Characterization of A Novel Virus Infected Watermelon in Indonesia Based on Viral Particle Using Electron Microscope

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ABSTRACT

Most of the cucurbits diseases in Indonesia are caused by plant viruses. Tobamovirus is one of the viral genera recently infected cucurbits. The members of cucurbits-infecting Tobamovirus are *Cucumber green mottle mosaic virus* (CGMMV), *Kyuri green mottle mosaic virus* (KGMMV), *Cucumber fruit mottle mosaic virus* (CFMMV), *Zucchini green mottle mosaic virus* (ZGMMV), and *Cucumber mottle virus* (CuMoV). A research on the occurrence of cucurbits-infecting Tobamovirus in Indonesia was carried out in 2011. Based on the result, a new characteristic of viral particle were found in watermelon. The viral particle was previously identified as CGMMV based on electron microscope investigation revealed presence of rod-shaped and not enveloped virions. The virions were straight about 300 nm long and 28–30 nm in diameter. However, in some electron micrograph shows rare characteristic of the viral particle. The viral particles could joint each other to bend a new form. Two viral particles were able to joint and formed an angle. Moreover, some of viral particles could joint and form a longer viral particle (800-1100 nm), two to four times longer than CGMMV particle, but the both diameter of the virus are similar. These characteristics indicate that the viral particle is a novel virus, different from CGMMV or other Tobamovirus members.

Key Words: Viral particle, electron microscope, watermelon, novel virus

INTRODUCTION

Cucurbit crops, including melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* L.), cucumber (*Cucumis sativus* L.), angled loofah (*Luffa acutangula* L.) and bitter gourd (*Momordica charantia* L.), produced throughout Yogyakarta, a province in Indonesia, are

important vegetable fruit crops. Java is one of the main cucurbit growing areas of Indonesia and more than 80% of national production is produced by five provinces in Java i.e. East Java, Central Java, Jakarta, West Java, and Yogyakarta (BSCI, 2003). The yield of the crop of cucurbit plants depends not only on agricultural and meteorological condition, but also on the diseases including those caused by plant viruses. Plant viruses have become a problem in cucurbits production in Indonesia. Daryono *et al.* (2005) reported that melon and cucumber plants grown in Java, Indonesia during 2000-2001 were severally affected by a new kind of mosaic disease.

The genus *Tobamovirus* is one of the biologically and molecularly best-characterized groups with many species and strains varying widely in host range and its interaction with host plants (Yoon *et al.*, 2002). Phylogenetic analysis of recognized and tentative tobamovirus species has revealed four sub-groups within the genus, which corresponded well with groupings based on host range. These four tobamovirus sub-groups are those that infect (1) Solanaceous plants; (2) brassicas; (3) Cucurbit or legumes, and (4) Malvaceous (Adkins *et al.*, 2003).

Tobamoviruses constitute a group of plant viruses with single positive-sense RNA molecules as genomes and rod-shaped virions consisting of viral RNA surrounded by a single coat protein (CP). Members of the genus *Tobamoviruses* form rigid, rod-shaped particles approximately 300 nm in length and 18 nm in width. The genomes are capped at the 5' end and have a tRNA-like structure at the 3' end (Lartey *et al.*, 1996). Tobamoviral RNAs encoded four polypeptides. Two large polypeptides, 130 and 180-kDa proteins, function in viral RNA replication, and are translated from the same 5' proximal start codon. The 30-kDa movement protein (MP) required for cell-to-cell movement and the 17-kDa CP are translated from 3 co-terminated sub-genomic mRNAs derived from the 3' portion of the tobamoviral RNAs (Lewandowski, 2000).

Cucumber green mottle mosaic virus (CGMMV) is a member of the genus *Tobamovirus*, a serious disease agent of cucurbit crops and causes significant economical losses in several countries (Tan *et al.*, 2000). This virus was firstly found in Great Britain (Ainsworth, 1935).

Surveys of viral diseases of cucurbitaceae plants conducted in Trisik, Kulonprogo, Indonesia during July – September 2011 showed that among cucurbit plants observed, watermelon crops grown in the area were severely infected by CGMMV symptomatically. The infected plants showed mottle and mosaic on the leaves.

The objectives of this study were to find out the characteristic of viral particle of a novel virus found in watermelon by electron microscope and molecular detection using RT-PCR detection.

MATERIALS AND METHODS

Virus source and maintenance. Watermelon leaves showing mosaic mottle and suggestive of virus infection were collected in the traditional fields in Trisik, Kulonprogo-Yogyakarta, Indonesia. The leaves were homogenized in 10mM sodium phosphate buffer (pH 7.0) to transmit the viral agent to *Cucurbita pepo* leaves mechanically. Mosaic and mottle appearing in inoculated *C. pepo* were used as inoculum source and for maintenance.

Electron microscopy. Leaf tissue samples were removed from watermelon showing mosaic symptoms, ground and then were placed on carboncoated copper grids and negatively stained with 2% phosphotungstic acid (Hitchborn and Hills, 1965) and examined with an electron microscope.

RNA extraction and reverse transcription and polymerase chain reaction (RT-PCR). Viral RNA was extracted from a novel virus-infected *C. pepo* leaves by incubation in 0.3% SDS following phenol chloroform extraction (Rosner et al., 1983). For first-strand cDNA synthesis, RNA preparations were mixed with a reverse primer, followed by adding 1 μ l DTT, 5 μ l bulk first-strand kit (Amersham Biosciences, Little Chalfant, UK), incubated at 37°C for 1 h. First-strand cDNA was then amplified by PCR using Takara Ex TaqTM PCR buffer (Takara Biomedicals, Kyoto, Japan) with universal primer for genus *Tobamovirus*. PCR was carried out for 30 cycles of 1 min at 94°C, 1 min at 48°C and 1 min at 72°C. The amplified products were fractionated on 1.5% agarose gel in Tris-Acetate-EDTA (TAE) buffer and ethidium bromide stained bands of interest were excised and electro-eluted using standard procedures (Sambrook *et al.*, 1989).

RESULT AND DISCUSSION

Electron micrograph of the virus particle. The viral particle was previously identified as CGMMV based on electron microscope investigation revealed presence of rod-shaped and not enveloped virions. The virions were straight about 300 nm long and 28–30 nm in diameter. However, the electron micrograph shows rare characteristic of the viral particle of CGMMV. The viral particles could joint each other to bend a new form. Two viral particles were able to joint and formed an angle (Figure 1; left). Some of viral particles could joint and form a longer viral particle (800-1100 nm), two to four times longer than CGMMV particle (Figure 1; right), but the both diameter of the virus are similar (18-20 nm). These characteristics indicate that the viral particle is a novel virus, different from CGMMV or other Tobamovirus members.

Figure 1. Electron micrograph of CGMMV with the joint of two viral particles (left) and CGMMV with the joint of three to four viral particles (right)

RT-PCR Detection.

A forward universal primer for Tobamovirus (Tob-F) and the reverse primer (Tob-R) were used for the detection of watermelon strain and the PCR product was obtained in 897 bp. The PCR product was constantly shown only in watermelon sample (Fig. 2A). This result indicates that isolate from watermelon was infected by new Tobamovirus and not CGMMV. On the other hand, the expected amplification band in 697 bp was observed for CGMMV-Indonesian Loofah isolate or CGMMV-ILa (Fig. 2B), indicating they are not similar virus.

Figure 2. Gel electrophoresis of single RT-PCR detection using universal Tobamovirus and CGMMV primers (B). M: 100 bp DNA marker (Promega); H: healthy zucchini; W: CGMMV-IW; C: CGMMV-ILa. A single arrow shows a new Tobavirus (A) and CGMMV (B).

CONCLUSION

The viral particles of virus found in watermelon could joint each other to bend a new form that two to four times longer than CGMMV common particle, but the both diameter of the virus are similar. These characteristics indicate that the viral particle is a novel virus, different from CGMMV or other Tobamovirus members and it is proposes as Tobavirus for the virus.

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