Immunological detection of bean common mosaic virus in French bean (*Phaseolus vulgaris* L.) leaves

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Abstract Bean common mosaic potyvirus (BCMV) is an important seed borne pathogen of French bean. Differential inoculation with bean common mosaic virus at cotyledonary trifoliate leaf stage and pre-flowering stage of crop growth revealed that cotyledonary leaf infection favored maximum disease expression. Under immunosorbent electron microscopy (ISEM) the virus particles of filamentous structure having a diameter of 750 nm (l) and 15 nm (w) were observed. These particles gave positive precipitin tests with polyclonal antiserum of Potato virus Y.

Keywords Bean common mosaic virus · French bean

Introduction

Bean common mosaic virus (BCMV) is a widely distributed destructive pathogen on bean (*Phaseolus vulgaris* L.) [1]. BCMV belongs to the Potyvirus group, the largest group of plant viruses known [2]. The virus is transmitted at a high frequency through seeds [3, 4]. Up to 98% yield losses have been reported due to BCMV infection [5, 6]. BCMV is detected by growing on test [7] and by serological techniques [1, 3], such as immunosorbent electron microscopy (ISEM), ELISA and dot immuno-binding assay are employed for the diagnosis of BCMV.

Monoclonal antibodies have been successfully employed for the detection of BCMV antigens [8, 9]. However, the high cost in their production makes it essential to use an alternative system for the detection. Polyclonal antiserum of Potato virus Y (PVY) has also been used for the detection of BCMV [10]. Since it recognizes all members of the family Potyviridae, it is not suitable for the routine detection of a particular virus. Therefore in the present study, the physical properties along with serological behavior are used for detection of BCMV.

Materials and methods

Virus source and inoculum

Young infected leaves of French bean with distinct mosaic symptoms under field condition were collected and ground in a mortar and 0.1 M phosphate buffer (pH = 7.0) was added in 1:1 ratio (w/v). The slurry was squeezed through muslin cloth. Sap was centrifuged at 3,000 rpm for 5 min. The supernatant thus obtained was used as standard inoculum (SI).

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Culture was maintained on French bean plant in insect free condition.

Inoculation

Mechanical inoculation using SI and celite powder was done at three leaf stage. Inoculation was done by gently rubbing leaves with forefinger dipped into SI.

Growth conditions

All plants were grown in earthen pot of 30 cm diameter containing mixture of sandy loam soil and compost (1:1) in glasshouse conditions.

Fig. 1 Electron micrograph showing filamentous particles in purified preparation

Fig. 2 ISEM of BCMV particles showing decorated virus particles

Physical properties

The studies on dilution endpoint (DEP), thermal inactivation point (TIP) and longevity *in vitro* (LIV) were carried by following the procedure outlined by Noordam [11].

Seed transmission

Seeds were collected from virus infected French bean plant and inoculated to healthy plant in insect – proof glasshouse. Two hundred and twenty-five mature seeds obtained from healthy and virus infected plant were periodically plucked and were inoculated on young seedlings of French bean PDR – 14 to check latent infection, if any.

Electron microscopy

A drop of 1:100 dilution of purified virus preparation was placed on carbon coated grids, incubated for 30 seconds, washed with distilled water and stained with 2% uranyl acetate. Excess stain was removed by filter paper and grids were examined under JEOL –100-CX-11 electron microscope. Similarly, for leaf dip preparation, infected French bean leaves were ground in a few drops of 0.05 M phosphate buffer (pH = 7.0). Sap thus extracted was centrifuged at low speed in an Eppendorf centrifuge for 2 min. A drop of resultant supernatant was placed on carbon coated grids and the same procedure was followed as with purified preparation.

Serology

Serological relationships of the viruses associated with mosaic symptoms of French bean in India were determined with other filamentous viruses infecting French bean elsewhere. These studies were performed in ISEM decoration tests. The different antisera used were bean yellow mosaic virus, passion fruit woodiness virus, peanut mottle virus, clover yellow vein virus and Cowpea aphid-borne mosaic virus.

Results and discussion

The initial symptoms of BCMV infection appear on trifoliate leaves. The infected seedlings show symptoms like mosaic, vein-bending and downward rolling of the leaf margins and reduced leaf size sequential decrease in the seed transmission was observed in plants inoculated at first/second trifoliate leaf stage and pre-flowering stage. Our studies corroborate with the findings of Davis

and Hampton [12] who observed that bean seedlings inoculated with cucumber mosaic virus (CMV) showed high concentration of the virus in the primary leaves, whereas the young trifoliate and portions below the primary leaves exhibited intermediate concentration of the virus as determined by ELISA.

A study of physical properties establish DEP of 10^{-3} – 10^{-4} , TIP as 60°C and LIV of 4 days at room temperature. Transmission study showed that the present virus is transmitted through sap and seeds. A reference has been made by Schmidt [4] that bean common mosaic is transmitted through seed, up to 93%. Capoor et al. [13] also published a note indicating 42% seed transmission in a commercial seed sample of French bean.

Filamentous flexuous virus particles having a diameter of 750 nm (l) and 15 nm (w) were observed under a leaf dip preparation test [Fig. 1]. The virus particles reacted positively and gave positive precipitin tests with polyclonal antiserum of PVY [Fig. 2]. BCMV is also detected by ELISA [3, 7]. The serological relationships of present virus isolate with PVY were further confirmed by ISEM tests. In ISEM, polyclonal antiserum of PVY reacted strongly with the virus under study and a uniform decoration was obtained in ISEM tests with the antiserum of PVY.

The positive serological reaction of the virus isolated under study in leaf dip preparation tests and ISEM tests with PVY antiserum also suggested that the isolate under study was a strain of BCMV.

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