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Use of enzymatic cDNA amplification as a method of detection of bean yellow mosaic virus

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Abstract

The C-terminal region of bean yellow mosaic virus (BYMV) coat protein gene was selected for the design of oligonucleotide primers. Reverse transcription of viral RNA present in sap of virus-infected plants followed by polymerase chain reaction (RT-PCR) was performed for amplification of a 449 baise pairs fragment. These primers supported BYMV amplification, but did not support amplification on both bean common mosaic virus (BCMV) and potato virus Y (PVY) templates.

Additional keywords: detection, bean common mosaic virus, RT-PCR, potato virus Y.

BYMV is a member of the potyvirus group (Bos, 1970), the largest and most economically important group of plant viruses. Potyviruses have 750–950 nm long rod-shaped particles, induce characteristic cylindrical inclusions in host cells, and most members are transmitted by aphids in a nonpersistent manner (Edwardson and Christie, 1986; Milne, 1988).

In general, plant virus diagnosis is based on symptom development in test plants and on detection of viral protein using serological techniques, such as enzyme-linked immunosorbent assay (ELISA). Recently, an enzymatic procedure named the polymerase chain reaction (PCR) has been described as a sensitive, nonradioactive and quick assay (Saiki et al., 1985; Saiki et al., 1988; Sambrook et al., 1989). This technique has been successfully used to detect very low amounts of plant viral nucleic acids (Rybicki and Hughes, 1990; Vunsh et al., 1990; Jones et al., 1991; Korschineck et al., 1991; Wetzel et al., 1991; Borja and Ponz, 1992).

In this study we report on the suitability of PCR, using oligonucleotide primers selected from a region of the BYMV coat protein gene (Hammond and Hammond, 1989) to amplify viral RNA after reverse transcription from crude plant extracts, without the need for nucleic acid purification.

The viral isolates used were: an isolate of BYMV collected from Villa del Prado, Madrid (Castro et al., 1992), BYMV (PV 89) and BCMV (PV 28) isolates purchased from the American Type Culture Collection (ATCC) and our isolate of BCMV serotype B (J-8) in *Phaseolus vulgaris* from Pedrajas (Valladolid, Spain) (Castro et al., 1991). BYMV was purified from leaves of systemically infected broad bean (*Vicia faba*) 15 days after inoculation, using method 2 of Moghal and Francki (1976) as modified by Randles et al. (1980). The RNA was phenol/ethanol extracted. Preparation of crude sap of infected and healthy broad bean and *Nicotiana benthamiana* tissue and RT-PCR were performed as described by Borja and Ponz (1992). Ten-fold dilutions were carried out in plant dilution buffer (PDB 10X = 100 mM Tris-HCl, 15 mM $MgCl_2$, 0.5% Tween 20, 0.1% gelatin, 500 mM KCl) from 1/10 to 1/1000 (w/v). A 23-mer primer 5'-CCAAGCATGGTGTGCATAT-

CACG-3' complementary to nucleotides 814–836 of a partial sequence of BYMV (Hammond and Hammond, 1989) was used to prime reverse transcription. PCR was performed using the previously described primer together with a 22-mer primer 5'-GGCCT-TATGGTGTGGTGCATAG-3' homologous to nucleotides 388–409. Products of PCR were ethanol precipitated, and the DNA resuspended in TE (10 mM Tris-HCl, 1 mM EDTA) pH 8.0 and subsequently analyzed by electrophoresis in a 1.1% agarose gel and stained with ethidium bromide.

RNA extracted from viral particles purified from broad bean plants inoculated with isolate J-10 were amplified in a RT-PCR assay carried out in the presence of two primers specific for the BYMV coat protein region. The PCR product (Fig. 1, lane E) of the BYMV (J-10)-RNA was a DNA band corresponding to the expected size (449 bp) deduced from the sequence data (Hammond and Hammond, 1989). Amplification from crude sap extracts of two isolates of BYMV (J-10 and PV 89) was as good as that from purified RNA in RT-PCR (Fig. 1, lanes C, D, H and I). Negative controls (Fig. 1, lanes A, B and G) from healthy broad bean and *N. benthamiana* showed no PCR product. No evidence of amplification was obtained when extracts of PVY-infected plants (lane J) and two BCMV isolates (J-8 and PV 28) were used as templates of RT-PCR These results were consistent through replications.

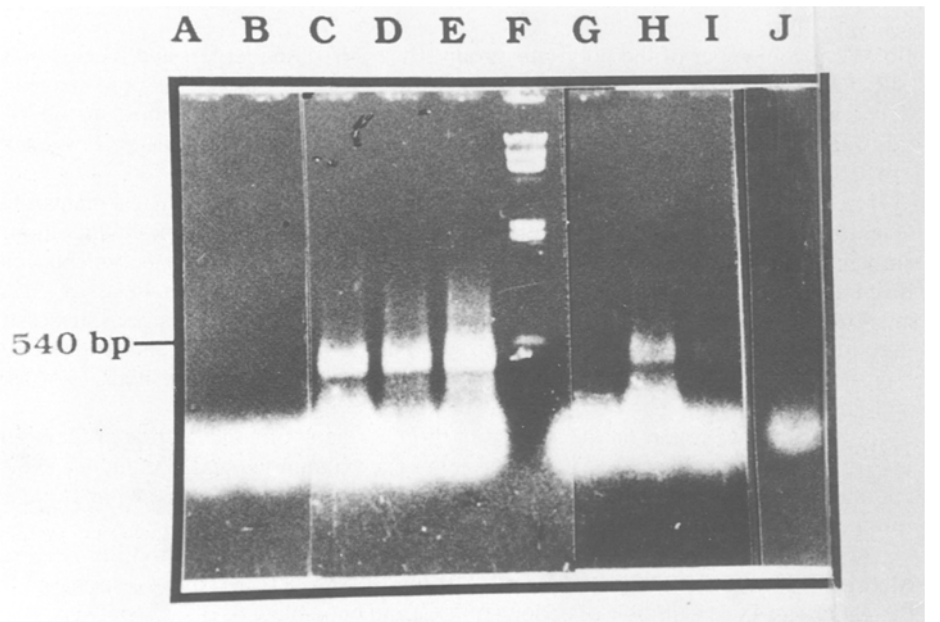

Fig 1. Electrophoresis of PCR products in 1.1% agarose gel (stained with ethidium bromide). The lanes contain amplification products from leaf extracts from: (A) and (B), healthy broad bean diluted 1/100 and 1/1000 respectively; (C) and (D), broad bean infected with BYMV J-10 diluted 1/100 and 1/1000 respectively; (E), 10 ng of purified BYMV-J10-RNA; (F), DNA size marker (phage lambda DNA, Hind III digested); (G), healthy tobacco diluted 1/100; (H) and (I), *N. benthamiana* (dried leaves) infected with BYMV (PV 89) diluted 1/100 and 1/1000 respectively; (J), PVY infected tobacco diluted 1/100.

Lanes were not all adjacent in the original gel. Lane J contain less sample than the other lanes.

Nucleic acid amplification by PCR was shown to be a sensitive, quick and specific assay, requiring small amounts of target DNA. In comparative tests conducted by others, RT-PCR was more sensitive than ELISA for the detection of cherry leafroll (Borja and Ponz, 1992), plum pox (Korschineck et al., 1991) and tomato spotted wilt (De Haan et al., 1991) viruses. We were able to amplify a portion of the viral genome from crude sap of infected broad bean, French bean or tobacco plants for the detection of BYMV without any further treatment, as previously described by Borja and Ponz (1992). These results show that the plant extracts at the dilutions used do not inhibit the enzymatic amplification reactions. We were able to amplify viral cDNA from both infected fresh leaves and extracts from dried leaves.

RT-PCR in our conditions works properly since the length of PCR products correlates with the theoretical length derived from the sequence data, and no PCR product is detected from any healthy plants. The test appears to be very sensitive, because the concentrations of template RNA in the diluted samples were well above the lower minimum detection limit of PCR. Borja and Ponz (1992) detected a dilution of 2.5×10^9 in wCLRV-infected tobacco plants. Using the primers for the nucleotide sequence of the C-terminal region of BYMV coat protein gene, we did not amplify others potyviruses: BCMV (both our isolate J-8 serotype B and an isolate from the ATCC, PV-68) and PVY.

This RT-PCR assay can be useful in those cases where a high sensitivity is required, such as detection of viruses in seeds. We are currently sequencing the BCMV capsid protein gene in order to design specific primers that would allow a differential detection of bean potyviruses.

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