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Identification and Molecular Diversity of *Rice Ragged Stunt Virus* and Rice Grassy Stunt Virus in Java, Indonesia

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Abstract

An outbreak of pest attack of the planthopper on rice crop in Indonesia nowadays has always been followed a virus disease that it transmits. This research aimed to identify the viruses that cause the disease transmitted by the brown planthopper (BPH) that attack rice crop in a number of regions, and to find out their molecular diversity. The research was preceded by a survey in a number of regions stricken with BPH in *DIY* (Special Region of Yogyakarta), Central Java, and West Java. Detection, identification, and characterization were carried out through the symptoms, vectorial transmission, and molecular detection by RT-PCR and sequencing. Results of the research showed that the diseases that attacked the rice crop in the planthopper-stricken regions were two kinds, i.e. *Rice ragged stunt virus* (RRSV) and *Rice grassy stunt virus* (RGSV). The sequence of nucleotide of all the RRSV from those regions showed a high percentage of similarity (more than 97%), while the sequence of nucleotide of RGSV isolates showed a similarity level of more than 95%. All the RRSV isolates from Java–Indonesia have the closest affinity with AF486811-Philippines, whereas the RGSV isolates have a closer affinity with GQ329710.1- Vietnam.

Keywords: identification; molecular diversity; RRSV; RGSV; Java–Indonesia.

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1. Introduction

As a result of today's climate change, a pest attack on rice crop is followed by virus attack on rice crop causing the resultant damage to redouble. The disease-causing viruses, which were originally not economically important viruses, become major viruses in rice, and some of them are newly emerging viruses in a region/country. Among the viruses in rice, *Rice ragged stunt virus* (RRSV) and *Rice grassy stunt virus* (RGSV) have lately become problems in several countries such as China, Vietnam, the Philippines, and Thailand [1, 2, 3, 4]. The two viruses are transmitted by the brown planthopper (BPH), *Nilaparvata lugens* Stal.. In China and Vietnam, there emerged a disease called *Southern rice black–streaked dwarf virus* (SRBSDV) transmitted by white back planthopper (WBPH) (*Sogatella furcifera*) [5]. SRBSDV has been in existence since 2001 and spreading rapidly from southern China to northern Vietnam, and become an important pathogen in rice crop. In 2009 this disease attacked 300.000 ha and 15.000 ha of rice crop in China and Vietnam respectively. The virus was naturally transmitted by WBPH and could also infect maize crop [6]. The BPH attack in 2010 can be considered an international outburst because all countries in South-East Asia, South Asia, and part of Central Asia were hit by BPH; The BPH attack rice crop had a direct effect by sucking the liquid of plant cells, which caused crop failure. An indirect effect of BPH attack was transmitting the virus, especially RRSV and RGSV; The BPH attack in several countries in Central Asia and South-east Asia was aggravated by an attack of the white back planthopper (WBPH), which spread the SRBSDV disease. The BPH attack in Indonesia occurred after the achievement of the P2BN program (Increased Production of National Rice) [7].

The damage and loss of yield resulting from the BPH attack were quite great. Besides its role as the main pest, the BPH transmitted several viruses to rice crop such as RRSV and RGSV [8]. From 2005 to 2010 RRSV and RGSV diseases were always found in Indonesia where the most serious attack in 2005 covered 1.588 ha, 550 ha of which led to crop failure, whereas the most serious attack of RRSV in 2010 hit an area of 6.094 ha, 20 ha of which caused crop failure [9]. In general, the visible symptoms of virus attack that followed planthopper pest in the past few years were stunted and yellowing crop. The result of identification by a serological test showed that the yellowing symptom in rice crop in Sukamandi–Subang was a complex of RRSV and RGSV diseases [10], whereas yellowing symptom in rice crop in Klaten (Central Java) there was a mixture of virus infection between Rice tungro spherical virus (RTSV), Rice tungro bacilliform virus (RTBV) transmitted by green leafhoppers (*Nephotettix virescens*) and Rice ragged stunt virus (RRSV) which emerged after a BPH attack since January 2010 [11].

Efforts to control virus disease transmitted by BPH have been oriented to the control of its vector. Control of RRSV in disease-ridden regions with insecticides only worked well if it was conducted during the time when planthopper population was scarce. It did not work during economic threshold or when there was a symptom of RRSV [12]. Vector control was done by using a resistant variety as one of the most effective ways in the BPH control program [13]. Identification of a disease-causing virus and characterization of its genetic diversity are essential for determining further control measures. The aims of this research was to identify the disease-causing viruses transmitted by BPH that attacked rice crop in several regions in Java, and to discover their molecular diversity.

2. Materials and Method

2.1. Survey and samples taking

The survey were done in the rice producing centers in the Special Region of Yogyakarta, Central Java, and West Java. The research was carried out in 2012 – 2014. The survey was conducted to obtain samples of diseased plants and information on the disease incidence and rice variety planted by farmers. The samples of diseased plants were then taken to the greenhouse to be reared, multiplied, and used for further tests. The leaf samples of diseased crop for RNA extraction were kept in a freezer at -80°C.

2.2. Detection and identification

The research was conducted in a greenhouse and the Virology Laboratory of the Faculty of Agriculture, Gadjah Mada University, Indonesia. Identification of symptom-based causes of disease was done by observing the visible symptoms on rice crop attacked by BPH and they were distinguished according to the criteria of the kinds of possible disease.

The rearing of BPH was done in an insect cage in the greenhouse. The adult BPH was obtained from Ambarketawang field (Gamping–Sleman). As many as twenty pairs of imagoes after the pre-oviposition period were put into a insect cage measuring 50 cm x 50 cm x 80 cm containing rice crop of Taichung Native 1 (TN1) cultivar aged 45 days after seedling (DAS) in pots as a source of food. The insect pairs were let to lay eggs for one week, then removed into another box for the next egg-laying. In this way, insects with a uniform age were obtained in one rearing cage [13].

The BPH was used as vector to transmit the virus diseases. Transmission of disease was done by artificial inoculation on TN1 cultivar using a test tube transmission method. Artificial inocculation was done by allowing instar 2 of BPH to acquire a virus in the inocculum of a diseased plant for 1 – 4 days; then the BPH was transferred to a healthy plant, after 7 – 10 days of the incubation period; the viruliverus BPH was allowed to inoculate the TN1 variety aged 7 – 10 days for 24 hours with a population density of three BPH/tube. Inocculation was done in a test tube with one plant/tube. Afterwards, the plant was grown in a pot and reared in an insect-free greenhouse.

Molecular detection of the sample of diseased plants from the field was done using a RT-PCR method. To determine the cause of disease, sequencing was done to determine the variance of the virus genetics. In order to detect the virus that causes yellowing syndrome on rice crop, an RNA extraction was done using isogen (Easy Blue) according to the manufacturer's instructions.

The total RNA extraction needs to be converted into cDNA through an RT PCR process using reagent Power cDNA Synthesis Kit from iNtRon Biotechnology. The RNA template was taken from the total result of the RNA extraction. In this research, reagent PCR in the form of Master Mix Royal (MMR) was used. The implementation of reaction was done according to the directions from the manufacturer.

Two pairs of primer were used in PCR, each for RRSV and RGSV. For RRSV a pair of primer was used, i.e.: RRSV F3: 5'-GACTAG GGATGTGCGTTC-3', RRSV B3: 5'-TGTAATCGACGTTCGCTC-3' with the amplification target of 218 bp (Segment 8 RRSV) [15], whereas for RGSV a primer was used i.e. RGSV NCP-F: 5'-CTATACACTACGCTAAAGGCT-3' and RGSV NCP-R: 5'-GTGTAAGATGGGT AAAGTGCA-3' with the amplication target of 1021 bp [16], but later was shortened to 450 bp by replacing the primer forward using RGSV NCP-F1: 5' GGCTTATGATAGTCTGTGATTTG-3' which was designed according to the sequence complete genome RGSV GQ329710.1 Isolate Longan–Vietnam. PCR was done by denaturation for three minutes at 95°C. The cycle of amplication through denaturation lasted one minute at 94°C, annealing for one minute at 53°C, and DNA synthesis (extension) for one minute at 72°C. The cycle was repeated 35 times continued with completion of synthesis for five minutes at 72°C. The PCR result was analyzed on gel agarose with electrophoresis. Gel coloring was done by soaking in ethidium bromide for five minutes. The DNA ribbon that formed in the gel agarose was observed under UV light using UV transluminator.

2.3. Molecular diversity

Sequencing was done to the sample of amplified PCR that was positively infected with virus. The sample was sent in the form of cDNA to PT Genetika Science Indonesia. Analysis of the sequenced DNA was compared with the sequenced DNA of another virus that had been put into the GenBank database using ClustalW with MEGA 6.0. software program. The result of homological searching for DNA and analysis of phylogenetic tree were used as a basis to determine the virus species and its affinity.

3. Results

3.1. The presence of virus disease in some BPH stricken regions

A survey and sample taking of diseased plants have been done in several rice production centers in Java in which BPH attack was endemic, particularly in Yogyakarta (Sleman and Kulon Progo), Central Java (Klaten, Sukoharjo, Magelang, and Banyumas) and West Java (Subang and Cirebon). The result of survey showed that attacks of planthoppers (BPH and WBPH) on the rice crop were followed by the emergence of the symptoms of virus diseases that they transmitted.

It was ascertained from the typical symptoms found in the field that there were two kinds of virus disease transmitted by BPH, namely RRSV and RGSV. The Virus disease was prevalent in high regions such as Gamping – Sleman (Yogyakarta) where 70% was found in IR64 variety, Banaran–Kulon Progo (Yogyakarta) where 45% was found in Situ Bagendit variety, Juwiring – Klaten (Central Java) where 70% was found in Pepe variety, Mungkid – Magelang (Central Java) where 75% was found in Ciherang variety, and Ciberes–Subang (West Java) where 75% was found in Inpari 10 variety and 90% in Ciherang variety (Table 1). There are not many rice varieties planted by farmers. The varieties that are commonly planted are IR64, Ciherang, Situ Bagendit, Inpari 13, Inpari 10, Cisedane, Mekongga, Pepe, Sidenok, and local varieties such as Menthik Wangi and Ketan.

Table 1: Rice Virus diseases and rice varieties that are commonly planted by farmers in some rice production centers in Java

Province	Location	Variety	Vector population/ clump	Disease Incidence
DI Yogyakarta	Gamping, Sleman	IR64	2-8 BPH	RRSV and RGSV 70%
		Menthik Wangi	3-5 BPH	RGSV <1%
		Situ Bagendit	3-5 BPH	RGSV <1%
		Cisedane	3-5 BPH	RGSV < 1%
	Banaran, Kulon Progo	Situ Bagendit	2-35 WBPH	like RRSV 45%
		Ciherang	5-33 WBPH	like RRSV 40%
	Kalibawang, Kulon Progo	Ciherang	1-20 WBPH	like RRSV 10%
Central Java	Bendosari, Sukoharjo	IR 64	1-8 BPH	RRSV <5%
	Juwiring, Klaten	Situ Bagendit	8-15 BPH	RRSV and RGSV 50%
		IR64	10-20 BPH	RRSV and RGSV 20%
		Ketan	20-30 BPH	RRSV and RGSV 25%
		Pepe	2-6 BPH	RRSV and RGSV 70%
	Mungkid, Magelang	Ciherang	1-5 BPH	RRSV and RGSV 75%
		Inpari 13	1-2 BPH	RRSV and RGSV 17%
	Purwokerto, Banyumas	Ciherang	1-5 BPH	RRSV 15%
			2-10 WBPH	
West Java	Pabuaran, Subang	Ciherang	0-1 BPH	RRSV and RGSV <1%
	Karanganyar, Subang	Ciherang	0-1 BPH	RRSV<1%
	Ciberes, Subang	Inpari 10	3-16 BPH	RRSV and RGSV 75%
		Ciherang	2-11 BPH	RRSV and RGSV 90%
		Mekongga	1-2 BPH	RRSV and RGSV <1%
		Sidenok	1-2 BPH	RRSV and RGSV <1%
	Cisaat, Cirebon	Ciherang	1-5 BPH	RRSV and RGSV 20%
			1-20 WBPH	

3.2. Virus detection

The result of field observation showed differences in the types of symptoms of yellowing syndrome in rice transmitted by BPH (Table 2). Upon close observation, there were two kinds of typical symptoms from the samples of diseased plants taken from the field, i.e. symptoms such as RRSV and RGSV.

Diseases with RRSV symptoms: stunted plants, dark green short leaves, with serrated leaf edge and sometimes ragged leaves, twisted at the end, the ragged part was yellowish and brownish yellow, sometimes the leaf or stem veins swelled, the leaf flag was twisted and short, the flowers or malai were impaired/came out partially from the leafstalk and usually empty (Figure 1). These symptoms were found in the samples of diseased crop from Bendosari–Sukoharjo, Juwiring–Klaten, Gamping–Sleman, Pabuaran–Subang, Karanganyar–Subang, Mungkid–Magelang, Cisaat–Cirebon, and Ciberes–Subang.

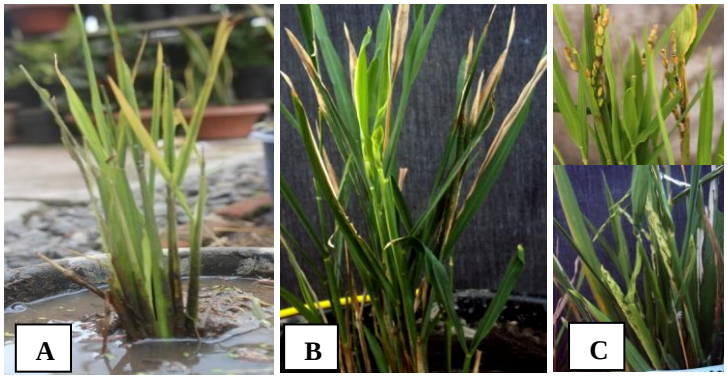

Figure 1: Rice crop with typical symptoms of RRSV disease; (A) Stunted, short leaves; (B) Dark green leaves and crop, with serrated edges and sometimes ragged leaves, twisted at end, the ragged leaves were yellowish and brownish yellow; (C) The leaf flag twisted and short, impaired flowers coming out partially from the leafstalk and empty.

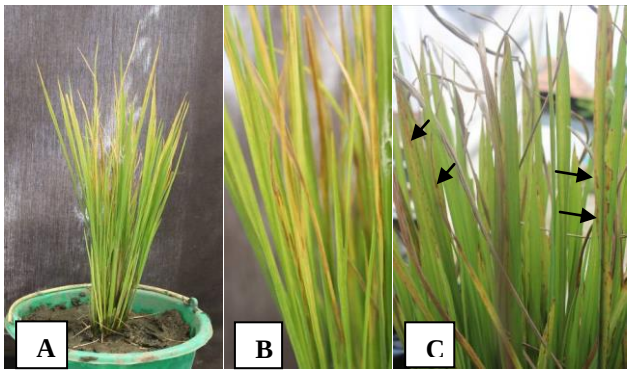

Figure 2: Rice crop with typical symptoms of RGSV; (A) Stunted with many tillers, erect, look like grass with many rosettes; (B) Narrow leaves, short, yellow; (C) Sometimes there are rust spots at leaf edge, and no panicle despite age.

Symptoms of RGSV were stunt with many shoots, with upright growth, like grass with the emergence of many rosettes, narrow leaves, short and yellow sometimes with spots of rust at end of leaf, no malai (Figure 2). The typical symptoms of RGSV were found in the samples from Juwiring–Klaten, Gamping–Sleman, Pabuaran–Subang, Mungkid–Magelang, Cisaat–Cirebon,and Ciberes–Subang.

The result of transmission from the samples with RRSV and RGSV symptoms in the field showed that not all the locations that were attacked by BPHs were positively infected with BPH transmitted virus.

There were eight samples of diseased crop from different locations that positively showed RRSV symptoms, i.e. samples from Gamping–Sleman, Bendosari–Sukoharjo, Juwiring–Klaten, Mungkid–Magelang, Pabuaran–Subang, Ciberes–Subang, and Cisaat–Cirebon. As for the RGSV symptoms that were transmissible, they were found in only five locations, i.e. Gamping–Sleman, Juwiring–Klaten, Mungkid–Magelang, Ciberes–Subang, and Cisaat–Cirebon (Table 2).

Table 2: Results of transmission from diseased plants with RRSV and RGSV symptoms from various locations using BPH vector

No.	Location	Results of transmission on TN1 with BPH	
		RRSV	RGSV
1.	Gamping-Sleman	+	+
2.	Banaran-Kulon Progo	-	-
3.	Kalibawang-Kulon Progo	-	-
4.	Bendosari-Sukoharjo	+	-
5.	Juwiring-Klaten	+	+
6.	Mungkid-Magelang	+	+
7.	Purwokerto-Banyumas	ns	ns
8.	Pabuaran-Subang	+	-
9.	Karanganyar-Subang	-	-
10.	Ciberes-Subang	+	+
11.	Cisaat-Cirebon	+	+

Note: + = transmissible, - = not transmissible, ns = not subjected to transmission

Results of RT-PCR showed that rice crop with symptoms that were transmissible on TN1 could be detected positive molecularly, which were shown by the presence of one constituent of DNA according to the target of amplification of each primer. The results of RT-PCR from the samples of plants with RRSV symptoms showed that the samples of diseased plants from Pabuaran–Subang, Mungkid–Magelang, Cisaat–Cirebon, Bendosari–Sukoharjo, Ciberes–Subang, Juwiring–Klaten, Gamping–Sleman, and Purwokerjo–Banyumas were detected positive with RRSV virus, which was indicated by the a constituent of DNA measuring 218 bp (Figure 3). The difference in the thickness of the constituent showed the difference of different titer of the virus. Based on the complete sequence in NCBI accession AF486811.1, the target of primer amplification was base sequence of nucleotide between 504-721 (217 bp) part of the gene of structural protein P8 (S8). Samples from other regions that were not detected positive in the molecular test with RT-PCR were not displayed. Results of PCR from the samples of plants with RGSV symptoms showed that the samples of diseased crop from Mungkid–Magelang, Cisaat–Cirebon, Ciberes–Subang, Juwiring–Klaten, and Gamping–Sleman were detected positive as RGSV, which was indicated by one constituent of DNA measuring ± 450 bp.

The samples from Pabuaran–Subang and Bendosari–Sukoharjo were not detected as RGSV (Figure 4). Based on the complete sequence in NCBI accession number AF290947.1, the target of primer amplification was base sequence of nucleotide between 2071-2521 (450 bp) part of the RNA5 segment and CP gene.

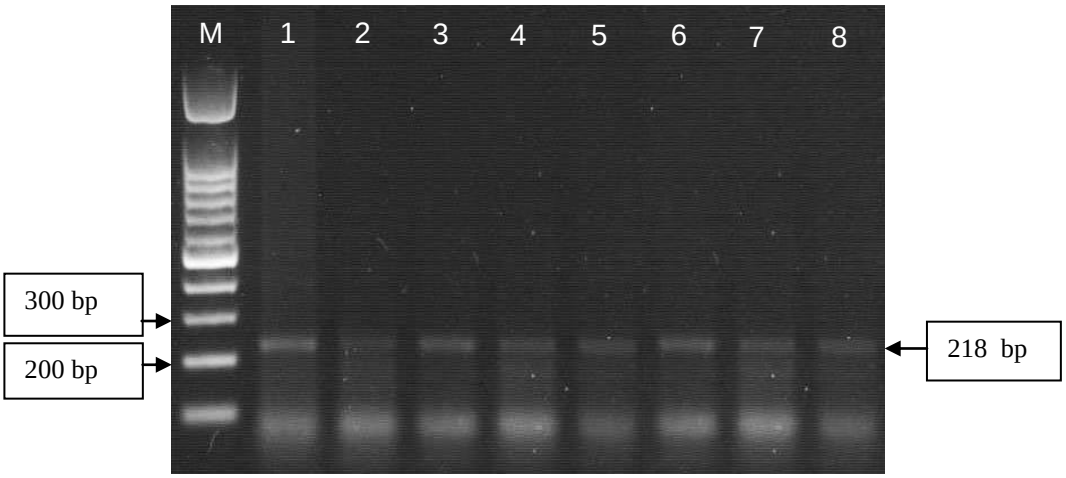

Figure 3: Electrophoresis of analysis with RT-PCR of RRSV isolates from several regions with BPH attack. M= Marker 100 bp DNA ladder; 1= Pabuaran–Subang; 2= Mungkid–Magelang; 3= Gamping–Sleman; 4= Cisaat–Cirebon; 5= Bendosari–Sukoharjo; 6= Ciberes–Subang; 7= Juwiring–Klaten; 8= Purwokerto–Banyumas

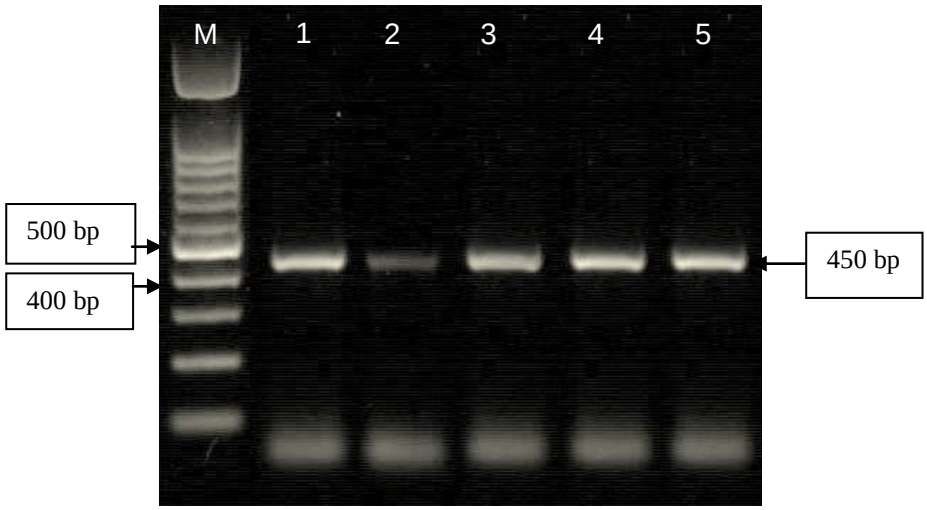

Figure 4: Electrophorosis of analysis with RT-PCR of RGSV isolates from several regions attacked by BPH. M= Marker 100 bp DNA ladder; 1= Mungkid–Magelang; 2= Gamping–Sleman; 3= Cisaat–Cirebon; 4= Ciberes–Subang; 5= Juwiring–Klaten

3.3. Molecular diversity of virus

Results of alignment of some DNA sequence of several samples showed that all isolates that were RRSV positive in the molecular test with RT-PCR, after sequencing and blas with NCBI, showed they were identical with the sequence order of RRSV nucleotide accession no.

AF486811 from the Philippines, accession no. HM125566-China and FR696625-Vietnam. The isolates in Java had a close affinity with a similarity percentage above 97%. The RRSV isolate from Mungkid – Magelang was even 100% identical with RRSV isolate from Bendosari – Sukoharjo. Almost all the isolates also had a high similarity level of more than 97%. In comparison with RRSV isolate accession no. HM125566-China and FR696615-Vietnam had a similarity percentage of 92.3 – 94.7% (Table 3).

Table 3: Similarity percentage of nucleotide sequence from some genes that encoded CP RRSV isolates from several regions in Java and several other RRSV isolates that have been published on the NCBI database

	Sequence	1	2	3	4	5	6	7	8	9	10
1	RRSV Gamping Sleman	ID									
2	RRSV Bendosari-Sukoharjo	98.2	ID								
3	RRSV Juwiring-Klaten	97.6	97.0	ID							
4	RRSV Mungkid-Magelang	98.2	100	97.0	ID						
5	RRSV Pabuaran-Subang	98.8	99.4	97.6	99.4	ID					
6	RRSV Ciberes-Subang	99.4	97.6	98.2	97.6	98.2	ID				
7	RRSV Cisaat-Cirebon	98.8	98.2	98.8	98.2	98.8	99.4	ID			
8	RRSV AF486811 Philippines	99.4	98.8	98.2	98.8	99.4	98.8	99.4	ID		
9	RRSV HM125566-China	93.5	92.9	93.5	92.9	93.5	94.1	94.7	94.1	ID	
10	RRSV FR696615-Vietnam	92.9	92.3	92.9	92.3	92.9	93.5	94.1	93.5	99.4	ID

The dendrogram of molecular affinity of RRSV isolates from several regions in Java and several other RRSV isolates that have been published in NCBI database showed that RRSV isolate from Mungkid–Magelang belongs to the same group as the isolate from Bendosari–Sukoharjo and is close to the degree of affinity with the isolate from Pabuaran–Subang. The isolate from Ciberes–Subang has the highest degree of affinity with the isolate from Gamping–Sleman, whereas the RRSV isolates from Cisaat–Cirebon and Juwiring–Klaten are rather separate from the other isolates. The isolates from Java generally have close affinity with AF486811 from the Philippines. RRSV accession no. HM125566-China has close affinity with FR696615-Vietnam and is in a separate group (Figure 5).

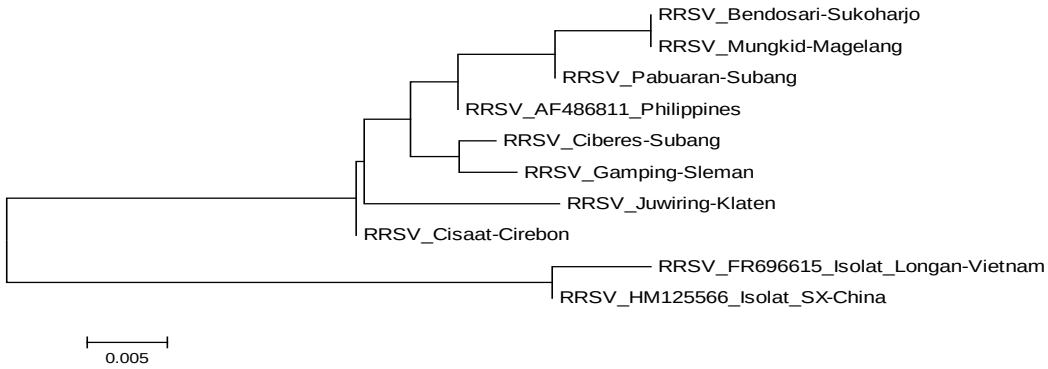

Figure 5: Dendrogram of molecular affinity of RRSV isolates from several regions in Java and several other isolates that have been published in NCBI database.

Results of alignment of some DNA sequence of RGSV from several isolates showed that all the isolates taken from several regions were identical with RGSV accession no. AF290947 China, AB000403.1 Japan, RGSV AB 023779.1 Philippines, and GQ329710.1 Vietnam. The RGSV isolates from Java had similarity percentage of nucleotide more than 95%. Compared with other isolates that have been published in NCBI, all the isolates had similarity percentage of nucleotide more than 93%. Even when compared with RGSV isolate GC329710.1 from Vietnam, isolates from Mungkid–Magelang, Gamping–Sleman, Cisaat–Cirebon, and Ciberes–Subang had similarity percentage of nucleotide more than 97% (Table 4).

Table 4: Similarity percentage of nucleotide base from some CP sequence of RGSV isolates from several regions in Java and several other RRSV isolates that have been published in NCBI database

	Sequence	1	2	3	4	5	6	7	8	9
1	RGSV Gamping-Sleman	ID								
2	RGSV Juwiring-Klaten	95.4	ID							
3	RGSV Mungkid-Magelang	99.3	96.1	ID						
4	RGSV Ciberes-Subang	97.4	98.0	97.4	ID					
5	RGSV Cisaat-Cirebon	98.3	96.1	98.3	97.4	ID				
6	RGSV GQ329710.1 Vietnam	99.0	95.4	99.0	97.4	98.7	ID			
7	RGSV AF290947 China	97.4	93.8	97.4	95.7	97.0	97.7	ID		
8	RGSV AB000403.1 Japan	97.7	94.1	97.7	96.1	97.4	98.0	99.0	ID	
9	RGSV AB023779.1 Philippines	98.0	94.4	98.0	96.4	97.7	98.3	99.3	99.6	ID

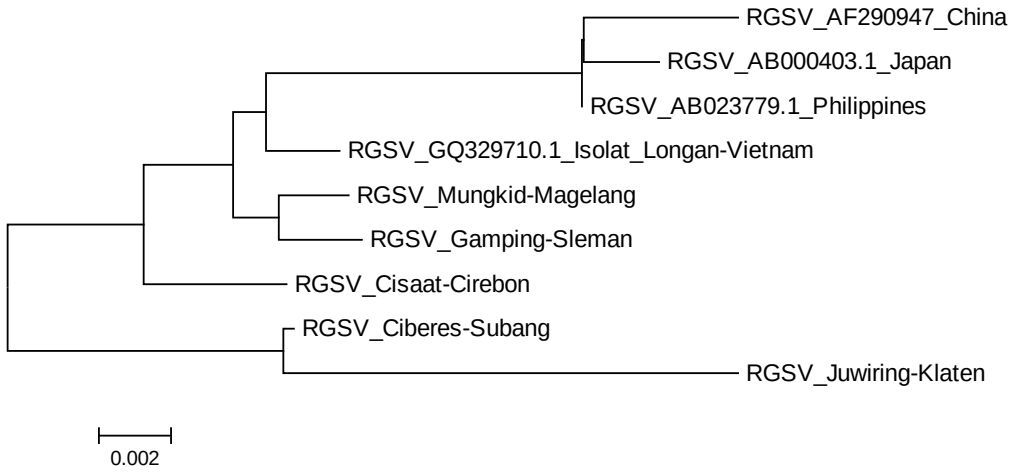

Figure 6: Dendrogram of molecular affinity of RGSV isolates from several regions in Java and several other RGSV isolates published in NCBI database

According to dendrogram of molecular affinity of RGSV isolates from several regions in Java and several other RGSV isolates published in NCBI database (Figure 6), the RGSV isolates from Mungkid–Magelang, Gamping–Sleman, and Cisaat–Cirebon can be classified into the same group as isolates from Ciberes–Subang and Juwiring–Klaten in another group.

The isolates published in NCBI showed that RGSV isolates with accession numbers AF290947 China, AB000403.1 Japan, and RGSV AB023779.1 Philippines were classified into the same group. RGSV GQ329710.1 Vietnam was closer to one group with Mungkid–Magelang, Gamping–Sleman, and Cisaat–Cirebon.

4. Discussion

In general, the symptom that indicates BPH attack is yellowing syndrome, which is a mixture of several virus diseases. The symptom of yellowing syndrome in the field in detail can be distinguished into two typical symptoms, each of which characterizes RRSV and RGSV diseases. The results of field survey in several regions with endemic diseases in Java showed that there was a high prevalence of disease in several varieties. The rice varieties that were commonly grown by farmers were Ciherang, IR64, and Situ Bagendit. Ciherang was the variety mostly grown by farmers and was found in almost all the survey locations. In this variety, the presence of virus diseases were always found following the BPH attack with the level of prevalence from low to high. In fact, the highest prevalence was found in Ciherang variety in Ciberes – Subang, reaching 90%.

There were two kinds of disease with typical symptoms identical with the typical symptoms reported previously by Hibino [17, 8] and typical symptoms identical with RGSV typical symptoms reported earlier by Ling [18]. The result of transmission test based on the typical symptoms of the two kinds of disease (RRSV and RGSV) showed that not all the samples of diseased plants from all locations attacked by BPH and which showed yellowing symptoms resembling RRSV or RGSV were positively transmissible to healthy plants. This was because the symptoms were not caused by one of the two kinds of virus. A number of samples of diseased plants that were not transmissible to healthy plants was found in the samples from Banaran–Kulon Progo, Kalibawang–Kulon Progo, and Karanganyar–Subang. Specifically for locations in Banaran–Kulon Progo and Kalibawang–Kulon Progo, it was assumed that the symptoms were not indicative of disease transmitted by BPH because the two locations were regions attacked by WBPH and BPH was not found.

Results of virus disease detection based on symptom identification, transmission test, and molecular test with RT-PCR showed that RRSV disease was detected in diseased plants from Gamping-Sleman, Bendosari–Sukoharjo, Juwiring–Klaten, Mungkid–Magelang, Purwokerto–Banyumas, Pabuaran–Subang, Ciberes–Subang, and Cisaat–Cirebon. The result of detection and identification also showed that RGSV disease was found in diseased plants from Gamping–Sleman, Juwiring–Klaten, Mungkid–Magelang, Ciberes–Subang, and Cisaat–Cirebon. This showed that the two kinds of disease transmitted by BPH did not always exist together, but the existence of RRSV was more dominant after BPH attack. In general, the result of alignment of some sequence of RRSV DNA, all the isolates from Java had close affinity, and likewise the result of alignment for RGSV sequence. The molecular diversity of RRSV and RGSV from several regions in Java did not definitely correlate with difference in geographical location. The molecular affinity of RRSV and RGSV isolates from Java with some isolates published in NCBI database showed that RRSV isolates from Java could be classified into one group with isolates from the Philippines, whereas the RGSV isolates from Java had the closest affinity and could be classified into one group with isolates from Vietnam.

5. Conclusion

Based on the survey results and the samples of diseases as well as detection and identification, it is found that there are two kinds of disease that strike rice plants in several regions in Java where there are BPH attack, namely *Rice ragged stunt virus*, found in Gamping–Sleman, Bendosari–Sukoharjo, Juwiring–Klaten, Mungkid–Magelang, Purwokerto–Banyumas, Pabuaran–Subang, Ciberes-Subang, and Cisaat–Cirebon; and *Rice grassy stunt virus*, found in Gamping–Sleman, Juwiring–Klaten, Mungkid–Magelang, Ciberes–Subang, and Cisaat–Cirebon.

The sequence of nucleotide of all RRSV isolates in several regions in Java shows that a high percentage of similarity (more than 97%), whereas the sequence of nucleotide of RGSV isolates show a similarity level of more than 95%. The molecular diversity found in RRSV and RGSV isolates does not definitely correlate with difference in geographical position. All RRSV isolates from Java have the closest affinity with isolate AF486811 from the Philippines, whereas RGSV isolates from Java have the closest affinity with RGSV isolate GC329710.1 from Vietnam.

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