First Report of Tobacco rattle virus Associated with Ring Spot and Line Pattern Disease of Peony in Ohio

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Tobacco rattle virus (TRV) is the type member of the Tobravirus genus which also includes pea early browning and pepper ringspot viruses. The TRV genome is linear, single-stranded, and has a positive sense RNA divided into two segments, RNA 1 and RNA 2 (1). RNA 1 is ~6.8 Kb in length and encodes a replicase, intercellular movement, and pathogenicity proteins. RNA 2 ranges from 1.8 to 4.5 Kb depending on the isolate, and encodes the coat protein and those required for nematode transmission (1). TRV has a broad host range which includes ornamental, vegetable, and weed hosts, and is transmitted by nematodes in the genera Trichodorus and Paratrichodorus (1). The ring spot disease of peony associated with TRV has been reported in Europe and Asia (4) and recently in Alaska (3) but the literature is sparse regarding reports of the disease in the US.

In the spring of 2009 several peony (Paeonia spp.) plants displaying concentric yellow ringspots and line patterns (Fig. 1) were submitted to the Ohio Plant Diagnostic Network for analysis. The samples tested negative for Alfalfa mosaic, Arabis mosaic, Cucumber mosaic, Impatiens necrotic spot, Tobacco mosaic, Tobacco ringspot, Tomato ringspot, and Tomato spotted wilt viruses by enzyme-linked immunosorbent assay (ELISA) using commercially available antibodies (Agdia Inc., Elkhart, IN). Double-stranded ribonucleic acid (dsRNA) was purified from symptomatic tissue essentially as previously described (5) resulting in a profile suggestive of a bipartite viral genome with major dsRNAs between 6 to 8 Kb and 2 to 2.5 Kb (Fig. 2). DsRNA was used as a template for first strand cDNA synthesis by mixing 9.5 µl (~1 µg) purified dsRNA with 0.5 µl 40 U/µl RNAse inhibitor, heat denaturing at 95°C for 10 min, and immediately icing. The entire 10 µl volume of denatured dsRNA was mixed with 4 µl 5× MMLV buffer, 2 µl 5 mM dNTPs, 0.5 µl 0.5 µg/µl random hexamers, 1 µl 1 mg/ml BSA, 1 µl 0.1 mM dithiothreitol, 0.5 µl RNAsin, 1 ul 200 units/µl MMLV-RT (Promega Inc., Madison, WI) and incubated for one hour at 42°C. 5 µl cDNA or sterile water was used for PCR with published (2) tobavirus group (TobraF3 5’-GGTGGKCAATGGTCTTWTTGG-3’, TobraR2 5’-GTCAGCTGYTGATCAGATAACC-3’) and novel TRV specific (TRVF683 5’-GCTATTGGTGATCAAGCTAGAAG-3’, TRVR1439 5’-GCHGCCCCGTTWATGAAAYARGAC-3’) primer pairs (IDT Inc., Coralville, IA) to amplify regions in the 194 K RNA polymerase gene (1,2). Amplification was done in 25 µl reactions [2.5 mM MgCl2, 0.2 mM dNTP mix, 0.2 µM primer pair, 0.625 units Taq polymerase (Promega)], with the following cycling parameters: 94°C (2 min), 30 cycles of 94°C (45 sec), 52°C (30 sec), 72°C (60 sec), final extension 72°C (10 min). Gel purified PCR products were ligated into pGEM-T Easy plasmid (Promega), E. coli JM109 cells were transformed, and colonies were screened for an insert using M13 primers. Plasmid DNA was purified (Wizard Plus SV mini-prep kit, Promega), sequenced (Plant Microbe Genomics Facility, The Ohio State University), vector sequences were trimmed from raw sequences (Chromas v. 2.33), assembled, and subjected to pairwise and multiple sequence alignments (Vector NTI 11, Invitrogen).
Fig. 1. Ringspots (A) and line patterns (B) observed on TRV infected peony.

The tobravirus group primers and TRV specific primers both amplified clear products of expected size; approximately 830 bp and 780 bp, respectively (Fig. 3). Five clones of the TRV-specific and four of the tobravirus group amplicons were sequenced and the sequences deposited in GenBank (accession no. JX144382-JX144390). Clones generated with the tobravirus group primers were 830 bp, 98.9 to 99.5% identical to one another, and corresponded to nt 367-1197 of the TRV ORF 1 on RNA 1. A BLASTn search of the NCBI database produced a hit with 99% nucleotide identity (100% query coverage) with a TRV isolate from Michigan potatoes (accession no. GQ903771.1) for all four tobravirus group clones. Clones generated with the TRV specific primers were 779 bp, 98.8 to
99.7% identical to one another, and corresponded to nt 481-1260 of the TRV ORF 1. A BLASTn search of the NCBI database also produced a hit with 99% nucleotide identity (100% query coverage) with the same TRV isolate from Michigan for all five TRV specific clones.

These results represent the first confirmed report of TRV in Peony in Ohio, and expand the known geographic distribution of the virus. Strains of TRV are more than 99% identical with respect to their RNA 1 nt sequences (1) and these results show that the TRV isolate from Ohio Peony appears to be the same strain as that found in Michigan potato. These results also represent a reliable detection method that utilizes cDNAs synthesized from dsRNA coupled with PCR assays that target regions on RNA-1 of the TRV genome, and therefore can detect the unencapsidated NM variants as well as encapsidated M variants (1). These results benefit Ohio perennial growers by increasing awareness of the symptoms associated with TRV in Peony and provide a useful visual tool for scouting infected plants.

Fig. 3. PCR detection of TRV from cDNAs synthesized from dsRNA template with tobravirus group (Lane 1) and TRV specific (Lane 2) primers. Water controls with tobravirus group (Lane 3) and TRV specific (Lane 4) primers, and TRV positive control with tobravirus group primers (Lane 5). M = 1 kb DNA ladder (250, 500, 750, 1000 bp markers indicated). Electrophoresis was performed in 1% agarose at 100 volts for 60 min.
Literature Cited


