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Geminiviruses Associated with the Weed Species *Ageratum conyzoides*, *Centipeda minima, Porophyllum ruderale,* and *Spilanthes iabadicensis* from Java, Indonesia

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Geminivirus has a wide host range including cultivated plants and weeds. Infected weeds may play an important role in disease epidemic. Unfortunately, little is known about weeds species that may serve as alternative host for *Geminivirus*. This research was conducted to identify *Geminivirus* on weeds around chili pepper field to study their potential role as virus reservoir. Field surveys were conducted to chilli pepper growing area in West and Central Java Provinces, and The Special Province of Yogyakarta during 2009 to collect symptomatic weed plants. *Geminivirus* infection was detected using PCR technique from 9 weed samples, i.e. 5 samples *Ageratum conyzoides* from Bogor (AgrBgr), Sukabumi (AgrSkm), Magelang (AgrMgl), Sleman (AgrJgy), and Garut (AgrGrt); *Centipeda minima* from Magelang (CtpMgl); *A. boehmerioides* from Sleman (AcpJgy); *Porophyllum ruderale* from Bogor (PrlBgr); *Spilanthes iabadicensis* from Magelang (SplMgl). Further genetic analysis showed that those geminiviruses can be differentiated into 2 clusters, showing the possible genetic differences among them. They neither have a close relationship with other geminiviruses published earlier in the GenBank, indicating weed infecting *Geminivirus* collected in this study is possibly a distinct *Geminivirus*.

Key words: *Begomovirus*, *Geminivirus*, polymerase chain reaction, weed species

Geminivirus memiliki kisaran inang yang luas termasuk berbagai tanaman budi daya maupun gulma. Hingga saat ini pengetahuan tentang jenis-jenis gulma yang berpotensi menjadi sumber *Geminivirus* masih sangat terbatas. Penelitian ini dilakukan dengan tujuan mengidentifikasi *Geminivirus* pada gulma-gulma yang tumbuh di sekitar pertanaman cabai dalam upaya mempelajari perannya sebagai sumber inokulum virus. Survei lapangan telah dilakukan pada tahun 2009 ke beberapa daerah penanaman cabai di Provinsi Jawa Barat, Jawa Tengah, dan Daerah Istimewa Yogyakarta untuk mengumpulkan gulma-gulma yang menunjukkan gejala. Infeksi *Geminivirus* berhasil dideteksi menggunakan teknik PCR dari 9 sampel gulma, yaitu 5 sampel *Ageratum conyzoides* masing-masing dari Bogor (AgrBgr), Sukabumi (AgrSkm), Magelang (AgrMgl), Sleman (AgrJgy), dan Garut (AgrGrt), *Centipeda minima* dari Magelang (CtpMgl), *A. boehmerioides* dari Sleman (AcpJgy), *Porophyllum ruderale* dari Bogor (PrlBgr), *Spilanthes iabadicensis* dari Magelang (SplMgl). Analisis genetika lebih lanjut menunjukkan bahwa *Geminivirus* yang menginfeksi gulma-gulma tersebut dapat dibedakan menjadi 2 kelompok besar yang merupakan indikasi adanya keragaman genetika di antara mereka. *Geminivirus* yang menginfeksi spesies gulma yang berbeda tersebut tidak memiliki hubungan kekerabatan yang dekat dengan geminivirus lain yang dilaporkan dalam *GenBank* sehingga *Geminivirus* yang diidentifikasi dari spesies gulma tersebut merupakan *Geminivirus* yang berbeda..

Kata kunci: *Begomovirus*, *Geminivirus*, polymerase chain reaction, gulma

Geminiviruses are single-stranded DNA viruses with geminate particle morphology. They are classified into four genera (*Mastrevirus, Curtovirus, Topocuvirus*, and *Begomovirus*) on the basis of host range, insect vector and genome organization (Fauquet and Stanley 2005). Most of the geminiviruses are transmitted by whiteflies and belong to the genus *Begomovirus*. These species have been reported to cause significant economic yield losses to many crops in tropical and subtropical regions of the world. Member of *Begomovirus* was known to have enormous diversity resulting from their widespread geographic distribution and host adaptation (Varma and Malathi 2003).

Diseases caused by *Begomovirus* in Indonesia has been reported including those infecting tobacco (Trisusilowati *et al*. 1990), tomato (Sukamto *et al*. 2005; Kon *et al*. 2006; Santoso *et al*. 2008), chilli pepper (Sulandari *et al*. 2006; Hidayat *et al*. 2006; Trisno *et al*. 2009), and also a weed species *Ageratum conyzoides* (Haerani and Hidayat 2003; Kon *et al*. 2007). Among those diseases, the most concerned one is pepper yellow leaf curl disease which induces symptoms involving foliar chlorosis and curling, reduced leaf size, inhibited fruit set and abnormal fruit. Emergence of pepper yellow leaf curl disease in Indonesia was first reported in 1999 in West Java (Rusli *et al*. 1999), and in 2003 the disease had been widely spread in Java with the highest incidence and severity occurred particularly in Central Java (Sulandari *et al*. 2006). Census data for the period from 2001 - 2003 shows that the disease has undergone a 4.6 fold increase between 2001 and 2002 and a 2.5 fold increase between 2002 and 2003 (Indonesian Ministry of

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Agriculture 2003, unpublished data). It was believed that two main factors may contribute to the spread and distribution of the disease in Indonesia, i.e. fluctuation of whitefly population and the presence of alternative plants that may act as virus reservoirs.

It was reported previously that weed species may serve as alternative hosts for geminiviruses. Yang *et al*. (2008) identified a distinct *Begomovirus* species from *Emilia sonchifolia* from Fujian province, China; whereas Wu *et al*. (2007) was successfully isolated a monopartite geminivirus from *M. coromandelianum from Guangdong, China.* Three weed species (*Sida* spp., *Macroptilium lathyroides*, and *Wissadula amplissima*) were identified as potential *Begomovirus*'s alternative host in Jamaica (Roye and McLaughlin 1997); meanwhile in Indonesia Sulandari *et al*. (2006) reported that *Hyptis brevipes*, *Physalis floridana*, *Crotalaria juncea*, *Ageratum conyzoides* were very susceptible to geminivirus infection. Thus, to build more information on the potency of weed species as virus reservoirs especially for pepper yellow leaf curl disease in Java, surveys were carried out in 2009 in West and Central Java. To this aim, weed samples showing leaf curl and yellow vein symptoms were collected from different chilli pepper growing area. In this paper, we report the identification of geminivirus associated with yellow disease of 4 weed species: *A. conyzoides*, *Centipeda minima, Porophyllum ruderale,* and *Spilanthes iabadicensis*.

MATERIALS AND METHODS

Virus Sources and DNA Extraction. Naturally infected weed species with yellow vein symptoms were observed in West Java (Bogor, Sukabumi, Garut), Central Java (Magelang), and Yogyakarta (Sleman). The specific symptoms was observed in *Compositae* weed species (*A. conyzoides, C. minima, E. prostrate, P. ruderale*, *S. iabadicensis, G. peruviana*), *Euphorbiaceae* species (*Croton hirtus*), *Convolvulaceae* species (*Ipomoea triloba*), and *Onagraceae* species (*Ludwigia peruviana*). Viral DNA was extracted from all samples as described by Kon *et al*. (2002).

PCR-Based Detection Using Geminivirus Specific Primers. Total DNA was extracted from symptomatic leaves according to Kon *et al.* (2002). The DNA pellet was resuspended in 100 µL TE buffer. Amplification of geminivirus genome was proceeded using a pair of specific primers designed for the amplification of coat protein gene, CP Protein-V1 (5' TAATTCTAGATGTCGAAGCGACCCGCCGA 3') and CP Protein-C1(5' GGCCGAATTTCTTAATTTT GAACAGAATCA 3'). These specific primers were

Table 1 List of geminiviruses used for viral sequence analysis

Geminivirus	Acronim	Geographic Location	Sequence Length (bp)	GenBank Accession No.
Ageratum conyzoides -Bogor	AgrBgr	Indonesia : Bogor, West Java	864	NS
Ageratum conyzoides -Sukabumi	AgrSkm	Indonesia : Sukabumi, West Java	993	NS
Ageratum conyzoides -Magelang	AgrMgl	Indonesia : Magelang, Central Java	868	NS
Ageratum conyzoides -Sleman	AgrJgy	Indonesia : Sleman, Jogyakarta	843	NS
Spilanthes iabadicencis- Magelang	SplMgl	Indonesia : Magelang, Central Java	890	NS
Centipeda minima- Magelang	CtpMgl	Indonesia : Magelang, Central Java	756	NS
Porophyllum ruderale -Bogor	PrlBgr	Indonesia : Bogor, West Java	832	NS
Bean yellow dwarf virus	BYDV	South Africa	2566	DQ458791
Tomato leaf curl Java virus	TLCJV	Indonesia : Java	2752	AB100304
Tomato leaf curl Java virus-[Ageratum]	TLCJV-[Ageratum]	Indonesia : Java	2747	AB162141
Tomato leaf curl Malaysia virus	TLCV	Malaysia	2754	AF327436
Tomato leaf curl Laos virus	TLCV	Laos	2748	AF195782
Ageratum yellow vein Taiwan virus	AYVV	Taiwan	2734	AF307861
Ageratum yellow vein China virus-[Hn2]	AYVV	China	2768	AJ495813
Chilli leaf curl virus-[Multan]	ChilCVA	Pakistan	2754	AF336806
Pepper leaf curl Bangladesh virus	PepLCV	Bangladesh	2753	AF314531
Pepper yellow leaf curl Indonesia virus	PepYLCV	Indonesia : West Java	1560	AB189849
Sida yellow vein Vietnam virus	SiYVVNV	Vietnam	2753	DQ641696
Mimosa yellow leaf curl virus	MiYLCV	Vietnam	2757	DQ641695
Malvastrum yellow vein virus-[Y47]	MYVV	China	2731	AJ457824
Malvastrum yellow vein Yunnan virus	MYVVNV	China	2747	AJ786711

NS, the sequence has not been submitted to GenBank

obtained from Asian Vegetable Research and Development Center (AVRDC), Taiwan. Amplification with PCR technique was carried out in a 25 µL reaction mixture containing 1 µL (200 ng) of sample DNA solution and 1 µL (0.2 µM) of each primer using Ready To Go™ PCR kit (Amersham Life Science). PCR was performed in thermalcycler Gen Amp PCR System9700 (Perkin Elmer) with 30 cycles of melting, annealing and DNA extension at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min, respectively. PCR products were then analysed by electrophoresis in 1% agarose gels in Tris-EDTA buffer (0.5x) and visualized under UV transilluminator (Maniatis *et al*. 1982).

Sequence analysis. DNA fragments of approximately 760 bp, as a product of PCR amplification, was sent to Macrogen Inc. (South Korea) for DNA sequencing by the dideoxy nucleotide chain termination method. Sequence data were then assembled and analyzed with the aid of Bioedit Programme and PAUP version 4.0 (Swofford 2002). *Geminivirus* sequences available from GenBank were used for phylogenetic analysisis (Table 1). The cladogram was set up with a quantitative cladistic maximum parsimony using heuristic methods. A hundred bootstrap iterations were performed.

RESULTS

Amplification and Sequencing of Coat Protein Gene. PCR using specific primers CP Protein-V1/CP Protein-C1 was successfully amplified a ~ 760 base pairs of coat protein fragments from 9 symptomatic samples (Fig 1) i.e. 5 samples *A. conyzoides* from Bogor (AgrBgr), Sukabumi (AgrSkm), Magelang (AgrMgl), Sleman (AgrJgy), and Garut (AgrGrt); *C. minima* from Magelang (CtpMgl); *A. boehmerioides* from Sleman (AcpJgy); *P. ruderale* from Bogor (PrlBgr); *S. iabadicensis* from Magelang (SplMgl). DNA fargments were not obtained from other weed samples (*G. parviflora* from Garut, *E. prostrata* from Brebes, *I. triloba* from Garut, and *L. peruviana* from Cianjur). Major constraint for PCR-based detection using weed samples occurred on viral DNA extraction. Field samples tend to easily damage thus required immediate processing. In addition, inhibitor and secondary metabolites components found in weed tissues may inhibit the amplification process using PCR.

Nucleotide sequence data was obtained from 7 virus samples (Table 1). Nucleotide's length of the virus that was successfully sequenced and used for sequence comparison was in the range of 756 to 993 bp, which contains parts of geminivirus coat protein gene (Santoso *et al*. 2008).

Analysis of Genetic Relationship. Coat protein fragment analysis showed that weed-infecting geminiviruses collected from this study can be differentiated into 2 groups (Fig 2). The first group consists of 6 weed-infecting geminiviruses from this study (AgrBgr, AgrSkm, AgrJgy, AgrMgl, SplMgl, and CtpMgl) with a 100 bootstrap value, and the second group consists of one weed-infecting geminivirus from this study (PrlBgr) and other geminiviruses previously reported in GenBank with a 92 bootstrap value.

Further more, the virus isolates in the first group can be differentiated into 4 subgroups each consisting of AgrBgr and AgrSkm, AgrJgy alone, AgrMgl and SplMgl, and CtpMgl alone. In the second group the virus isolate PrlBgr was placed in the different sub group with other weed-infecting geminiviruses. None of the weed infecting geminiviruses collected from this study has close relationship with geminiviruses previously reported from Java, Indonesia (TLCV, TLCV-[Ageratum], PepYLCV).

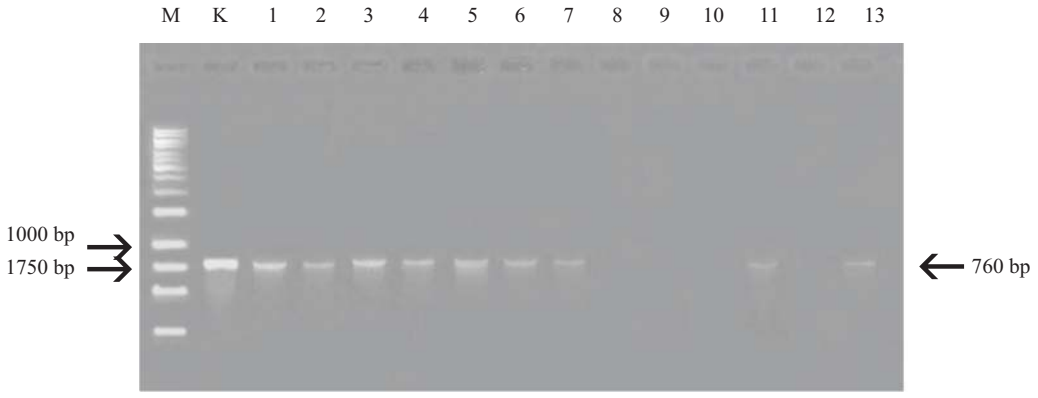

Fig 1 Amplification product of geminiviral DNA fragment using specific primers CP Protein-V1/CP Protein-C1. The samples consist of weed-infecting geminiviruses : K. Positive control (artificially inoculated geminivirus from *A. conyzoides*); 1. *A. conyzoides*-Bogor; 2. *A. conyzoides*-Sukabumi; 3. *A. conyzoides*-Magelang; 4. *A. conyzoides*-Sleman; 5. *A. conyzoides*-Garut; 6. *S. iabadicencis*-Magelang; 7. *C. minima*-Magelang; 8. *E. prostrata*-Brebes; 9. *G. parviflora*-Garut; 10. *I. triloba*-Garut; 11. *A. conyzoides*-Jogyakarta, 12; *L. peruviana*- Cianjur; 13. *P. ruderale*-Bogor; M. 1 kb DNA *ladder.*

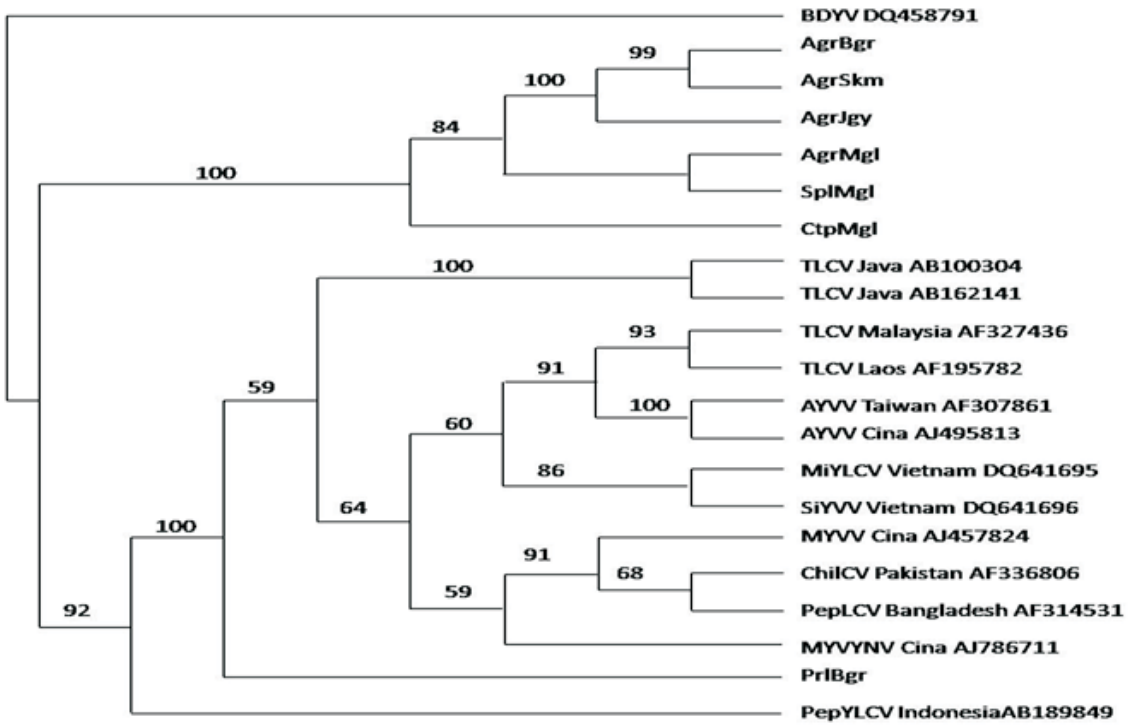

Fig 2 Cladogram showing the interrelationship among weed-infecting geminiviruses and relative geminivirus' species based on allignment of part of coat protein gene's nucleotide sequence. Boothstrap value (100 replication) are shown on the branch of cladogram. Bean Yellow Dwarf Virus (BYDV) is included as outgroup. AgrBgr = *A. conyzoides*-Bogor, AgrSkm = *A. conyzoides*-Sukabumi, AgrMgl = *A. conyzoides*=Magelang, AgrJgy= *A. conyzoides*-Sleman, SplMgl = *S. iabadicencis-Magelang, CtpMgl =C. minima-Magelang, PrlBgr = P. ruderale-Bogor,* BYDV = *Bean yellow dwarf virus* from South Africa (DQ458791), TLCV Java = T*omato leaf curl Java virus* from Java, Indonesia (AB100304), TLCV Java = *Tomato leaf curl Java virus*-[Ageratum] from Java, Indonesia (AB162141), TLCV Malaysia = T*omato leaf curl Malaysia virus* segment A from Malaysia (AF327436), TLCV Laos = *Tomato leaf curl Laos virus* from Malaysia (AF195782), AYVV Taiwan = *Ageratum yellow vein Taiwan virus* from Taiwan (AF307861), AYVV China = *Ageratum yellow vein China virus* from China (AJ495813), ChilCVA = *Chili leaf curl virus*-[Multan] from Pakistan (AF336806), PepLCV = *Pepper leaf curl Bangladesh virus* segment A from Bangladesh (PAF314531), PepYLCV = P*epper yellow leaf curl Indonesia* virus from West Java, Indonesia (AB189849), SiYVV = *Sida yellow vein virus* from Vietnam (SiYVV DQ641696), MiYLCV = *Mimosa yellow leaf curl virus* from Vietnam (DQ641695), MYVV = *Malvastrum yellow vein virus* from China (AJ457824), MYVYnV = *Malvastrum yellow vein Yunnan virus* from China (Aj786711).

DISCUSSIONS

Weeds are potential sources of primary inoculum of viruses and play an important role in their persistence and spread (Hallan *et al*. 1998). Weed infecting geminiviruses has been reported from different geographic location especially in the region where the geminivirus infection causing significant yield loss in important crops. Roye and McLaughlin (1997) reported a distinct geminivirus species from *Sida* spp., *Macroptilium lathyroides*, and *Wissadula amplissima* when they studied the role of weed species in the establishment of tomato and pepper diseases due to *Geminivirus* infection in Jamaica. Artificial inoculation using *Bemisia tabaci* involving several weed species was conducted by Sulandari *et al*. (2006) to determine the host range of pepper infecting geminivirus and concluded that *H. brevipes*, *P. floridana*, *C. juncea*, *A. conyzoides* were very susceptible to PepYLCV infection. Yellow vein symptom or leaf netting is commonly found associated with geminivirus infection on weed species. During field survey to chilli pepper growing area in 2009, we easily found *A. conyzoides* showing yellow vein symptom. Evidence of *Geminivirus* infection on *A. conyzoides* in Indonesia has been reported previously by Haerani and Hidayat (2003), Sukamto *et al*. (2005), and Kon *et al*. (2007). New *Geminivirus* infection on weed species in Indonesia was reported in this paper, i.e. *Geminivirus* infecting *C. minima, P. ruderale,* and *S. iabadicensis.*

Preliminary reports indicated that the primary *Geminiviruses* infecting weeds are not the same ones that infect crops (Gilbertson *et al*. 1991; Mc Laughlin *et al*. 1994), although it has been speculated that a number of common weeds may serve as alternate hosts for crop-infecting geminiviruses. Based on phylogenetic analysis, Roye and McLaughlin (1997) concluded that weed-infecting geminiviruses are not host to crop-infecting geminiviruses in Jamaica. Similarly, phylogenetic relationships of the weed-infecting viruses collected in this study with other geminiviruses indicate that crop- and weed-infecting geminiviruses from Java, Indonesia are distinct, and highly diverse. Despite all seven weed-infecting geminiviruses were collected from endemic area of pepper yellow leaf curl disease in Java, none of them has a close relationship with PepYLCV Indonesia. Based on our phylogenetic analysis, it is evident that *A. conyzoides*, *C. minima, P.*

ruderale, and *S. iabadicensis* are not reservoirs for geminiviruses important on chilli pepper and tomato in Java. However, our host range study showed that PepYLCV was able to infect *Ludwigia peruviana*, *A. conyzoides*, *S. iabadicensis*, *P. ruderale*, *Synedrella nodiflora*, and *Galinsoga parviflora* (data not published). Earlier, Kon *et al*. (2007) found evidence for interspecies recombination between *Tomato leaf curl Java virus* (ToLCJV) and a strain of *Ageratum yellow vein virus* (AYVV - [Java]). Therefore, the importance of weeds as alternative hosts for crop-infecting geminiviruses in Indonesia will need further investigation. These results may significantly affect the development of strategies for managing the spread of these geminiviruses.

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