JerseyKJ849137NJ10New JerseyKJ849100OR1OregonKJ849103OR2OregonKJ849103OR4OregonKJ849105OR4OregonKJ849105OR4OregonKJ849107OR6OregonKJ849108OR7OregonKJ849113OR10OregonKJ849113OR11OregonKJ849113OR12OregonKJ849113OR4OregonKJ849113OR5OregonKJ849113OR10OregonKJ849113OR10OregonKJ849113OR11OregonKJ849114OR12OregonKJ849122<	AR3	Arkansas	KJ849100
KY1KentuckyKJ849127KY2KentuckyKJ849128KY3KentuckyKJ849129KY4KentuckyKJ849130M11MichiganKJ849092M12MichiganKJ849093M13MichiganKJ849094M14MichiganKJ849095M15MichiganKJ849097M14MichiganKJ849097NJ1New JerseyKJ849131NJ2New JerseyKJ849132NJ3New JerseyKJ849133NJ4New JerseyKJ849135NJ5New JerseyKJ849136NJ7New JerseyKJ849136NJ7New JerseyKJ849136NJ7New JerseyKJ849137NJ8New JerseyKJ849138NJ9New JerseyKJ849139NJ10New JerseyKJ849130NJ11New JerseyKJ849103OR2OregonKJ849103OR3OregonKJ849105OR4OregonKJ849106OR5OregonKJ849112OR6OregonKJ849112OR6OregonKJ849112OR9OregonKJ849112OR10OregonKJ849112OR11OregonKJ849112OR12OregonKJ849112OR4OregonKJ849112OR5OregonKJ849114OR16OregonKJ849114OR16OregonKJ849124OR16OregonKJ849124<	AR4	Arkansas	KJ849101
KY2KentuckyKJ849128KY3KentuckyKJ849129KY4KentuckyKJ849130M11MichiganKJ849092M12MichiganKJ849093M13MichiganKJ849094M14MichiganKJ849095M15MichiganKJ849097M11New JerseyKJ849097NJ1New JerseyKJ849131NJ2New JerseyKJ849133NJ3New JerseyKJ849134NJ4New JerseyKJ849135NJ6New JerseyKJ849137NJ7New JerseyKJ849137NJ8New JerseyKJ849137NJ8New JerseyKJ849137NJ8New JerseyKJ849137NJ8New JerseyKJ849137NJ8New JerseyKJ849137NJ8New JerseyKJ849137NJ10New JerseyKJ849137NJ11New JerseyKJ849137NJ8New JerseyKJ849137NJ11New JerseyKJ849107OR1OregonKJ849107OR2OregonKJ849107OR3OregonKJ849107OR6OregonKJ849107OR6OregonKJ849112OR10OregonKJ849112OR10OregonKJ849112OR12OregonKJ849112OR13OregonKJ849112OR14OregonKJ849112OR15OregonKJ849114OR16OregonK	AR5	Arkansas	KJ849102
KY3KentuckyKJ849129KY4KentuckyKJ84903M11MichiganKJ849092M12MichiganKJ849093M13MichiganKJ849096M14MichiganKJ849096M15MichiganKJ849096M16MichiganKJ849097NJ1New JerseyKJ849132NJ3New JerseyKJ849133NJ4New JerseyKJ849133NJ5New JerseyKJ849135NJ6New JerseyKJ849136NJ7New JerseyKJ849137NJ8New JerseyKJ849138NJ9New JerseyKJ849138NJ0New JerseyKJ849136NJ10New JerseyKJ849139NJ11New JerseyKJ849103OR2OregonKJ849103OR2OregonKJ849105OR4OregonKJ849105OR5OregonKJ849106OR5OregonKJ849107OR6OregonKJ849113OR10OregonKJ849113OR11OregonKJ849113OR12OregonKJ849113OR4OregonKJ849113OR5OregonKJ849113OR10OregonKJ849113OR12OregonKJ849114OR14OregonKJ849117OR15OregonKJ849117OR16OregonKJ849117OR17OregonKJ849126OR14OregonKJ849120 <t< td=""><td>KY1</td><td>Kentucky</td><td>KJ849127</td></t<>	KY1	Kentucky	KJ849127
KY4     Kentucky     KJ849130       M11     Michigan     KJ849092       M12     Michigan     KJ849093       M13     Michigan     KJ849095       M14     Michigan     KJ849096       M14     Michigan     KJ849096       M15     Michigan     KJ849097       NJ1     New Jersey     KJ849132       NJ2     New Jersey     KJ849133       NJ3     New Jersey     KJ849135       NJ4     New Jersey     KJ849135       NJ5     New Jersey     KJ849136       NJ7     New Jersey     KJ849138       NJ7     New Jersey     KJ849133       NJ8     New Jersey     KJ849133       NJ9     New Jersey     KJ849130       NJ10     New Jersey     KJ849130       NJ11     New Jersey     KJ849140       NJ11     New Jersey     KJ849133       NJ10     New Jersey     KJ849133       NJ11     New Jersey     KJ849103       OR2     Oregon     KJ849103	KY2	Kentucky	KJ849128
MI1MichiganKJ849092M12MichiganKJ849093M13MichiganKJ849094M14MichiganKJ849096M15MichiganKJ849097M15MichiganKJ849097M11New JerseyKJ849131N12New JerseyKJ849132N33New JerseyKJ849133N14New JerseyKJ849133NJ5New JerseyKJ849135NJ6New JerseyKJ849136NJ7New JerseyKJ849137NJ8New JerseyKJ849138NJ9New JerseyKJ849137NJ10New JerseyKJ849137NJ11New JerseyKJ849138NJ9New JerseyKJ849133NJ10New JerseyKJ849141OR1OregonKJ849104OR2OregonKJ849104OR3OregonKJ849105OR4OregonKJ849106OR5OregonKJ849105OR4OregonKJ849113OR6OregonKJ849113OR10OregonKJ849112OR6OregonKJ849113OR11OregonKJ849114OR12OregonKJ849116OR5OregonKJ849113OR14OregonKJ849114OR15OregonKJ849113OR16OregonKJ849114OR17OregonKJ849114OR18OregonKJ849114OR19OregonKJ849114<	KY3	Kentucky	KJ849129
MI2     Michigan     KJ849093       MI3     Michigan     KJ849095       MI4     Michigan     KJ849095       MI5     Michigan     KJ849096       MI6     Michigan     KJ849097       NJ1     New Jersey     KJ849131       NJ2     New Jersey     KJ849133       NJ3     New Jersey     KJ849133       NJ4     New Jersey     KJ849135       NJ5     New Jersey     KJ849135       NJ6     New Jersey     KJ849135       NJ7     New Jersey     KJ849137       NJ8     New Jersey     KJ849138       NJ9     New Jersey     KJ849138       NJ10     New Jersey     KJ849133       NJ10     New Jersey     KJ849104       N11     New Jersey     KJ849103       OR2     Oregon     KJ849103       OR2     Oregon     KJ849104       OR3     Oregon     KJ849105       OR4     Oregon     KJ849107       OR6     Oregon     KJ849116	KY4	Kentucky	KJ849130
Mi3     Michigan     KJ849094       Mi4     Michigan     KJ849095       MI5     Michigan     KJ849097       NJ1     New Jersey     KJ849131       NJ2     New Jersey     KJ849132       NJ3     New Jersey     KJ849133       NJ4     New Jersey     KJ849133       NJ5     New Jersey     KJ849135       NJ6     New Jersey     KJ849137       NJ7     New Jersey     KJ849137       NJ8     New Jersey     KJ849137       NJ7     New Jersey     KJ849137       NJ8     New Jersey     KJ849140       NJ10     New Jersey     KJ849141       OR1     Oregon     KJ849105       OR2     Oregon     KJ849104       OR3     Oregon     KJ849105       OR4     Oregon     KJ849105       OR5     Oregon     KJ849112       OR6     Oregon     KJ849112       OR4     Oregon     KJ849113       OR10     Oregon     KJ849112	MI1	Michigan	KJ849092
MI4     Michigan     KJ849095       MI5     Michigan     KJ849097       MI6     Michigan     KJ849097       NJ1     New Jersey     KJ849131       NJ2     New Jersey     KJ849133       NJ4     New Jersey     KJ849133       NJ4     New Jersey     KJ849133       NJ5     New Jersey     KJ849136       NJ7     New Jersey     KJ849136       NJ7     New Jersey     KJ849137       NJ8     New Jersey     KJ849137       NJ8     New Jersey     KJ849137       NJ8     New Jersey     KJ849133       NJ9     New Jersey     KJ849133       NJ10     New Jersey     KJ849133       NJ11     New Jersey     KJ849103       OR2     Oregon     KJ849103       OR2     Oregon     KJ849103       OR4     Oregon     KJ849105       OR5     Oregon     KJ849113       OR6     Oregon     KJ849113       OR10     Oregon     KJ849114	MI2	Michigan	KJ849093
MI5MichiganKJ849096MI6MichiganKJ849097NJ1New JerseyKJ849131NJ2New JerseyKJ849132NJ3New JerseyKJ849133NJ4New JerseyKJ849133NJ5New JerseyKJ849136NJ7New JerseyKJ849136NJ7New JerseyKJ849137NJ8New JerseyKJ849137NJ8New JerseyKJ849139NJ10New JerseyKJ849133NJ11New JerseyKJ849140N111New JerseyKJ849141OR1OregonKJ849103OR2OregonKJ849104OR3OregonKJ849105OR4OregonKJ849106OR5OregonKJ849108OR7OregonKJ849108OR7OregonKJ849113OR8OregonKJ849112OR10OregonKJ849112OR11OregonKJ849112OR12OregonKJ849117OR13OregonKJ849117OR14OregonKJ849117OR15OregonKJ849117OR16OregonKJ849117OR17OregonKJ849111OR18OregonKJ849114OR19OregonKJ849114OR16OregonKJ849114OR17OregonKJ849122OR20OregonKJ849123OR16OregonKJ849124OR19OregonKJ849124 <td< td=""><td>MI3</td><td>Michigan</td><td>KJ849094</td></td<>	MI3	Michigan	KJ849094
MI6     Michigan     KJ849097       NJ1     New Jersey     KJ849131       NJ2     New Jersey     KJ849132       NJ3     New Jersey     KJ849133       NJ4     New Jersey     KJ849133       NJ5     New Jersey     KJ849135       NJ6     New Jersey     KJ849137       NJ7     New Jersey     KJ849137       NJ8     New Jersey     KJ849133       NJ7     New Jersey     KJ849133       NJ8     New Jersey     KJ849139       NJ10     New Jersey     KJ849104       N111     New Jersey     KJ849104       OR1     Oregon     KJ849104       OR1     Oregon     KJ849104       OR3     Oregon     KJ849106       OR7     Oregon     KJ849105       OR8     Oregon     KJ849113       OR10     Oregon     KJ849114       OR10     Oregon     KJ849116       OR11     Oregon     KJ849116       OR12     Oregon     KJ849112	MI4	Michigan	KJ849095
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     KJ849138       NJ8     New Jersey     KJ849138       NJ9     New Jersey     KJ849133       NJ10     New Jersey     KJ849133       NJ10     New Jersey     KJ849140       NJ11     New Jersey     KJ849103       OR1     Oregon     KJ849103       OR2     Oregon     KJ849105       OR4     Oregon     KJ849105       OR4     Oregon     KJ849107       OR6     Oregon     KJ849108       OR7     Oregon     KJ849113       OR8     Oregon     KJ849113       OR10     Oregon     KJ849112       OR12     Oregon     KJ849112       OR13     Oregon     KJ849112	MI5	Michigan	KJ849096
NJ2New JerseyKJ849132NJ3New JerseyKJ849133NJ4New JerseyKJ849133NJ5New JerseyKJ849135NJ6New JerseyKJ849137NJ7New JerseyKJ849137NJ8New JerseyKJ849137NJ8New JerseyKJ849139NJ10New JerseyKJ849140NJ11New JerseyKJ849141OR1OregonKJ849103OR2OregonKJ849104OR3OregonKJ849105OR4OregonKJ849107OR6OregonKJ849107OR6OregonKJ849107OR6OregonKJ849103OR7OregonKJ849107OR6OregonKJ849107OR6OregonKJ849115OR8OregonKJ849113OR10OregonKJ849112OR11OregonKJ849112OR12OregonKJ849112OR13OregonKJ849112OR14OregonKJ849117OR15OregonKJ849110OR17OregonKJ849110OR19OregonKJ849126OR21OregonKJ849123OR10OregonKJ849124OR15OregonKJ849122OR20OregonKJ849124OR16OregonKJ849120OR21OregonKJ849122OR22OregonKJ849123Slo1SloveniaKJ849143Slo2	MI6	Michigan	KJ849097
NJ3New JerseyKJ849133NJ4New JerseyKJ849134NJ5New JerseyKJ849135NJ6New JerseyKJ849137NJ7New JerseyKJ849137NJ8New JerseyKJ849138NJ9New JerseyKJ849139NJ10New JerseyKJ849141OR1OregonKJ849103OR2OregonKJ849103OR3OregonKJ849105OR4OregonKJ849107OR6OregonKJ849107OR6OregonKJ849107OR6OregonKJ849108OR7OregonKJ849113OR8OregonKJ849113OR9OregonKJ849113OR10OregonKJ849109OR11OregonKJ849112OR12OregonKJ849112OR13OregonKJ849117OR14OregonKJ849118OR15OregonKJ849117OR16OregonKJ849118OR17OregonKJ849118OR16OregonKJ849118OR17OregonKJ849110OR20OregonKJ849110OR21OregonKJ849123Slo1SloveniaKJ849123Slo1SloveniaKJ849123Slo1SloveniaKJ849143Slo3SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147	NJ1	New Jersey	KJ849131
NJ4New JerseyKJ849134NJ5New JerseyKJ849135NJ6New JerseyKJ849136NJ7New JerseyKJ849137NJ8New JerseyKJ849138NJ9New JerseyKJ849138NJ10New JerseyKJ849139NJ11New JerseyKJ849103OR2OregonKJ849103OR2OregonKJ849105OR4OregonKJ849106OR5OregonKJ849107OR6OregonKJ849108OR7OregonKJ849108OR7OregonKJ849107OR8OregonKJ849113OR10OregonKJ849113OR10OregonKJ849112OR10OregonKJ849112OR11OregonKJ849112OR12OregonKJ849112OR13OregonKJ849113OR16OregonKJ849118OR16OregonKJ849118OR17OregonKJ849118OR18OregonKJ849114OR19OregonKJ849114OR10OregonKJ849126OR21OregonKJ849126OR22OregonKJ849123Slo1SloveniaKJ849123Slo1SloveniaKJ849143Slo3SloveniaKJ849144Slo4SloveniaKJ849146Slo6SloveniaKJ849146	NJ2	New Jersey	KJ849132
NJ5New JerseyKJ849135NJ6New JerseyKJ849136NJ7New JerseyKJ849137NJ8New JerseyKJ849139NJ9New JerseyKJ849139NJ10New JerseyKJ849140NJ11New JerseyKJ849103OR2OregonKJ849105OR4OregonKJ849105OR4OregonKJ849106OR5OregonKJ849107OR6OregonKJ849108OR7OregonKJ849107OR6OregonKJ849113OR7OregonKJ849113OR8OregonKJ849113OR10OregonKJ849113OR11OregonKJ849112OR9OregonKJ849112OR12OregonKJ849112OR13OregonKJ849117OR14OregonKJ849117OR15OregonKJ849118OR16OregonKJ849114OR17OregonKJ849114OR18OregonKJ849110OR20OregonKJ849114OR14OregonKJ849114OR15OregonKJ849122OR20OregonKJ849123Slo1SloveniaKJ849123Slo1SloveniaKJ849143Slo3SloveniaKJ849143Slo4SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147	NJ3	New Jersey	KJ849133
NJ6     New Jersey     KJ849136       NJ7     New Jersey     KJ849137       NJ8     New Jersey     KJ849138       NJ9     New Jersey     KJ849140       NJ10     New Jersey     KJ849140       NJ11     New Jersey     KJ849140       NJ11     New Jersey     KJ849103       OR1     Oregon     KJ849106       OR2     Oregon     KJ849106       OR3     Oregon     KJ849106       OR4     Oregon     KJ849106       OR5     Oregon     KJ849106       OR6     Oregon     KJ849107       OR6     Oregon     KJ849113       OR7     Oregon     KJ849113       OR10     Oregon     KJ849113       OR11     Oregon     KJ849113       OR12     Oregon     KJ849117       OR13     Oregon     KJ849117       OR14     Oregon     KJ849117       OR15     Oregon     KJ849110       OR16     Oregon     KJ849114       OR17<	NJ4	New Jersey	KJ849134
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     KJ849108       OR5     Oregon     KJ849108       OR7     Oregon     KJ849108       OR7     Oregon     KJ849115       OR8     Oregon     KJ849112       OR9     Oregon     KJ849112       OR9     Oregon     KJ849112       OR10     Oregon     KJ849112       OR11     Oregon     KJ849117       OR15     Oregon     KJ849117       OR16     Oregon     KJ849114       OR16     Oregon     KJ849114       OR17     Oregon     KJ849114       OR16     Oregon     KJ849114       OR16	NJ5		KJ849135
NJ8     New Jersey     KJ849138       NJ9     New Jersey     KJ849139       NJ10     New Jersey     KJ849140       NJ11     New Jersey     KJ849141       OR1     Oregon     KJ849103       OR2     Oregon     KJ849105       OR3     Oregon     KJ849105       OR4     Oregon     KJ849106       OR5     Oregon     KJ849107       OR6     Oregon     KJ849107       OR6     Oregon     KJ849107       OR6     Oregon     KJ849113       OR7     Oregon     KJ849113       OR9     Oregon     KJ849113       OR10     Oregon     KJ849113       OR11     Oregon     KJ849113       OR12     Oregon     KJ849116       OR13     Oregon     KJ849118       OR14     Oregon     KJ849118       OR15     Oregon     KJ849110       OR17     Oregon     KJ849114       OR18     Oregon     KJ849110       OR19	NJ6	New Jersey	KJ849136
NJ9     New Jersey     KJ849139       NJ10     New Jersey     KJ849140       NJ11     New Jersey     KJ849141       OR1     Oregon     KJ849103       OR2     Oregon     KJ849105       OR4     Oregon     KJ849105       OR4     Oregon     KJ849106       OR5     Oregon     KJ849107       OR6     Oregon     KJ849108       OR7     Oregon     KJ849103       OR8     Oregon     KJ849109       OR7     Oregon     KJ849113       OR8     Oregon     KJ849112       OR9     Oregon     KJ849109       OR11     Oregon     KJ849112       OR12     Oregon     KJ849112       OR13     Oregon     KJ849119       OR14     Oregon     KJ849119       OR17     Oregon     KJ849114       OR18     Oregon     KJ849110       OR19     Oregon     KJ849114       OR10     Oregon     KJ849120       OR20 <td< td=""><td>NJ7</td><td></td><td>KJ849137</td></td<>	NJ7		KJ849137
NJ10     New Jersey     KJ849140       NJ11     New Jersey     KJ849141       OR1     Oregon     KJ849103       OR2     Oregon     KJ849104       OR3     Oregon     KJ849106       OR4     Oregon     KJ849106       OR5     Oregon     KJ849106       OR6     Oregon     KJ849107       OR6     Oregon     KJ849107       OR6     Oregon     KJ849108       OR7     Oregon     KJ849109       OR8     Oregon     KJ849112       OR9     Oregon     KJ849112       OR10     Oregon     KJ849112       OR12     Oregon     KJ849112       OR13     Oregon     KJ849117       OR14     Oregon     KJ849119       OR17     Oregon     KJ849119       OR18     Oregon     KJ849114       OR19     Oregon     KJ849114       OR19     Oregon     KJ849120       OR20     Oregon     KJ849121       OR21     Or	NJ8	New Jersey	KJ849138
NJ11     New Jersey     KJ849141       OR1     Oregon     KJ849103       OR2     Oregon     KJ849104       OR3     Oregon     KJ849105       OR4     Oregon     KJ849107       OR5     Oregon     KJ849107       OR6     Oregon     KJ849107       OR7     Oregon     KJ849112       OR8     Oregon     KJ849113       OR10     Oregon     KJ849112       OR11     Oregon     KJ849112       OR12     Oregon     KJ849112       OR13     Oregon     KJ849112       OR14     Oregon     KJ849114       OR15     Oregon     KJ849114       OR16     Oregon     KJ849114       OR17     Oregon     KJ849120       OR22     Oregon     KJ849120       OR22     Oregon<	NJ9	New Jersey	KJ849139
OR1     Oregon     KJ849103       OR2     Oregon     KJ849104       OR3     Oregon     KJ849105       OR4     Oregon     KJ849106       OR5     Oregon     KJ849107       OR6     Oregon     KJ849108       OR7     Oregon     KJ849108       OR7     Oregon     KJ849115       OR8     Oregon     KJ849112       OR9     Oregon     KJ849112       OR10     Oregon     KJ849112       OR11     Oregon     KJ849112       OR12     Oregon     KJ849112       OR14     Oregon     KJ849116       OR15     Oregon     KJ849117       OR16     Oregon     KJ849114       OR16     Oregon     KJ849110       OR17     Oregon     KJ849110       OR18     Oregon     KJ849110       OR20     Oregon     KJ849114       OR21     Oregon     KJ849120       OR22     Oregon     KJ849142       DS02     Slovenia<	NJ10	New Jersey	KJ849140
OR2     Oregon     KJ849104       OR3     Oregon     KJ849105       OR4     Oregon     KJ849105       OR4     Oregon     KJ849107       OR5     Oregon     KJ849107       OR6     Oregon     KJ849115       OR7     Oregon     KJ849115       OR8     Oregon     KJ849113       OR9     Oregon     KJ849113       OR10     Oregon     KJ849116       OR11     Oregon     KJ849116       OR12     Oregon     KJ849116       OR13     Oregon     KJ849116       OR14     Oregon     KJ849117       OR15     Oregon     KJ849119       OR16     Oregon     KJ849110       OR17     Oregon     KJ849110       OR20     Oregon     KJ849124       OR21     Oregon     KJ849120       OR22     Oregon     KJ849120       BC2     Canada     KJ849120       BC2     Canada     KJ849143       Slo1     Slovenia </td <td>NJ11</td> <td>New Jersey</td> <td>KJ849141</td>	NJ11	New Jersey	KJ849141
OR3     Oregon     KJ849105       OR4     Oregon     KJ849106       OR5     Oregon     KJ849107       OR6     Oregon     KJ849107       OR6     Oregon     KJ849108       OR7     Oregon     KJ849113       OR8     Oregon     KJ849113       OR9     Oregon     KJ849113       OR10     Oregon     KJ849113       OR11     Oregon     KJ849112       OR12     Oregon     KJ849116       OR13     Oregon     KJ849117       OR14     Oregon     KJ849118       OR15     Oregon     KJ849118       OR16     Oregon     KJ849118       OR17     Oregon     KJ849119       OR17     Oregon     KJ849110       OR19     Oregon     KJ849122       OR20     Oregon     KJ849114       OR11     Canada     KJ849120       BC2     Canada     KJ849143       Slo1     Slovenia     KJ849145       Slo2     Sloven	OR1	Oregon	KJ849103
OR4     Oregon     KJ849106       OR5     Oregon     KJ849107       OR6     Oregon     KJ849107       OR6     Oregon     KJ849108       OR7     Oregon     KJ849113       OR8     Oregon     KJ849113       OR9     Oregon     KJ849113       OR10     Oregon     KJ849113       OR11     Oregon     KJ849112       OR12     Oregon     KJ849116       OR13     Oregon     KJ849117       OR14     Oregon     KJ849119       OR15     Oregon     KJ849119       OR16     Oregon     KJ849119       OR17     Oregon     KJ849119       OR18     Oregon     KJ849110       OR19     Oregon     KJ849110       OR20     Oregon     KJ849114       OR11     Canada     KJ849120       BC2     Canada     KJ849143       Slo1     Slovenia     KJ849143       Slo2     Slovenia     KJ849145       Slo4     Slo	OR2	Oregon	KJ849104
OR5     Oregon     KJ849107       OR6     Oregon     KJ849108       OR7     Oregon     KJ849115       OR8     Oregon     KJ849115       OR9     Oregon     KJ849113       OR10     Oregon     KJ849109       OR11     Oregon     KJ849112       OR12     Oregon     KJ849112       OR13     Oregon     KJ849116       OR14     Oregon     KJ849119       OR15     Oregon     KJ849119       OR16     Oregon     KJ849119       OR17     Oregon     KJ849119       OR18     Oregon     KJ849110       OR19     Oregon     KJ849110       OR20     Oregon     KJ849124       OR21     Oregon     KJ849120       DS2     Canada     KJ849123       Slo1     Slovenia     KJ849143       Slo2     Slovenia     KJ849145       Slo3     Slovenia     KJ849145       Slo4     Slovenia     KJ849145  Slo5     Sloven	OR3	Oregon	
OR6     Oregon     KJ849108       OR7     Oregon     KJ849115       OR8     Oregon     KJ849112       OR9     Oregon     KJ849113       OR10     Oregon     KJ849112       OR11     Oregon     KJ849112       OR12     Oregon     KJ849112       OR13     Oregon     KJ849112       OR14     Oregon     KJ849117       OR15     Oregon     KJ849118       OR16     Oregon     KJ849124       OR17     Oregon     KJ849124       OR18     Oregon     KJ849124       OR16     Oregon     KJ849124       OR17     Oregon     KJ849124       OR18     Oregon     KJ849120       OR20     Oregon     KJ849120       OR21     Oregon     KJ849120       OR22     Oregon     KJ849142       Slo1     Slovenia     KJ849142       Slo2     Slovenia     KJ849143       Slo3     Slovenia     KJ849145       Slo4     <	OR4	Oregon	KJ849106
OR7     Oregon     KJ849115       OR8     Oregon     KJ849121       OR9     Oregon     KJ849113       OR10     Oregon     KJ849112       OR11     Oregon     KJ849112       OR12     Oregon     KJ849116       OR13     Oregon     KJ849116       OR14     Oregon     KJ849117       OR15     Oregon     KJ849117       OR16     Oregon     KJ849114       OR17     Oregon     KJ849114       OR18     Oregon     KJ849114       OR19     Oregon     KJ849111       OR16     Oregon     KJ849111       OR17     Oregon     KJ849110       OR20     Oregon     KJ849110       OR21     Oregon     KJ849120       BC2     Canada     KJ849120       BC2     Canada     KJ849142       Slo2     Slovenia     KJ849143       Slo3     Slovenia     KJ849145       Slo4     Slovenia     KJ849146       Slo4 <t< td=""><td></td><td>Oregon</td><td></td></t<>		Oregon	
OR8OregonKJ849121OR9OregonKJ849113OR10OregonKJ849109OR11OregonKJ849116OR12OregonKJ849116OR13OregonKJ849117OR14OregonKJ849117OR15OregonKJ849119OR16OregonKJ849119OR17OregonKJ849111OR18OregonKJ849111OR19OregonKJ849110OR20OregonKJ849122OR21OregonKJ849120BC2CanadaKJ849120BC2SloveniaKJ849143Slo3SloveniaKJ849143Slo4SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147			
OR9     Oregon     KJ849113       OR10     Oregon     KJ849109       OR11     Oregon     KJ849112       OR12     Oregon     KJ849112       OR13     Oregon     KJ849116       OR14     Oregon     KJ849117       OR15     Oregon     KJ849118       OR16     Oregon     KJ849118       OR16     Oregon     KJ849119       OR17     Oregon     KJ849111       OR18     Oregon     KJ849112       OR19     Oregon     KJ849122       OR20     Oregon     KJ849122       OR22     Oregon     KJ849122       OR22     Oregon     KJ849123       Slo1     Slovenia     KJ849143       Slo2     Slovenia     KJ849143       Slo3     Slovenia     KJ849145       Slo5     Slovenia     KJ849145       Slo6     Slovenia     KJ849147			
OR10     Oregon     KJ849109       OR11     Oregon     KJ849112       OR12     Oregon     KJ849112       OR13     Oregon     KJ849116       OR14     Oregon     KJ849117       OR15     Oregon     KJ849118       OR16     Oregon     KJ849118       OR16     Oregon     KJ849119       OR17     Oregon     KJ849111       OR18     Oregon     KJ849110       OR19     Oregon     KJ849122       OR20     Oregon     KJ849114       BC1     Canada     KJ849120       BC2     Canada     KJ849123       Slo1     Slovenia     KJ849143       Slo2     Slovenia     KJ849143       Slo3     Slovenia     KJ849145       Slo5     Slovenia     KJ849145       Slo6     Slovenia     KJ849147		-	
OR11     Oregon     KJ849112       OR12     Oregon     KJ849116       OR13     Oregon     KJ849115       OR14     Oregon     KJ849117       OR15     Oregon     KJ849119       OR16     Oregon     KJ849119       OR16     Oregon     KJ849119       OR17     Oregon     KJ849111       OR18     Oregon     KJ849110       OR19     Oregon     KJ849124       OR20     Oregon     KJ849120       OR21     Oregon     KJ849120       DSC2     Canada     KJ849123       Slo1     Slovenia     KJ849143       Slo2     Slovenia     KJ849143       Slo3     Slovenia     KJ849145       Slo4     Slovenia     KJ849145       Slo5     Slovenia     KJ849145       Slo6     Slovenia     KJ849147		-	
OR12     Oregon     KJ849116       OR13     Oregon     KJ849125       OR14     Oregon     KJ849117       OR15     Oregon     KJ849118       OR16     Oregon     KJ849124       OR17     Oregon     KJ849124       OR18     Oregon     KJ849111       OR19     Oregon     KJ849124       OR20     Oregon     KJ849120       OR21     Oregon     KJ849120       OR22     Oregon     KJ849120       BC1     Canada     KJ849120       BC2     Canada     KJ849123       Slo1     Slovenia     KJ849143       Slo2     Slovenia     KJ849143       Slo3     Slovenia     KJ849145       Slo4     Slovenia     KJ849145       Slo5     Slovenia     KJ849146       Slo6     Slovenia     KJ849147		C C	
OR13     Oregon     KJ849125       OR14     Oregon     KJ849117       OR15     Oregon     KJ849117       OR16     Oregon     KJ849119       OR16     Oregon     KJ849119       OR17     Oregon     KJ849111       OR18     Oregon     KJ849111       OR19     Oregon     KJ849110       OR20     Oregon     KJ849122       OR21     Oregon     KJ849120       BC1     Canada     KJ849120       BC2     Canada     KJ849123       Slo1     Slovenia     KJ849142       Slo2     Slovenia     KJ849143       Slo3     Slovenia     KJ849145       Slo4     Slovenia     KJ849145       Slo5     Slovenia     KJ849146       Slo6     Slovenia     KJ849147		-	
OR14     Oregon     KJ849117       OR15     Oregon     KJ849118       OR16     Oregon     KJ849119       OR17     Oregon     KJ849119       OR17     Oregon     KJ849110       OR18     Oregon     KJ849110       OR19     Oregon     KJ849126       OR20     Oregon     KJ849126       OR21     Oregon     KJ849120       BC1     Canada     KJ849120       BC2     Canada     KJ849123       Slo1     Slovenia     KJ849143       Slo2     Slovenia     KJ849143       Slo3     Slovenia     KJ849145       Slo5     Slovenia     KJ849145       Slo6     Slovenia     KJ849147		-	
OR15OregonKJ849118OR16OregonKJ849119OR17OregonKJ849124OR18OregonKJ849111OR19OregonKJ849110OR20OregonKJ849126OR21OregonKJ849122OR22OregonKJ849120BC1CanadaKJ849123Slo1SloveniaKJ849143Slo3SloveniaKJ849143Slo4SloveniaKJ849145Slo5SloveniaKJ849145Slo6SloveniaKJ849147			
OR16     Oregon     KJ849119       OR17     Oregon     KJ849124       OR18     Oregon     KJ849111       OR19     Oregon     KJ849110       OR20     Oregon     KJ849122       OR21     Oregon     KJ849124       OR22     Oregon     KJ849120       DR22     Oregon     KJ849120       BC1     Canada     KJ849123       Slo1     Slovenia     KJ849143       Slo2     Slovenia     KJ849143       Slo3     Slovenia     KJ849145       Slo4     Slovenia     KJ849145       Slo5     Slovenia     KJ849145       Slo6     Slovenia     KJ849147			
OR17     Oregon     KJ849124       OR18     Oregon     KJ849111       OR19     Oregon     KJ849110       OR20     Oregon     KJ849124       OR21     Oregon     KJ849124       OR22     Oregon     KJ849124       BC1     Canada     KJ849123       Slo1     Slovenia     KJ849142       Slo2     Slovenia     KJ849143       Slo3     Slovenia     KJ849145       Slo5     Slovenia     KJ849145       Slo6     Slovenia     KJ849145		-	
OR18OregonKJ849111OR19OregonKJ849110OR20OregonKJ849126OR21OregonKJ849122OR22OregonKJ849120BC1CanadaKJ849120BC2CanadaKJ849123Slo1SloveniaKJ849142Slo2SloveniaKJ849143Slo3SloveniaKJ849145Slo5SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147		-	
OR19OregonKJ849110OR20OregonKJ849126OR21OregonKJ849122OR22OregonKJ849114BC1CanadaKJ849123Slo1SloveniaKJ849123Slo2SloveniaKJ849142Slo3SloveniaKJ849143Slo4SloveniaKJ849144Slo4SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147		0	
OR20OregonKJ849126OR21OregonKJ849122OR22OregonKJ849114BC1CanadaKJ849120BC2CanadaKJ849123Slo1SloveniaKJ849142Slo2SloveniaKJ849143Slo3SloveniaKJ849144Slo4SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147		e e	
OR21OregonKJ849122OR22OregonKJ849114BC1CanadaKJ849120BC2CanadaKJ849123Slo1SloveniaKJ849142Slo2SloveniaKJ849143Slo3SloveniaKJ849144Slo4SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147		-	
OR22OregonKJ849114BC1CanadaKJ849120BC2CanadaKJ849123Slo1SloveniaKJ849142Slo2SloveniaKJ849143Slo3SloveniaKJ849144Slo4SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147			
BC1CanadaKJ849120BC2CanadaKJ849123Slo1SloveniaKJ849142Slo2SloveniaKJ849143Slo3SloveniaKJ849144Slo4SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147			
BC2CanadaKJ849123Slo1SloveniaKJ849142Slo2SloveniaKJ849143Slo3SloveniaKJ849144Slo4SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147		-	
Slo1SloveniaKJ849142Slo2SloveniaKJ849143Slo3SloveniaKJ849144Slo4SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147			
Slo2SloveniaKJ849143Slo3SloveniaKJ849144Slo4SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147			
Slo3SloveniaKJ849144Slo4SloveniaKJ849145Slo5SloveniaKJ849146Slo6SloveniaKJ849147			
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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 dodecylsulphate, 10 mM ethylene diamine tetra-acetic acid [EDTA], 1% sodium deoxycholate, 1% NP-40, and 1% 14 M  $\beta$ -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<sup>®</sup> reverse transcriptase, RiboLock<sup>™</sup> RNase inhibitor and random hexamer primer, UltraPure<sup>™</sup> 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-TRTAYAGCACRGTTAAACA-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., 2013). Download English Version:

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