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Virus-mealybug relationship in the transmission of piper yellow mottle virus

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Abstract

In the current study, the piper yellow mottle virus (PYMoV)-mealybug (*Ferrisia virgata*) relationship in the transmission of PYMoV in black pepper was determined. The mealybug *F. virgata* collected from healthy black pepper plants was reared on pumpkin in the laboratory and used to determine the acquisition access period (AAP), retention period (RP) and inoculation access period (IAP) for the transmission of PYMoV. The crude extracts isolated from black pepper plants and mealybugs were tested through PCR using PYMoV-specific primers. The study determined 30 min, 8 h, and 30 min as the AAP, RP, and IAP respectively, indicating the semipersistent type of transmission of PYMoV.

Keywords: Acquisition access period, black pepper, inoculation access period, mealybug, PCR, PYMoV, retention period

Introduction

Black pepper (*Piper nigrum*) (family: Piperaceae) known as the King of Spices is mostly cultivated in South India's coastal regions, the plantation is now spread around the world, including Vietnam, Sri Lanka, Malaysia, Indonesia, China, and Brazil. Crop loss due to diseases and insect pests is one of the major limitations of pepper production in all pepper-growing regions. Stunt disease also referred to as mottle disease, caused by piper yellow mottle virus (PYMoV) (*Badnavirus*: *Caulimoviridae*) is one of the main diseases of this crop (Lockhart *et al*., 1997; De Silva *et al*., 2002; Bhat *et al*., 2023). The infected plants exhibit symptoms such as yellow mottling and mosaic, vein clearing, leaf deformation, and stunted growth. In addition to black pepper, PYMoV also infects other related *Piper* species in various countries including India. It is classified as a pararetrovirus since it has non-enveloped, bacilliform-shaped virions and contains a closed double-stranded DNA genome that

reproduces through an intermediate RNA (Hohn *et al*., 2008).

As black pepper is vegetatively propagated, the virus spreads primarily through the infected cuttings for planting and secondarily through insects such as mealybugs (*Ferissia virgata*, *Planococcous citri*, *P. minor* and *P. elisae*) and black pepper lace bug (*Diconocoris distanti*) (Lockhart *et al*., 1997; De Silva *et al*., 2002; Bhat *et al*., 2003; 2005). Based on the characteristics of virus–vector interactions, the modes of virus transmission are classified as nonpersistent, semipersistent, and persistent. The virus transmission involves three stages namely, acquisition (the process by which the vector acquires virus from the infected plant), retention (retention of the acquired virus within the vector), and inoculation (the process by which retained virus is released into the host plant to initiate infection) (Ng & Perry, 2004). Though previous studies have shown that mealybug, *F. virgata* transmits PYMoV, the vector-virus relationship is not determined. In the present study, we determined the AAP, RP, and IAP by *F. virgata* for the transmission of PYMoV in black pepper.

The adult mealybug, *F. virgata* collected from healthy black pepper plants was reared on a matured pumpkin under *in vitro* conditions. After three generations on the pumpkin, non-viruliferous young adult mealybugs were used as the vector for transmission of PYMoV. Non-viruliferous nature of mealybugs, healthy and PYMoV-infected black pepper plants were confirmed through PCR as described below. PCR-identified PYMoV-infected plants were used as source plants for transmission of the virus using mealybug. The crude extract was isolated using NaOH method by grinding the sample in 0.5 N NaOH (1:10), centrifuged for 30 sec at 5000 rpm and supernatant diluted 10 times with buffer, 0.1 M Tris-HCl (pH 8) (Mohandas & Bhat, 2020). PCR was performed using the crude extract of plants and mealybugs as a template. The forward primer AIB 254 (5′-TTTGTCAAGCCAAGAGACCAC-3′) and reverse primer AIB 255 (5′-TTGAGTGATTTGGTCCTCCAC-3′), representing a portion of the open reading frame (ORF) 2 region of the PYMoV genome were used as primers in the PCR. The amplified product obtained at 352 base pairs indicated a positive PCR reaction. The PCR components contained 12.5 µl of 2x PCR Master Mix (Takara Bio Inc, Japan), 0.5 µl (10 µM) each of forward and reverse primers, 2 µl of template, and 9.5 µl of sterile water. The thermal cycler was programmed as follows: Initial denaturation for 3 min at 94^0C, followed by 35 cycles that included 94^0C for 30 sec for denaturation, 54^0C for 30 sec for annealing, 72^0C for 30 sec for extension, and final extension phase at 72^0C for 10 min. The PCR reaction products were subjected to agarose gel electrophoresis and analyzed using a Gel Doc system.

To determine the AAP, virus-free mealybug cultured on the pumpkin were picked individually using a camel brush and transferred onto PYMoV-infected black pepper leaf placed over a Petri dish containing moist blotting paper that was wrapped in a black cloth. Mealybugs were allowed to feed on the infected leaf for

different periods such as 25 min, 30 min, 1 h, 2 h, and 3 h. After each AAP, mealybugs were collected separately in Eppendorf tubes, crude extracts were prepared and subjected to PCR. To determine the RP of PYMoV by *F. virgata*, the mealybugs were allowed to feed on PYMoV-infected leaf overnight to acquire PYMoV and thereafter were moved to another Petri dish having healthy black pepper leaf using a camel brush. They were then allowed to feed on the healthy leaf for different periods such as 30 min, 1 h, 6 h, 7 h, 8 h, 9 h, and 10 h. After each period, mealybugs were collected separately in Eppendorf tubes, crude extracts were prepared and subjected to PCR. To determine the IAP of PYMoV by *F. virgata*, the mealybugs were allowed to feed on PYMoV-infected leaf overnight to acquire PYMoV, and on the next day, ten mealybugs each were inoculated to five healthy black pepper plants for different inoculation periods such as 0 h, 25 min, 30 min, 1 h, 2 h, 3 h, 4 h, and 6 h. After each IAP, insecticide (0.5 g/L of acetamiprid 20% SP) was sprayed on the plants to kill the mealybugs and placed in an insect-proof greenhouse. PCR was performed using the crude extracts prepared from the black pepper plants three weeks after inoculation. All the experiments were repeated three times.

The healthy and PYMoV-infected black pepper and mealybugs were identified through PCR (Fig. 1). PYMoV-infected plants were used as source plants for transmission experiments using mealybug. The non-viruliferous mealybugs permitted to feed on PYMoV-infected black pepper leaf for different time periods when subjected to PCR using PYMoV-specific primers, showed a positive reaction for PYMoV in mealybugs fed for 30 min to 3 h while no amplification was seen in mealybugs fed for 25 min indicating that the minimum time required by *F. virgata* to acquire the PYMoV is 30 min (Fig. 2). The viruliferous mealybugs that fed on the healthy black pepper leaf for different time periods when subjected to PCR, showed the presence of PYMoV in *F. virgata* that were allowed to feed till 8 h while no PYMoV could be detected in mealybugs fed for >9 h indicating that *F. virgata* could retain the PYMoV till 8 h (Fig. 3). The viruliferous mealybugs when fed on healthy black pepper plants for different IAP showed positive reactions in plants that were given IAP of 30 min and above, indicating that IAP of PYMoV by *F. virgata* is 30 min (Fig. 4). None of the plants given 25 min of IAP showed positive reaction in PCR (not shown).

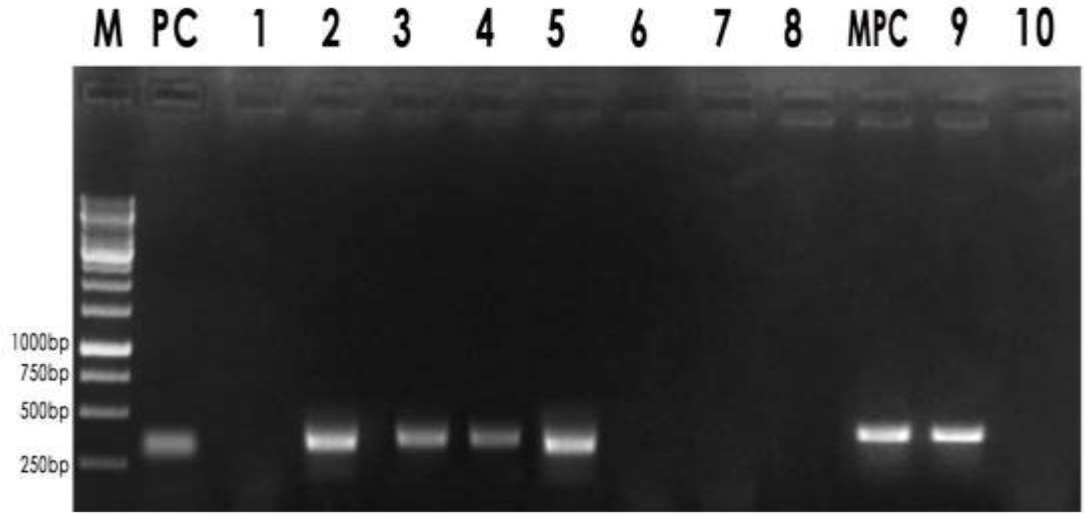

Fig. 1. Detection of PYMoV-infected and PYMoV-free black pepper plants and mealybugs by PCR. Lane M: Molecular markers; Lane PC: Known PYMoV-infected black pepper plant (Positive control); Lanes 1-8: Test plants of black pepper; Lane MPC: Known PYMoV-infected mealybug; Lanes 9-10: Test mealybugs.

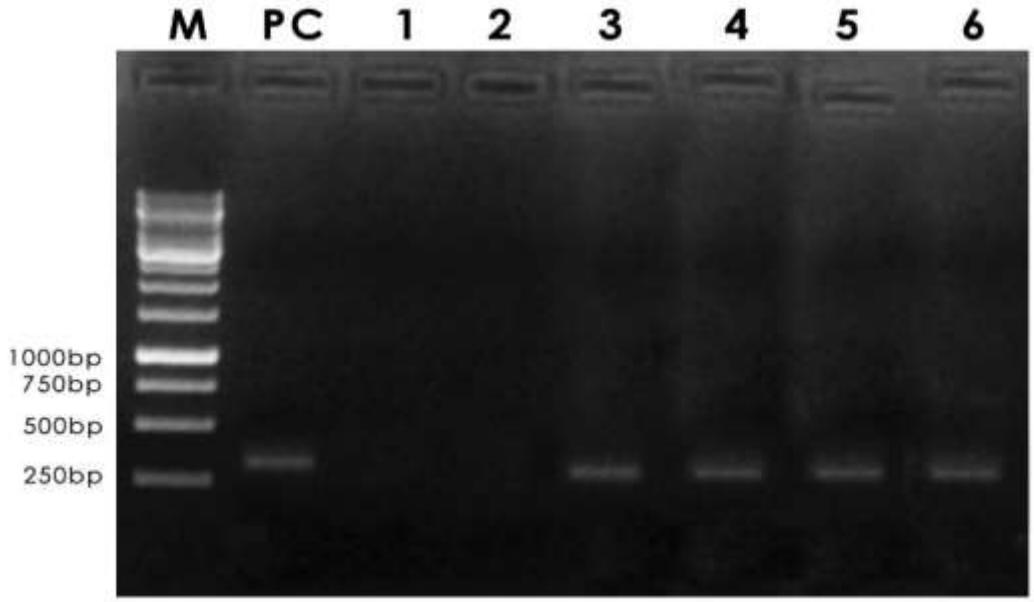

Fig. 2. Determination of acquisition access period (AAP) of PYMoV by *F. virgata* through PCR. Lane M: Molecular markers; Lane PC: Positive control; Lane 1: Non-viruliferous mealybug; Lanes 2-6: Mealybugs allowed for different AAP such as 25 min, 30 min, 1 h, 2 h, 3 h.

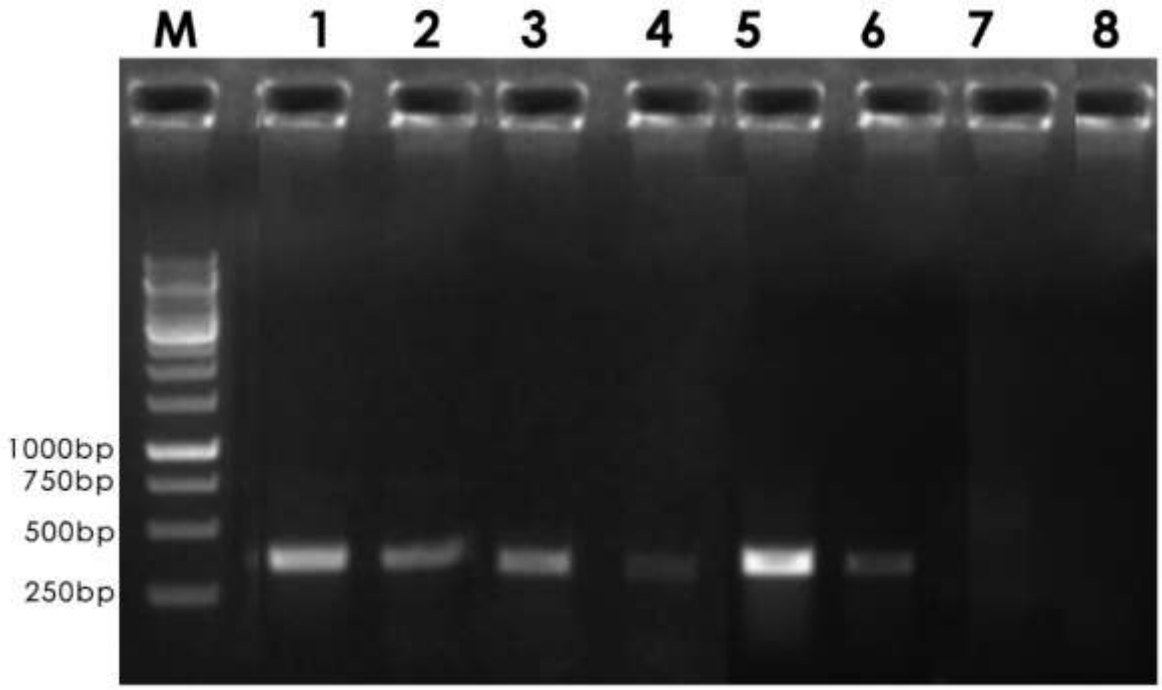

Fig. 3. Determination of retention period of PYMoV by *F. virgata* through PCR. Lane M: Molecular marker; Lane 1: Control (viruliferous mealybug); Lanes 2-8: Viruliferous mealybugs fed on healthy plants for 30 min, 1 h, 6 h, 7 h, 8 h, 9 h, 10 h.

Based on the virus-vector relationship, viruses are classified into different groups. Nonpersistent viruses survive in their vectors for only a short period and have short acquisition and inoculation periods. Semipersistent viruses need more time for acquisition and transmission while persistent viruses survive in their vectors for longer periods, often for weeks or months (Raccah & Fereres, 2009). Mealybugs transmit viruses mainly through semipersistent transmission and they serve as vectors of several badnaviruses, closteroviruses, and trichoviruses (Nayudu, 2008; Bhat *et al*., 2023). The current study determined the AAP of PYMoV by *F. virgata* as 30 min. It is reported that *Pseudococcus longispinus* requires an AAP of only 15 min to transmit grapevine virus A (GVA) (La Notte *et al*., 1997) while *Planococcus ficus* needs an AAP of 1 h for the transmission of grapevine leaf roll-associated virus 3 (GLRaV-3) (Tsai *et al.,* 2008). Posnette & Robertson (1950) reported that the cacao swollen shoot virus (CSSV) transmitted by the vector *Pseudococcus njalensis* needs 20 min of AAP. The present study determined RP of PYMoV by *F. virgata* as 8 h. CSSV persists for <3 h in the vector (Posnette & Robertson, 1950) while *Planococcus citri* retains GLRaV-3 for 24 h (Cabaleiro & Segura, 1997). *Phenacoccus aceris* retains GVA for 5 days and GLRAV-1 for 7 days while GLRaV-3 persists for 8 days in *Planococcus ficus* (Krüger *et al*., 2015). The current study determined the IAP as 30 min

for the transmission of PYMoV. The IAP for the transmission of GVA by *P. longispinus* was 30 min (La Notte *et al*., 1997) while it was 1 h for the transmission of GLRaV-3 by *P. ficus* (Tsai *et al*., 2008). Based on the AAP, RP, and IAP, it is concluded that PYMoV-*F. virgata* relationship is semipersistent.

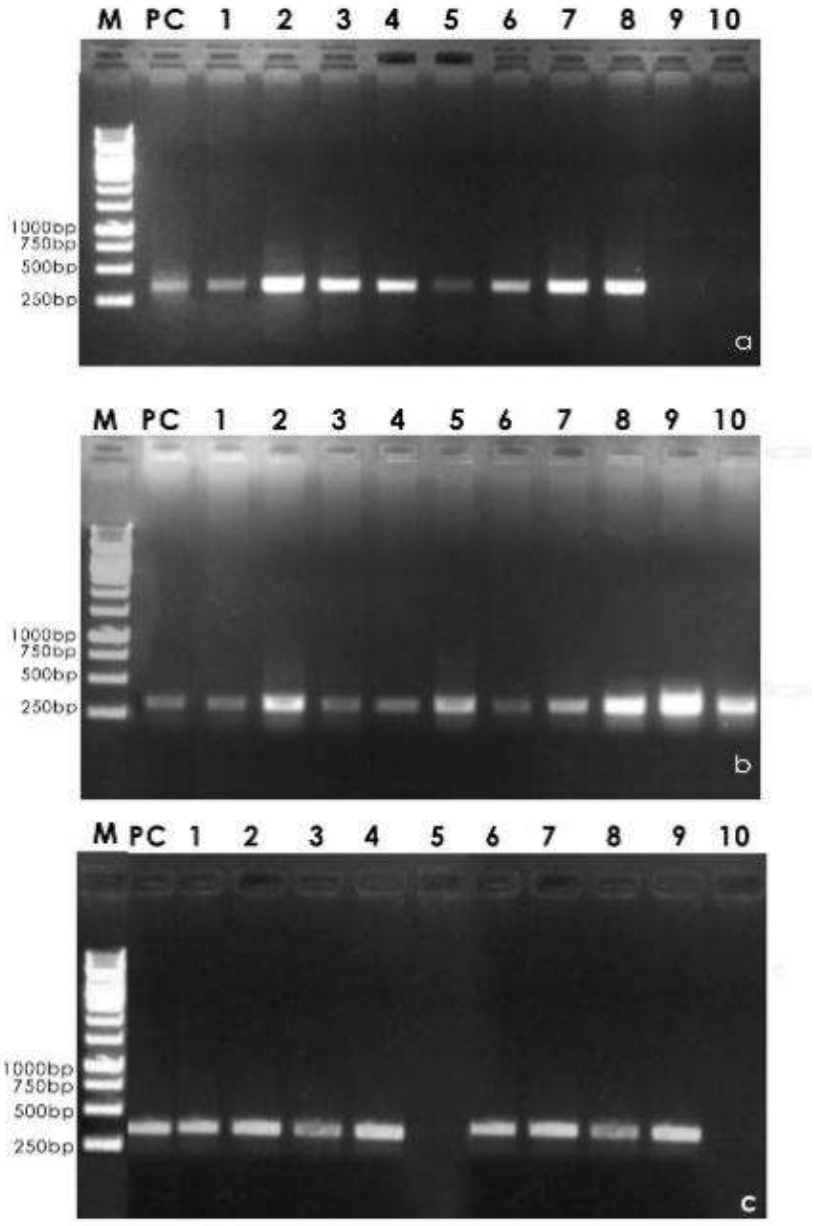

Fig. 4. Determination of inoculation access period (IAP) of PYMoV by *F. virgata* through PCR. Lane M: Molecular markers; Lane PC: Positive Control; (a) Lanes 1-5: IAP of 30 min; Lanes 6-10: IAP of 1 h (b) Lanes 1-5: IAP of 2 h; Lanes 6-10: IAP of 3 h (c) Lanes 1-5: IAP of 4 h; Lanes 6-10: IAP of 6 h.

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