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A Mosaic Disease Infecting Yam Bean; Molecular Detection and Transmisions

Tri Asmira Damayanti and Siti Nurlaelah

Department of Plant Protection, Bogor Agricultural University, Bogor, Indonesia

INTRODUCTION

Yam bean (*Pachyrhizus erosus* (L.)) Urban is one of the horticulture crop in several areas in Indonesia. The role of yam bean in Indonesia such as a source of essential nutrients, pickles, utilized in cosmetic industrial, its seed and leaves utilized as botanical pesticide. Due to its ability as symbion with nitrogen fixation bacteria, yam bean could act as a good nitrogen supplier in soil [7]. In several yam bean fields in Bogor area, West Java and in Prembun Central Java, we found yam bean with severe mosaic with leaf malformed, and green vein-banding symptoms. The mosaic symptom also present in infected beans. In Bogor area, the incidence up to 100%. Based on the phenotype symptoms, the causal of mosaic disease is a Poty-like virus. As one of beneficial legume, the presence of uncharacterized disease might become a serious problem not only for yam bean, also for other related legumes in the field and also in determining the management strategies. Here, we report the transmission modes and its partial molecular characters of the virus as a basic information of the disease.

MATERIALS AND METHODS

Source of Inoculum.

We surveyed several yam bean fields in Darmaga, Bogor and Prembun measured the disease incidence and the infected plants from Bogor collected the as source of inoculum.

Electron Microscopy.

To examined the viral particles, we investigated the particles morphology and size under microscope electron (TEM) by using crude extract of infected sap (leaf dip) method at Eikjman Research Institute, Jakarta.

Transmission modes

Transmission modes were conducted by inoculated healthy plants mechanically, by insect vectors (*Aphis glycines*, *A. craccivora* and *A. gossyppii*) and serologically detection of seeds obtained from infected plants using general antisera for *Potyvirus* (DSMZ, Deutsche Samlung von Mikroorganismen un Zellkulturen, Germany).

Molecular Detection.

Total RNA was extracted from infected leaves by grinding in glycine buffer premix (0.1M Glycine, 0.1M NaCl, 01M EDTA : 10% SDS : 5% bentonite with ratio 100:10:1). The supernatant treated twice with P/C/I (25:24:1) and centrifugation at 14.000 rpm for 5 minutes. RNA was precipitated by adding 99% ethanol 1.5 times of supernatant volume and 10% 3M Natrium acetate and centrifugation, then pellet was washed with 70% ethanol and suspended in 50 ul RNAse free water (Kroner *et al.*, 1992). cDNA was constructed by using reverse primer for 3' terminal region M4T (5'-GTTTTCCCAGTCACGACT(15)-3') using reverse transcriptase Superscript III. RT-PCR was carried out by using a universal forward primer for *Potyvirus* Sprimer (5'-GGNAAYAAYAGYGGNCARCC-3'; N= A,C,G or T; Y = C or T; R = A or G) and M4T with PCR was performed for 30 cycles each of 0.5 min at 94°C, 1 min at 50°, 2 min at 72°C followed by an extension step 72°C for 10 min (Chen *et al.*, 2001). The amplified DNA (1700 bp) was separated in 1% gel electrophoresis (TAE) and the sea plaque extracted DNA used as template for bulk sequencing. Additional primers were constructed for sequencing the 1700 bp DNA. DNA sequencing was conducted at Laboratory of Plant Pathology, Graduate School of Agriculture, Kyoto University, Japan. The homology of nucleotide sequences of coat protein gene (CP) and 3'untranslated region (3'UTR) with other *Potyviruses* were analyzed by using DNASIS software for Macintosh Computer system.

RESULTS AND DISCUSSION

Virus-like symptoms were observed during the survey in all yam bean fields in Bogor, West Java and Prembun, Central Java. The symptoms such mosaic with green vein-banding, leaf malformation were observed severely in Bogor with incidence range from 14%-100%, while in Prembun was low incidence. Based on symptom appearances showed that the causal of virus-like symptom was Poty-like virus. The electron microscopy of leaf infected sap (lip dip method) confirmed the *Potyvirus* viral particles morphology and size. *A. potyvirus*, for which the name *Yam bean Mosaic Virus* (YbMV) is proposed. The transmission modes of virus was investigated by mechanical inoculation, through 3 species of aphids (*Aphis craccivora*, *A. glycines* and *A. gossypii*) and detection of seeds obtained from infected plants by ELISA using *Potyvirus* general antiserum. The results showed that the virus transmitted mechanically and transmitted by all of tested aphids efficiently in non-persistent manner with efficiency 100% of *A. craccivora* and *A. gossypii* and 70% of *A. glycines*. Detection of seeds obtained from infected plants showed positively some of seed infected by virus, suggesting that virus could transmitted through seeds. Total RNA extracted from infected leaves were detected molecularly by using universal primer Sprimer and M4T which designed from consensus sequences that code for conserve sequence GNNSGQP in the NIb region of the family *Potyviridae*. RT-PCR successfully amplified the DNA (1700 bp), indicating the *Potyviridae* identity. Further, the RT-PCR product was isolated and used as template for bulk sequencing analysis. Additional primers used for primer extension method for sequencing were designed. The

sequence homology of the 3'-region of CP excluding the poly (A) stretch were compared with other Potyviruses. Both nucleotide sequences of YbMV showed the highest identities with those of *Bean Common Mosaic Virus* (BCMV) strain peanut stripe (77.4% and 78.6% for CP and 3'UTR, respectively). The identity of YbMV CP and 3'UTR with others Potyviruses (AzMV, BYMV, SMV, CabMV, TuMV, YMV, WMV, ZYMV) ranged from 49.1% to 77.4% and 44.6% to 78.7% , respectively. According to Shukla and Ward (1989), the CPs of individual potyviruses show over 90% similarity, while Frenkel *et al* (1989) reported that the degree of homology in nucleotide sequences of the 3'UTR between strains of a potyvirus was in the range 83-%-99%, and sequences from distinct viruses had identities in the range 39%-53%. Based on those classification, the YbMV is a new Potyvirus; the homology of CP and 3'UTR were less than 90% and 83%. However, Adams *et al* (2005) reviewed from many studies related with *Potyviruses* and showed that demarcation of the CP nucleotide identity optimal was 76-77% and CP amino acid identity over 79.6% in same species. The sequences of the CP, amino acid and 3'UTR of YbMV showed the highest homology (77.4%, 76.8% and 78.6%, respectively) with those of BCMV strain peanut stripe compared to other potyviruses, suggesting that the virus must have some close evolutionary relations. We conclude that YbMV is a distinct strain of BCMV and/or a new potyvirus because identity between the CPs amino acid and the 3'UTR rather low (less than 79.6% and 83%) and no data support that YbMV and BCMV belong to the same virus species. Desmiarti (2006) reported that the virus was systemically infect *Lycopersicon esculentum*, *Vigna unguiculata*, *Phaseolus vulgaris* and latent infection in *Gomphrena globosa*. Generally, BCMV infect related legumes, but rarely infects tomato. However, the YbMV infected all of tested *L. esculentum* and *G. globosa* systemically, suggesting distinct biological characteristics. Further study of the fully identity of the nucleotide sequences of the virus and its biological characteristics are now underway.

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