Diagnostic Tip of the Month

Double-Stranded RNA Analysis as a Tool for Diagnosing Plant Viruses

J.R. Fisher
Plant Pathologist
Ohio Dept. of Agriculture

Diagnosing plant viruses can be a challenging endeavor. The number of viruses infecting plants is formidable and previously uncharacterized viruses are being described regularly, thereby adding to the challenge.

How, then, does a diagnostician confronted with virus-like symptoms approach identifying the causal agent? Viral symptoms are often striking, but rarely are they specific to a particular virus. Likewise, a particular virus often produces very different symptoms on different hosts.

Fortunately tools such as ELISA (enzyme-linked immunosorbent assay) and PCR (polymerase chain reaction) exist which are very useful for virus identification, but they require that you at least have some idea of which virus(es) you are dealing with. ELISA and PCR as the first line of virus identification are a shot in the dark at best due to the specificity of the assays. They also may allow a mixed infection to go undetected due to their specificity. So where does one turn?

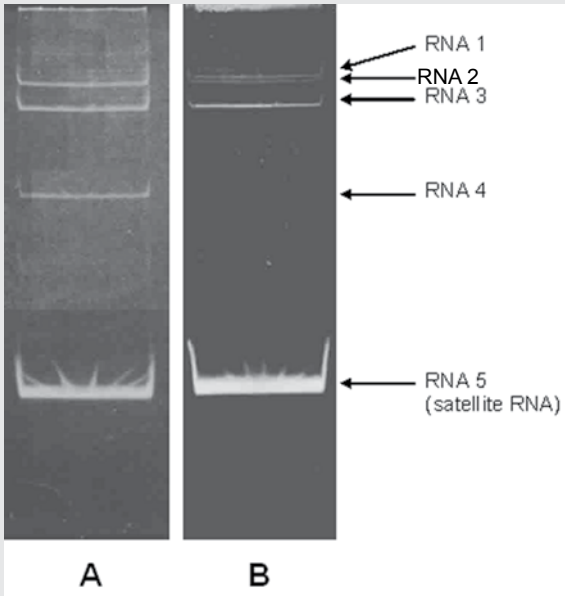

Fig.1. dsRNA profiles from: A) cucumber mosaic virus (CMV) infected *Lobelia* spp. and B) CMV infected *Ajuga reptans*. CMV genomic dsRNAs 1-3 and subgenomic dsRNA 4 indicated by arrows (Note: RNA 4 appears absent in gel B). A satellite RNA (dsRNA 5) is also indicated. The Lobelia sample is also likely infected with at least one other virus as suggested by additional dsRNAs in the gel. Gel is 5% polyacrylamide stained with ethidium bromide. Electrophoresis was performed at 125 volts for 90 minutes.

Double-stranded ribonucleic acid (dsRNA) analysis is a non-specific alternative to more precise assays like ELISA and PCR. The majority of viruses infecting plants (>95%) are composed of single-stranded messenger sense (+) RNA genomes. When these ssRNA+ viruses replicate in the host cell they go through an intermediate double-stranded stage where a negative sense (-) RNA strand is copied from the sense strand. This antisense strand serves as the template for synthesis of more messenger sense RNA. It is the intermediate double-stranded RNA molecule that is useful as a diagnostic tool.

Continued on page 4...

Diagnostic Tip of the Month

Continued from page 3...

Interpretation of the results is the key. In the case of a virus with a multi-partite genome the dsRNA banding profile may provide a clue as to the viral genus. This is true of the cucumoviruses (Fig. 1) and alfamoviruses. Likewise, the relative molecular weight of a monopartite genome may point you toward the viral genus or group.

It is important to be aware of what you might be seeing in a dsRNA gel. A monopartite virus may produce one or more sub-genomic dsRNAs in addition to the genomic dsRNA. The same is true for a multipartite virus. Even if the dsRNA banding profile isn't familiar to you, at least it is indicative of a viral infection and you may be able to narrow the field by searching the literature for clues. Or you may not see anything. In that case the plant may still be infected with a virus but it could be a DNA virus or a ssRNA- virus. It is important to note that although most uninfected plants do not produce dsRNAs there are some, like the cucurbits, that produce indigenous dsRNA molecules. It is therefore imperative to include 'healthy tissue' controls so you don't mistake indigenous dsRNAs for viral dsRNAs. As with many techniques, the more experienced you become with dsRNA profiles the more comfortable you will become interpreting the results.

dsRNA analysis has advantages and disadvantages. Advantages include it is relatively inexpensive, making use of fairly common lab reagents and equipment. It is also relatively quick, a typical sample being prepared from start to finish in two days (there are tricks to shorten the time frame). The dsRNA molecule is very stable, so precautions that one would take when working with messenger RNA (ie. RNAse-free environment) need not be so stringent. The purified dsRNA molecule can also be used as template for cDNA synthesis and subsequent PCR.

A disadvantage is that this procedure would generally not be used to process a large number of samples at a time (16 is the most I have attempted at once). Also, since dsRNA is related to replication of the virus the titre of the dsRNA molecule(s) may increase or decrease in the plant at certain times of the year, thereby influencing detection. Summer is generally an unfavorable time of year for plant growth and virus replication due to the heat. It's also important to note that this technique doesn't work for all ssRNA+ virus groups, notably the ilarviruses. It also doesn't work for the ambisense tospoviruses (Tomato Spotted Wilt and Impatiens Necrotic Spot viruses), but does work well for the cucumoviruses, alfamoviruses, tobamoviruses, potyviruses, potexviruses, and closteroviruses.

dsRNA analysis has become an unsung tool for diagnosing plant viruses, but one that is still useful because of its non-specific nature. Even given its limitations I believe it still has a place in the diagnostic and research laboratory. At the very least it provides a jumping-off point in the diagnostic process before proceeding to more specific techniques.

For protocol information contact:
J.R. Fisher, Plant Pathologist
Ohio Dept. of Agriculture
8995 East Main St.
Reynoldsburg, OH 43068
jfisher@agri.ohio.gov