A PCR-based Assay for Differentiation of Clover Yellow Vein Virus and Bean Yellow Mosaic Virus in Common Bean

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Clover yellow vein virus (CYVV) and *Bean yellow mosaic virus* (BYMV) (Family: *Potyviridae*) are important viruses of snap and dry bean. Serological differentiation of the two viruses has typically been difficult because of the similarity of their coat proteins. In addition, symptoms on bean are highly variable and this often results in erroneous identification of field isolates. Symptoms vary greatly according to virus strain, cultivar and growth stage at time of infection. Typical symptoms include mild to severe mosaic or yellow mosaic, leaf malformation and stunting. Selected strains of both viruses are reported to cause vein necrosis and apical or top necrosis resulting in plant death. Pod distortion or malformation may occur on plants infected with either virus but is generally more severe on those infected with CYVV.

In the Great Lakes region during the 2000-2003 growing seasons, snap beans were observed showing extensive pod necrosis or "chocolate pod" in many fields (3). CYVV was suspected as the causal agent based on preliminary host range studies but identity of the pathogen was not conclusive. Monoclonal antibodies have been produced that distinguish between BYMV and CYVV (1, 2) in ELISA, but these antisera are not readily available. Hence, a reverse transcription polymerase chain reaction (RT-PCR) assay has been developed that can unambiguously distinguish between CYVV and BYMV in single and mixed infections of bean.

Field isolates and nucleic acid preparation. Bean samples exhibiting mosaic symptoms and pod necrosis were collected from fields in Washington State, Idaho, and Wisconsin during 2000-2003. A strain of CYVV originating in Oregon and a strain of BYMV from bean in Washington were used as positive controls. Their identity was verified by comparing the nucleic acid sequence of their respective viral coat proteins and 3-prime non-translated regions to known sequence data available in GenBank. Total nucleic acid was extracted from bean samples using a modified method of Dellaporte *et al.* (4).

Primer design. DNA primers for CYVV and BYMV were designed using available sequence in GenBank including sequence date from the isolate of BYMV from Washington and the isolate of CYVV from Oregon. Target areas for forward and reverse primers were identified in the 5' and 3' regions of the viral coat protein genes, respectively.

CYVV-F 5'-TTGATGACAGCCAGATG-3'

CYVV-R 5'-AATCGTGCTCCAGCAATG-3'

BYMV-F 5'-GCGCTCAAGCACCTATACACT-3'

BYMV-R 5'-CTCGCTCTACAAAGATCAG-3'

RT-PCR conditions. Reverse transcription reactions were carried out using Malone Murine Leukemia Virus reverse transcriptase with the respective reverse primers for each virus, and incubated at 42 C for one hr. Two microliters of the cDNA product were added to the PCR mixture and amplified with 25 cycles of 94 C for 1 min, 58 C for 1 min, 72 C for 1 min, and a final extension at 72 C for 10 min. Amplification products were resolved through 1.4% agarose gels in TAE buffer.

Results and Discussion

RT-PCR using DNA primers designed for the detection of CYVV produced amplicons of the expected size (844 bp) from bean samples infected with CYVV but it did not amplify BYMV. Similarly, the BYMV primers amplified products (1113 bp) from plant samples infected with the homologous virus.

Each primer pair produced the expected amplicon from extracts infected with both CYVV and BYMV. These primers could be used in a multiplex reaction with no interference.

Primers designed to detect CYVV were used to demonstrate that this virus was directly associated with pod necrosis or "chocolate pod" symptoms on snap bean collected from production fields in Wisconsin. BYMV was not detected in leaves or pods of these samples. Neither *Cucumber mosaic virus* nor *Alfalfa mosaic virus* were detected in the symptomatic pods when evaluated using ELISA, although mixed infections have been known to occur. Interestingly, leaf samples of many plants bearing pods infected with CYVV did not contain detectable levels of this virus. In addition, when selected bean varieties were mechanically inoculated with leaf extracts from "chocolate pod" samples from Wisconsin, all inoculated plants were negative for CYVV, but plants inoculated with extracts of symptomatic pods taken from the same plants were positive for the virus. Thus, CYYV could be predominantly localized in the pods of diseased plants, while the virus was absent or present in very low titer in the vegetative portion of the same plants. BYMV was not detected in leaves or pods of any of the samples from Wisconsin infected with CYVV.

This work has demonstrated that the RT-PCR assay was able to unambiguously differentiate between BYMV and CYVV in single or in mixed infections in bean, and can be easily conducted in a single day. Furthermore, all samples of pods exhibiting typical 'chocolate pod' symptoms were positive for CYVV. This suggests that CYVV should be considered an important virus associated with the "chocolate pod" symptom in snap beans.

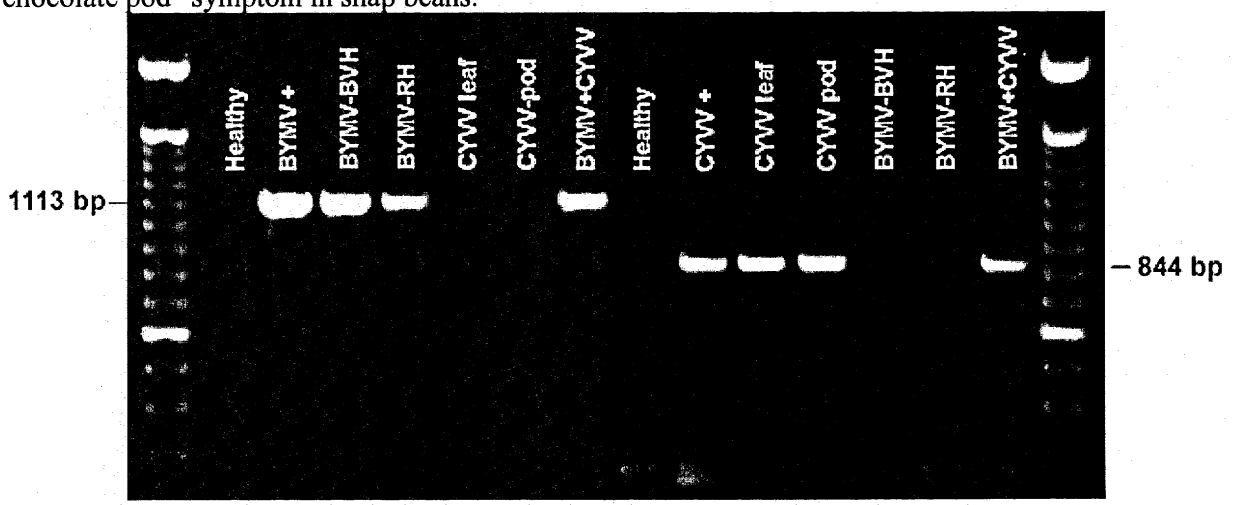

Figure. 1. Agarose gel showing amplicons of RT-PCR using primers specific to BYMV and CYVV. Total nucleic acid was extracted from bean tissue infected with BYMV (strains BVH and RH) or CYVV in naturally-infected snap bean from Wisconsin. BYMV+CYVV indicates samples with mixed infection.

References

1. Hewish, D. R., Xiao, X. W., Mishra, A., Gough, K. H., and Shuckla, D. D. 1993. Characterization and epitope analysis of monoclonal antibodies to virions of clover yellow veain and johnsongrass mosaic potyviruses. Arch. Virol. 127-141.
2. Sasaya, T., Shimizu, T., Nozu, Y., Nishiguchi, M., Inouye, N., and Koganezawa, H. 1997. Biological, serological, and molecular variabilities of clover yellow vein virus. Phytopathology 87:1014-1019.
3. Larsen, R. C., Grau, C. R., Eastwell, K. C., Mondjana, A. M., Miklas, P. N., and Stevenson, W. R. 2002. A virus disease epidemic of snap bean occurring in the Great Lakes region of the United States. Phytopathology S44.
4. Dellaporta, S. L. 1993. Plant DNA miniprep and microprep: Version 2.1-2.3. Pages 522-525 in: the Maize Handbook, M. Freeling and V. Walbot, eds. Springer-Verlag, New York. .