

AN INTEGRATED BADNAVIRUS INFECTS BLACKBERRY

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Summary

A new badnavirus, tentatively named as blackberry virus F (BVF), was discovered in plants displaying blackberry yellow vein disease (BYVD) symptoms. BVF was characterized at the molecular level and several epidemiological attributes including genome integration, distribution, host range, population structure and association with disease symptoms were assessed. This discovery changes the standards in the production of virus-free propagation material as there are not methods developed to eliminate the integrated genomes of badnavirus. A sensitive detection protocol was developed that could assist in the elimination of BVF-infected material from the propagation pipeline.

Virus Characterization

Fig. 2. A. Schematic representation of the Blackberry virus F (BVF) genome showing relative positions of tRNA^{MET} TATA-box (denoted by box), ORF1; ORF2; ORF3 with movement protein (MP), capsid protein zinc-finger domain (CP), pepsin-like aspartate protease (Pro), reverse transcriptase (RT) and RNase H (RNase H) motifs; and ORF4. **B.** Neighbor-joining phylogenetic tree showing relationship of BVF with other badnaviruses. Percent (%) bootstrap values are indicated at nodes.

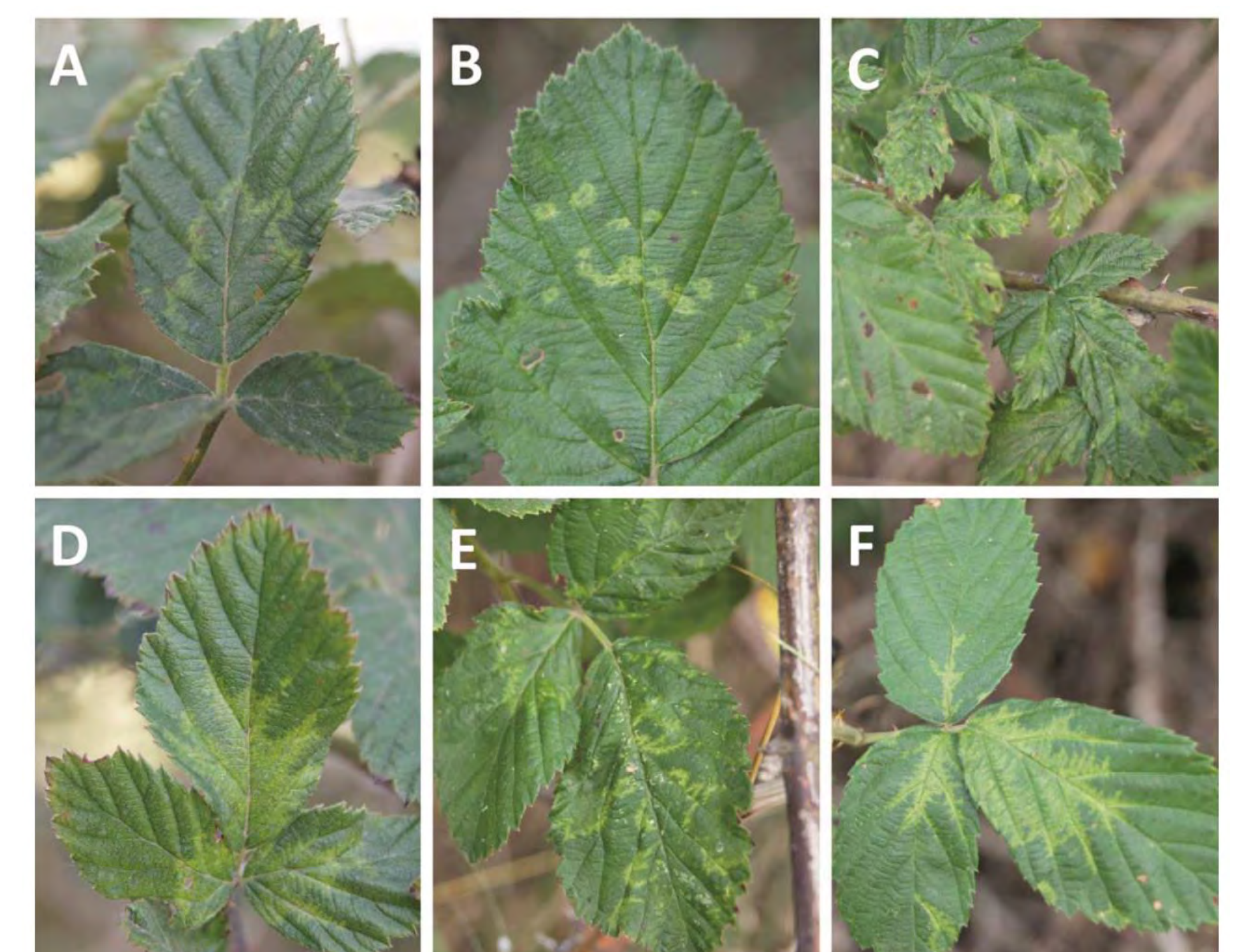
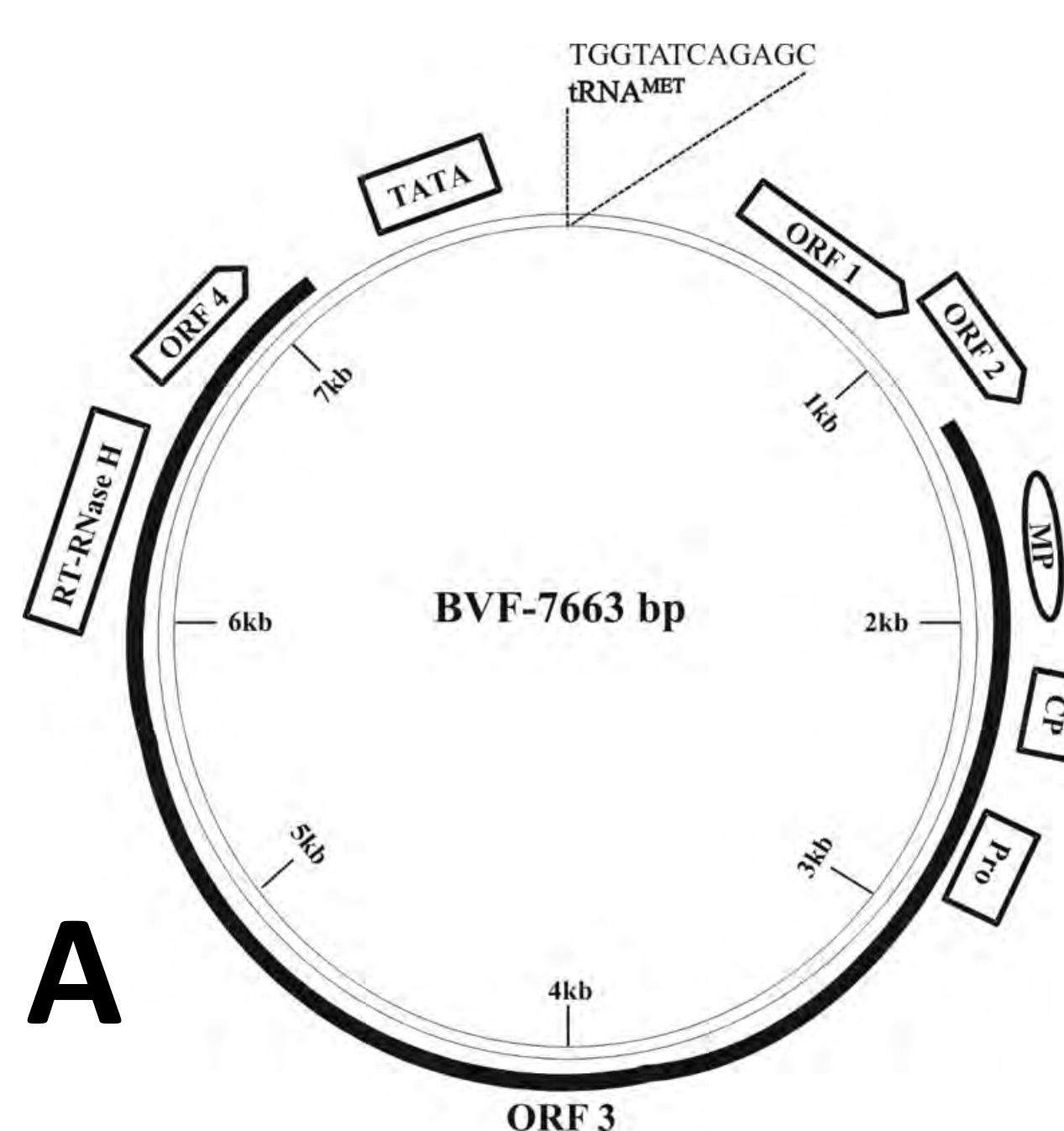
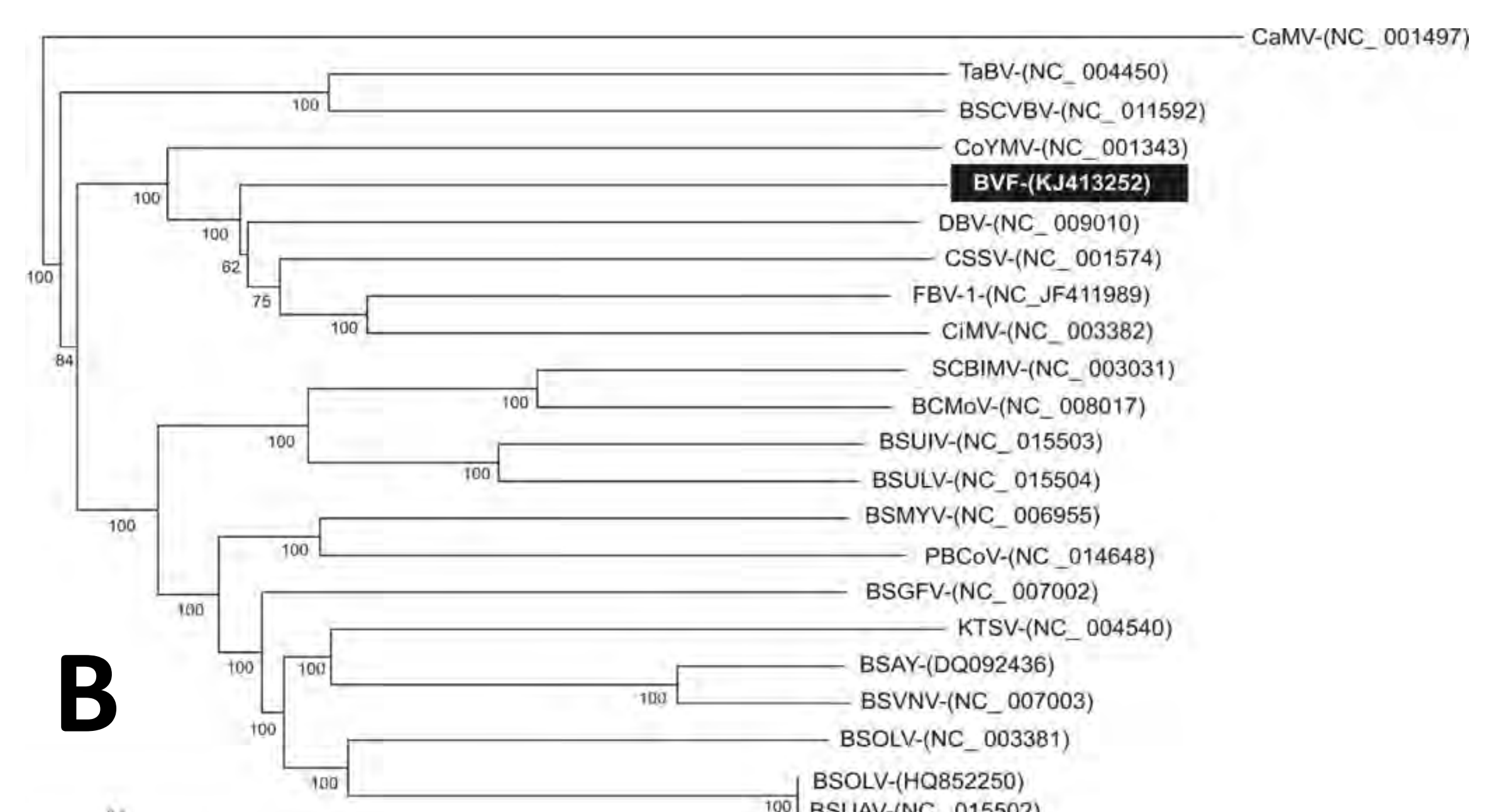


Fig. 1. Variety of symptoms of Blackberry yellow vein disease displayed on the same plant

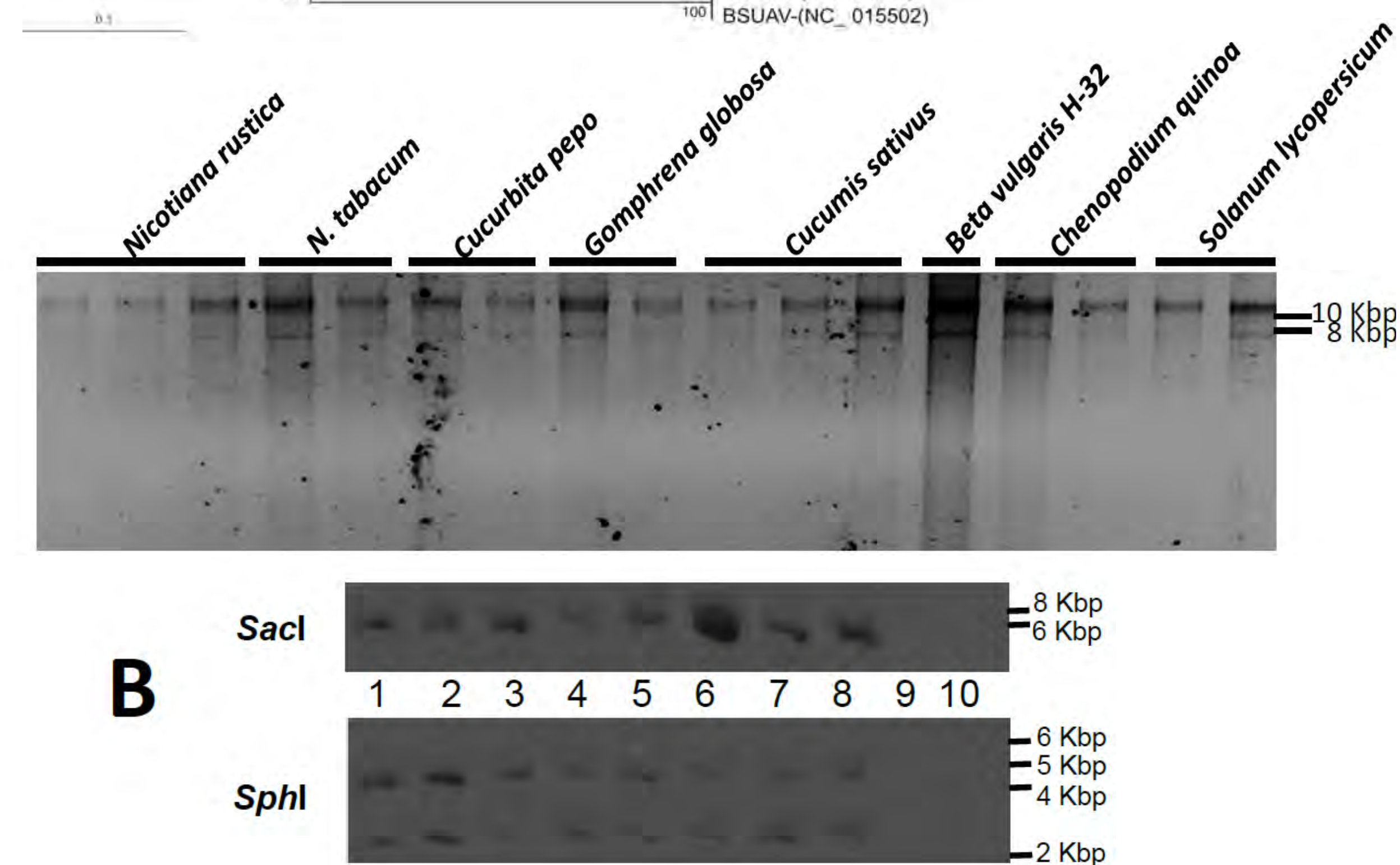


Alternative hosts

Species	Exp.1	Exp.2	Exp.3	BVF PCR-positives*
Beta vulgaris cv. Ruby Queen	3	6	3	0/12
Beta vulgaris, Swiss chard H-32	4	4	4	5/12
Brassica rapa subsp. pekinensis	2	4	5	0/12
Chenopodium quinoa	4	4	8	7/16
Cowpea cv. Elegance 801	5	5	4	2/14
Cucumis melo cv. Edisto 47 cantaloupe	4	6	3	3/13
Cucumis sativus cv. National Pickling	5	6	6	6/17
Glycine max cv. William 82	4	2	6	2/12
Gomphrena globosa	4	5	4	7/13
Nicotiana glauca	4	6	4	0/14
Nicotiana benthamiana	6	5	4	0/15
Nicotiana rustica	6	4	6	3/16
Nicotiana tabacum	6	6	8	5/20
Phaseolus vulgaris var. Black Valentine	4	2	3	0/9
Pisum sativum cv. Wando	5	4	7	6/17
Pumpkin; Cucurbita pepo cv. Connecticut field	4	4	2	3/10
Pumpkin; Cucurbita pepo cv. National Pickling	5	6	4	8/15
Solanum lycopersicum cv. Bradley	4	4	7	6/15
Spinach cv. meerkat	4	4	5	3/13
Vigna unguiculata cv. Monarch	5	3	4	0/12

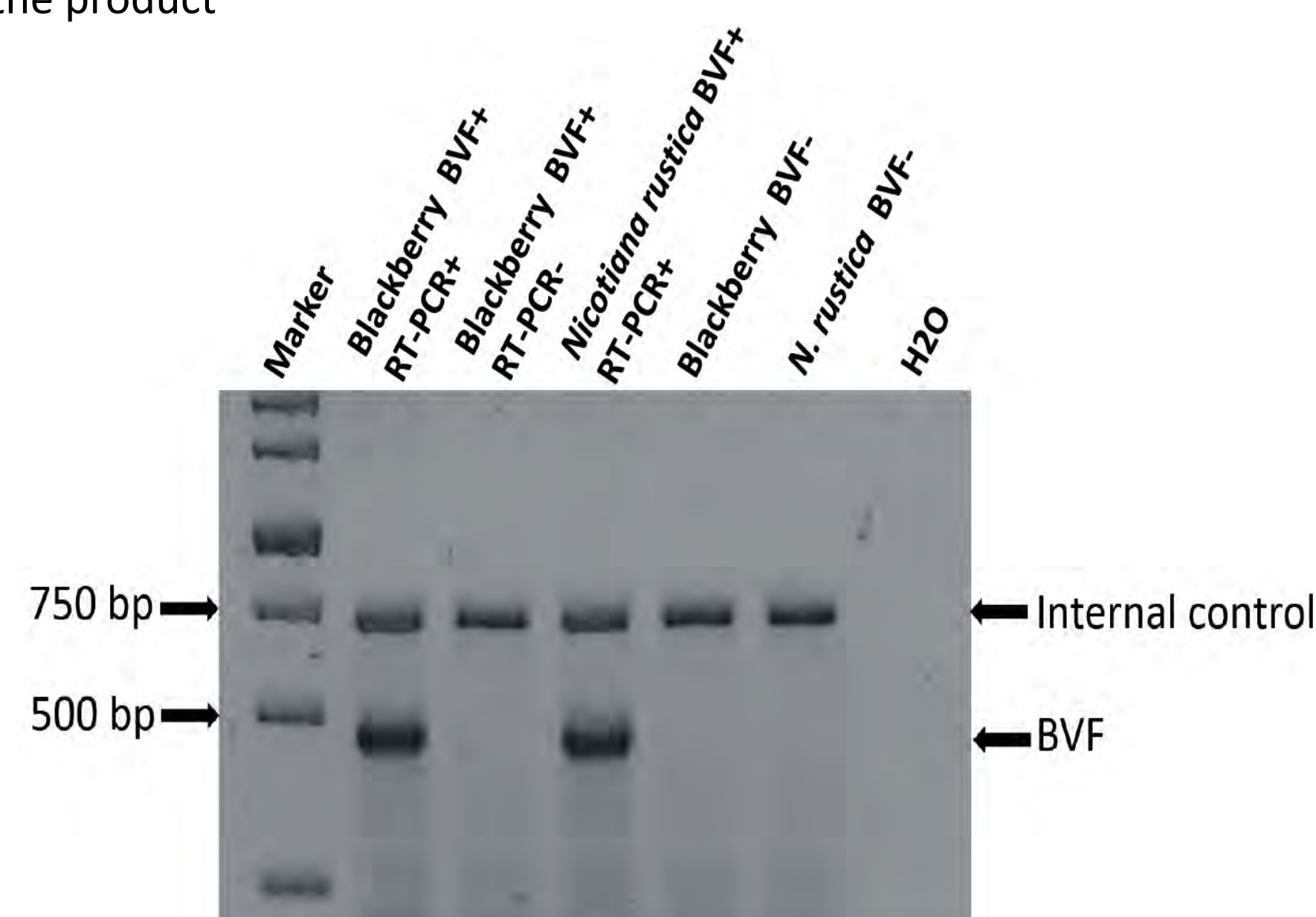
*All positive amplicons were confirmed by direct sequencing of the product

Fig. 3. A. Rolling circle amplification digested with *KpnI* (single restriction site in the virus genome) and B. Hybridization on the RCA products of BVF-infected indicator plants. RCA products were digested with *SacI* (single restriction site in the genome) or *SphI* (two restriction sites in the genome). Lanes 1-8 represent a RCA positive plant from each of the species presented in (A), Lanes 9 and 10 are BVF-free *Nicotiana rustica* and *N. tabacum* respectively.



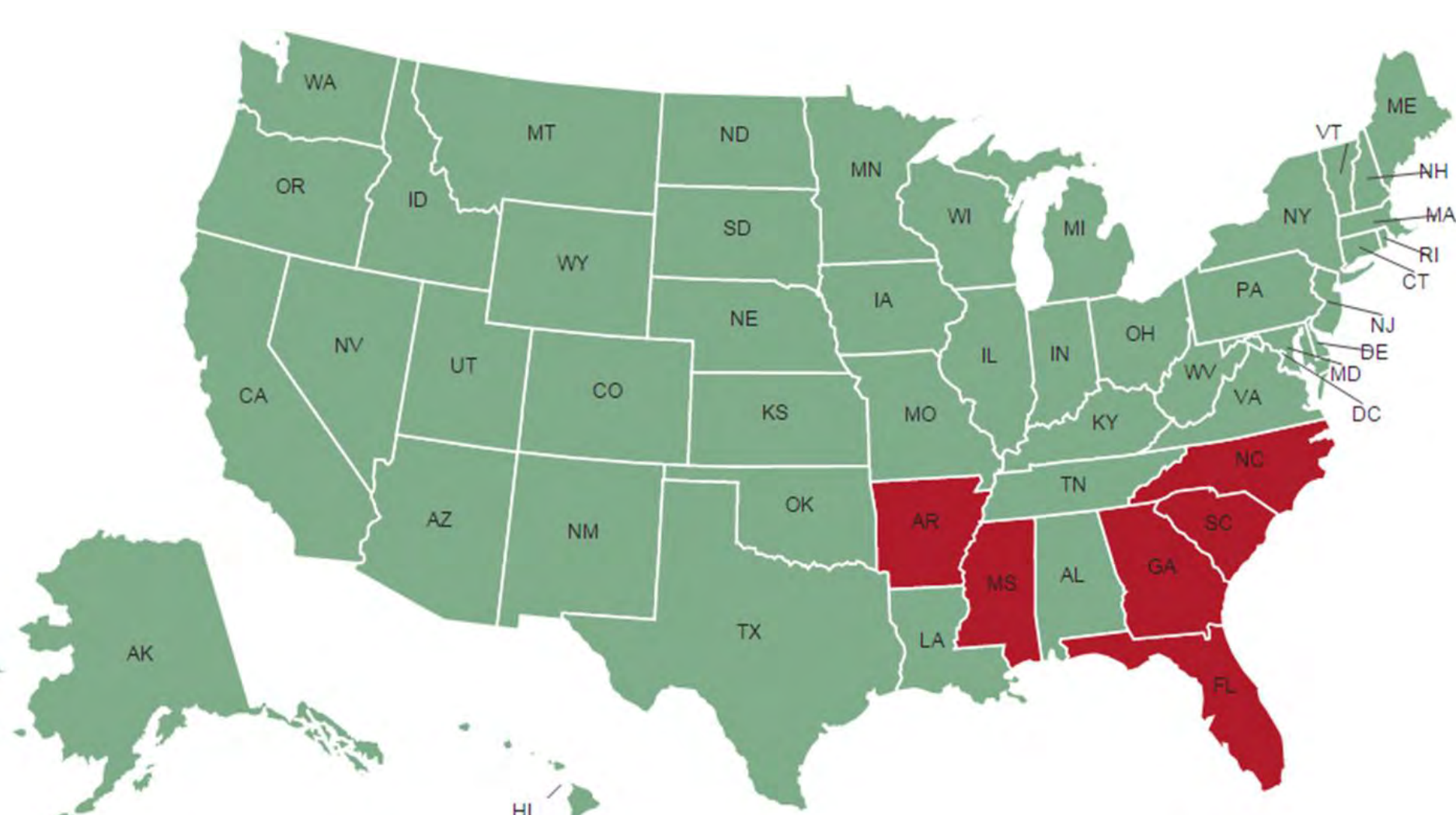
Virus Detection

Fig 4. Reverse transcription-PCR detection of Blackberry virus F with the NADH dehydrogenase ND2 subunit transcript used as an internal control.



Association with BYVD

Arkansas (219), Georgia (61), Florida (26), Mississippi (13), North Carolina (53) and South Carolina (22)



Conclusions

BVF is a putative new member of the genus *Badnavirus*. The population structure of the virus is homogeneous and recombination may be the driving evolutionary force for the virus. As BVF can integrate in the host genome it can affect the propagation pipeline. Blackberry virus diseases are often caused by virus complexes and infected material with a virus which may never be eliminated from the germline because of its integration in the host genome may serve as the trigger for epidemics as field plants are being infected by additional viruses.

Acknowledgments

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Fig. 5. Southern hybridization for discrimination of the episomal and integrated forms of Blackberry virus F (BVF) using blackberry and *Nicotiana rustica* digested with *KpnI* (single site in genome) or *SphI* (two sites in genome). 1, PCR/RT-PCR positive blackberry; 2, PCR positive/RT-PCR negative blackberry; 3, *N. rustica* positive 4; BVF-free blackberry and 5, BVF-free *N. rustica*. Asterisks (*) indicate the expected size of BVF genomic fragments after digestion.

