



Molecular Diversity of Rice Ragged Stunt *Oryzavirus* in Java and Bali, Indonesia

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Abstract: Rice ragged stunt virus (RRSV), a member of the Genus *Oryzavirus* in the Family *Reoviridae*, is one of the most important causal agent of rice viral diseases in Asia. This virus is transmitted by brown planthopper (*Nilaparvata lugens*) in a persistent manner. It was first discovered in 1967 in Pandeglang, Indonesia that RRSV infects another plant in Gramineae family. The disease incidences were rarely reported in high damage until major outbreak were observed during 2010 to 2011 in several rice growing regions. The purpose of this research was to provide scientific information of RRSV distribution and molecular diversity in Indonesia. About 16 samples with yellowing, ragged and stunting symptoms were collected from Special Region of Yogyakarta, East Java, West Java, Central Java and Bali. PCR assay was used to detect RRSV in samples using specific primer for Coat Protein (CP) gene. The results revealed that 14 out of 16 samples positively reacted with RRSV primers. The positive samples from D.I. of Yogyakarta, Central Java, and Bali were then sequenced, and the results exhibited that all samples from Indonesia had 99% similarity and were closely related to the isolate RRSV from Chanting, China (i.e., Acc.No.HM125546.1) based on the nucleotide and amino acid sequences of CP gene (segment 8).

Keywords: Brown planthopper, CP gene, RRSV, RT-PCR

1. INTRODUCTION

Rice is the most widely consumed staple food for more than a half of world's populations, mostly in Asian countries including Indonesia. In 2015 the average amount of national rice consumption per capita was 114 kg yr⁻¹ with 75.36 × 10⁶ t production [1]. Brown planthopper [*Nilaparvata lugens* (Stål, 1854)] is the most destructive global pest in rice and lately has become a problem in several rice growing countries in the world, such as China, Vietnam, Thailand, India, Pakistan, Malaysia, Philippines, Japan, and also Korea [2]. Brown planthopper (BPH) also acts as a vector for two viruses Rice ragged stunt virus (RRSV) and Rice grassy stunt virus (RGSV) [3] and can caused significant crop losses [4].

RRSV is a member of the family Reoviridae and species of the genus *Oryzavirus*. The virus is transmitted by BPH in a persistent manner, propagative in the vectors but not transmitted through eggs [4]. The virions are icosahedral, about 70 nm in diameter, with a polyhedral core about 50 nm in diameter. RRSV genome consists of ten segments of double-stranded RNA ranging from 1.2 kb to 3.9 kb [5] that encodes at least eight structural proteins (P1, P2, P3, P4A, P5, P8A, P8B, and P9) and three nonstructural proteins (Pns6, Pns7, and Pns10) [6].

RRSV was first discovered in Pandeglang, East Java, Indonesia in 1976, and the disease has become widespread in many countries where rice is grown. Major outbreaks of RRSV incidences

had not been reported in order countries until high levels of RRSV were observed in Vietnam in 2006 [7]. In Indonesia, RRSV had not been reported until outbreaks incidence of BPH in 2005, allegedly due to climate change effect that led to the emergence of new biotypes of BPH [8]. The incidences of RRSV and RGSV diseases increased and were always found since 2005 to 2010 in several BPH endemic regions in Indonesia with the most serious damage in 2010 with covered area up to 6.094 ha, in which 20 ha, leading to crop failure [9]. In 2010, Cabunagan and Choi reported that many plants collected in West Java were mixed infected with RRSV and RGSV, and many plants from Klaten, Central Java region suffered from a mixed infection with RRSV and tungro (RTSV & RTBV) [10].

Increasing RRSV individual attack and its mixed infection with RGSV and another rice viruses such as *Rice tungro spherical virus* (RTSV) & *Rice tungro bacilliform virus* (RTBV), need a serious attention in pest and disease control, especially from possibility of RRSV new strain emergence. Mixed-infection between all of these rice viruses can cause more severe damage and greater yield losses [11]. The molecular characterization is necessary in order to understand molecular diversity of RRSV in several epidemic regions in Indonesia. Previous

study showed that molecular diversity of RRSV in Java (Yogyakarta, Central and West Java) did not definitely correlate with difference in geographical location [9]. So this study will use different samples and primers with wider range of locations.

2. MATERIALS AND METHODS

2.1 Virus Source and Field Samples

Rice leaves were collected from several BPH endemic regions in Indonesia (Special Region of Yogyakarta, West Java, East Java & Central Java) since 2013 to 2015 (Table 1). Leaves sample from Bali belonged to Plant Virology Laboratory of Agriculture Faculty, Universitas Gadjah Mada, Indonesia. About 53 samples were collected, and fourteen was selected as representative of each location in Java. Sample from Padang, Sumatera Barat also added to determine the possibility of RRSV spread to Sumatera Island. Fourteen symptomatic rice leaves from plants suspected to be infected with RRSV were collected. RT-PCR was performed to determine the RRSV infection.

2.2 Detection and Identification

Detection and identification of RRSV were

Table 1. RT-PCR result from selected samples from several locations.

Region	Samples	Code	RRSV
Special Region of Yogyakarta	Gamping	G	+
	Imogiri	Mg	+
	Moyudan	AY	+
Central Java	Klaten	J	+
	Klaten	RK	+
	Boyolali	S1H	+
	Boyolali	S1R	+
	Garut	BK	+
West Java	Garut	Lg	+
	Subang	Sb	+
	Cirebon	Cb	+
	East Java	Ngawi	Ng
Bali	Tabanan	Tbn	+
	Tapaksiring	Tps	-
	Badung	Bdg	+
Sumatera	Padang	Pd	-

conducted in Plant Virology Laboratory of the Faculty of Agriculture, Universitas Gadjah Mada, Indonesia. Total RNA was extracted using GeneAid Plant Virus RNA Extraction Kit (Gene Aid Biotech Ltd., Taiwan). RT-PCR was performed to convert RNA into DNA complementary (cDNA), using RevertAid First Strand cDNA Synthesis (Thermo Scientific, Massachusetts, USA). OligodT was used as a primer of RRSV cDNA Synthesis.

One pair of primers was used as the PCR, based on genomic sequence of RRSV CP gene from segment 8 in GeneBank. The forward primer (5'-ACC GTC GTT GAG CTA CCA TCC ATT-3'), corresponding to position 890 nt to 913 nt and the reverse primer (5'-GGC GGG CCA CTC AAA CCA T-3'), corresponds to position 1366 nt to 1384 nt amplified a conserved segment with a single product of approximately 494 bp [12]. cDNA was immersed in a total of 25 μ L of PCR mix Kapa Extra Hotstart (KapaBiosystem Inc., Massachusetts, USA). The PCR program were performed as follow: pre-heating at 95 °C for 3 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 1 min. The PCR product was analyzed using 1.5 % agarose gel in electrophoresis at 100V for 30 min.

2.3 Sequencing and Molecular Diversity

The PCR product of RRSV-AY, RRSV-RK & RRSV-GB was sequenced by 1st Base Sequencing Int. (Selangor, Malaysia). BLAST was performed to find similarity of sequenced DNA and amino acid with reference data base in GeneBank NCBI.

2.4 Sequence Comparison and Phylogeny

Sequenced DNA and amino acid that found from BLAST was then used for Multiple Sequence Alignment using Clustal W program to get a conserved align of the sequences. Alignment result was used to performed Phylogenetic Tree using MEGA 6.0. software programe. The evolutionary distances were computed using Neighbor-Joining methods with the Bootstrap test (1000 replicates).

3. RESULTS

3.1 Detection and Identification

cDNA from RRSV infected plants was separated by 1.5 % agarose gel electrophoresis. Based on RT-PCR test, it is known that 14 out of 16 samples were positively reacted with RRSV CP gene primers (Fig. 1A and 1B) as was indicated by the appearance of

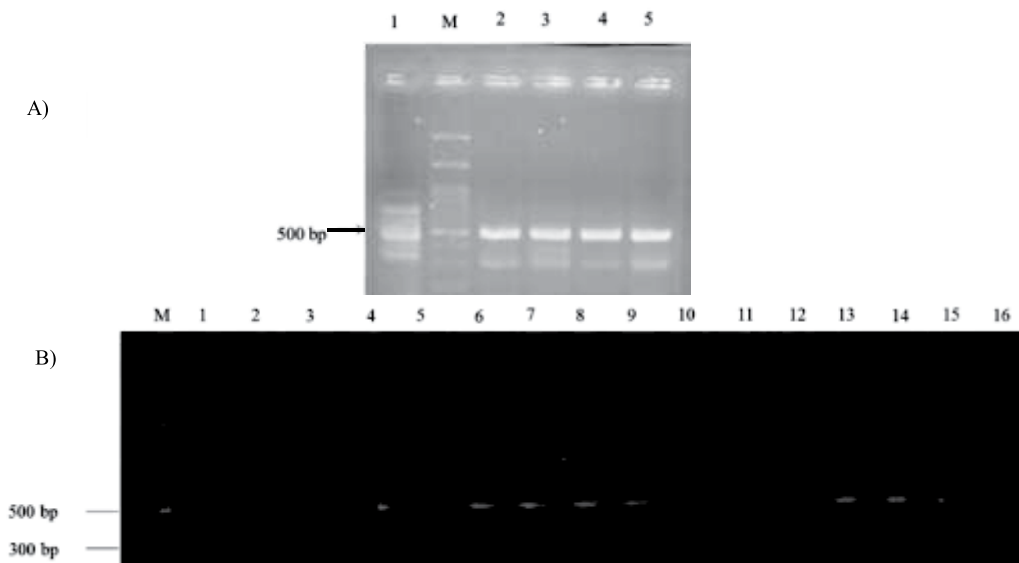


Fig. 1. Electrophoresis analysis of RT-PCR for RRSV CP gene in symptomatic rice leaves using 1.5 % agarose gel. PCR product A) M: 1 kB DNA marker, 1–5: Tbn, GB, AY, RK, S1H. B) M: 1kB DNA Marker. 1–16: Pd, Tps, Tbn, GB, AY, G, Mg, RK, J, S1H, Ng, Sb, BK, Lg, Cb, Positive control.

CLUSTAL O(1.2.4) multiple sequence alignment

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Fujian_RRSV      TACCGTTGTTGAATTACCATCCATTCGCCAGAGGA---CTCGTCAATTGAAGTGGCGA
RRSV_Yogya      TACCGTCTTGAGCTACCATCCATTCGCCAGAGGTAATCTCGTCAATTGAAGTGGCGA
RRSV_Bali       TACCGTCTTGAGS TACCATCCATTCGCCAGAGGA---CTCGTCAATTGAAGTGGCGA
RRSV_KLATEN     TACCGTCTTGAGCTACCATCCATTCGCCAGAGGA---CTCGTCAATTGAAGTGGCGA
Guandong_RRSV  TACCGTCTTGAGCTACCATCCATTCGCCAGAGGA---CTCGTCAATTGAAGTGGCGA
Chanting_RRSV  TACCGTCTTGAGCTACCATCCATTCGCCAGAGGA---CTCGTCAATTGAAGTGGCGA
Thailand_RRSV   TACCGTCTTGAGCTACCATCCATTCGCCAGAGGA---CTCGTCAATTGAAGTGGCGA
Philippines_RRSV TACCGTCTTGAGCTACCATCCATTCGCCAGAGGA---CTCGTCAATTGAAGTGGCGA
*****

Fujian_RRSV      CGCCGTCGCATGAGATGTTCTTTGATATCAACACCATGATATATACATAATGTGCCGTG
RRSV_Yogya      CGCCGTCGCACGAGACGTTCTTTGATATCAACACCATGATATACATATAATGTGHTGTG
RRSV_Bali       CGCCGTCGCACGAGACGTTCTTTGATATCAACACCATGATATACATATAATGTGHTGTG
RRSV_KLATEN     CGCCGTCGCACGAGACGTTCTTTGATATCAACACCATGATATACATATAATGTGCTGTG
Guandong_RRSV  CGCCGTCGCACGAGACGTTCTTTGATATCAACACCATGATATACATATAATGTGCTGTG
Chanting_RRSV  CGCCGTCGCACGAGACGTTCTTTGATATCAACACCATGATATACATATAATGTGCTGTG
Thailand_RRSV   CGCCGTCGCACGAGACGTTCTTTGATATCAACACCATGATATACATATAATGTGCTGTG
Philippines_RRSV CGCCGTCGCACGAGACGTTCTTTGATATCAACACCATGATATACATATAATGTGCTGTG
*****

Fujian_RRSV      GAAGCATCACTAATCCTATGATCCAGCGCTAAACGGTATCGTACCAGATACAACACGA
RRSV_Yogya      GAAGCATCACTAATCCTATGATCCAGCGCTAAACGGTATCGTACCAGATACAACACGA
RRSV_Bali       GAAGCATCACTAATCCTATGATCCAGCGCTAAACGGTATCGTACCAGATACAACACGA
RRSV_KLATEN     GAAGCATCACTAATCCTATGATCCAGCGCTAAACGGTATCGTACCAGATACAACACGA
Guandong_RRSV  GAAGCATCACTAATCCTATGATCCAGCGCTAAACGGTATCGTACCAGATACAACACGA
Chanting_RRSV  GAAGCATCACTAATCCTATGATCCAGCGCTAAACGGTATCGTACCAGATACAACACGA
Thailand_RRSV   GAAGCATCACTAATCCTATGATCCAGCGCTAAACGGTATCGTACCAGATACAACACGA
Philippines_RRSV GAAGCATCACTAATCCTATGATCCAGCGCTAAACGGTATCGTACCAGATACAACACGA
*****

Fujian_RRSV      CCAATTACGTGGTAAAGTTATCCTGATACTGACGATGGAAGGAAGAAGGCATTGGCTGAGC
RRSV_Yogya      CCAATTACGTGGTAAAGTTATCCTGATACTGACGATGGAAGGAAGAAGGCATTGGCTGAGC
RRSV_Bali       CCAATTACGTGGTAAAGTTATCCTGATACTGACGATGGAAGGAAGAAGGCATTGGCTGAGC
RRSV_KLATEN     CCAATTACGTGGTAAAGTTATCCTGATACTGACGATGGAAGGAAGAAGGCATTGGCTGAGC
Guandong_RRSV  CCAATTACGTGGTAAAGTTATCCTGATACTGACGATGGAAGGAAGAAGGCATTGGCTGAGC
Chanting_RRSV  CCAATTACGTGGTAAAGTTATCCTGATACTGACGATGGAAGGAAGAAGGCATTGGCTGAGC
Thailand_RRSV   CCAATTACGTGGTAAAGTTATCCTGATACTGACGATGGAAGGAAGAAGGCATTGGCTGAGC
Philippines_RRSV CCAATTACGTGGTAAAGTTATCCTGATACTGACGATGGAAGGAAGAAGGCATTGGCTGAGC
*****

Fujian_RRSV      GTGCGGTTATTACTGTGGACGGTAAGTATTATAAGTGTATAATGATATCAAGGCTGACA
RRSV_Yogya      GTGCGGTTATCACTGTGGACGGTAAGTACTATAAGTGTACAACGATATCAAGGCTGACA
RRSV_Bali       GTGCGGTTATCACTGTGGACGGTAAGTACTATAAGTGTACAACGATATCAAGGCTGACA
RRSV_KLATEN     GTGCGGTTATCACTGTGGACGGTAAGTACTATAAGTGTACAACGATATCAAGGCTGACA
Guandong_RRSV  GTGCGGTTATCACTGTGGACGGTAAGTACTATAAGTGTACAACGATATCAAGGCTGACA
Chanting_RRSV  GTGCGGTTATCACTGTGGACGGTAAGTACTATAAGTGTACAACGATATCAAGGCTGACA
Thailand_RRSV   GTGCGGTTATCACTGTGGACGGTAAGTACTATAAGTGTACAACGATATCAAGGCTGACA
Philippines_RRSV GTGCGGTTATCACTGTGGACGGTAAGTACTATAAGTGTACAACGATATCAAGGCTGACA
*****

Fujian_RRSV      CAGATAAGCGAAGAATTCTCAACCCAGCTGTATCAAAAGAGGTTATGATTAGTCTGCGTC
RRSV_Yogya      CAGATAAGCGAAGAATTCTCAACCCAGCTGTATCAAAAGAGGTYATGATTAGTCTGCGTC
RRSV_Bali       CAGATAAGCGAAGAATTCTCAACCCAGCTGTATCAAAAGAGGTCATGATTAGTCTGCGTC
RRSV_KLATEN     CAGATAAGCGAAGAATTCTCAACCCAGCTGTATCAAAAGAGGTCATGATTAGTCTGCGTC
Guandong_RRSV  CAGATAAGCGAAGAATTCTCAACCCAGCTGTATCAAAAGAGGTTATGATTAGTCTGCGTC
Chanting_RRSV  CAGATAAGCGAAGAATTCTCAACCCAGCTGTATCAAAAGAGGTTATGATTAGTCTGCGTC
Thailand_RRSV   CAGATAAGCGAAGAATTCTCAACCCAGCTGTATCAAAAGAGGTTATGATTAGTCTGCGTC
Philippines_RRSV CAGATAAGCGAAGAATTCTCAACCCAGCTGTATCAAAAGAGGTTATGATTAGTCTGCGTC
*****

Fujian_RRSV      ATTATTGGTGTAGTGCATTCATACAGAGAGGATGGAAGCACTCATATTAGTCAAG
RRSV_Yogya      ACTATTGGTGTAGTGCATTCATACAGAGAGGATGGAAGCACTCATATTAGTCAAG
RRSV_Bali       ACTATTGGTGTAGTGCATTCATACAGAGAGGATGGAAGCACTCATATTAGTCAAG
RRSV_KLATEN     ACTATTGGTGTAGTGCATTCATACAGAGAGGATGGAAGCACTCATATTAGTCAAG
Guandong_RRSV  ACTATTGGTGTAGTGCATTCATACAGAGAGGATGGAAGCACTCATATTAGTCAAG
Chanting_RRSV  ACTATTGGTGTAGTGCATTCATACAGAGAGGATGGAAGCACTCATATTAGTCAAG
Thailand_RRSV   ACTATTGGTGTAGTGCATTCATACAGAGAGGATGGAAGCACTCATATTAGTCAAG
Philippines_RRSV ACTATTGGTGTAGTGCATTCATACAGAGAGGATGGAAGCACTCATATTAGTCAAG
*****

Fujian_RRSV      TGTTCGCTTCTTATGGGAATATGCTACGGTGGTTAGATACCAAGAAGATCAGGTGTC
RRSV_Yogya      TGTTTTGTCTTCTGATGGGAATATGCTATGGTGGCTTAGATWYCARSAWATCAGGTGTC
RRSV_Bali       TGTXTTGTCTTCTGATGGGAATATGCTATGGTGGCTTAGATWYCARSAWATCAGGTGTC
RRSV_KLATEN     TGTTTTGTCTTCTGATGGGAATATGCTATGGTGGCTTAGATACHRAGAAGATCAGGTGTC
Guandong_RRSV  TGTTTTGTCTTCTGATGGGAATATGCTATGGTGGCTTAGATACCAAGAAGATCAGGTGTC
Chanting_RRSV  TGTTTTGTCTTCTGATGGGAATATGCTATGGTGGCTTAGATACCAAGAAGATCAGGTGTC
Thailand_RRSV   TGTTTTGTCTTCTGATGGGAATATGCTATGGTGGCTTAGATACCAAGAAGATCAGGTGTC
Philippines_RRSV TGTTTTGTCTTCTGATGGGAATATGCTATGGTGGCTTAGATACCAAGAAGATCAGGTGTC
*****

Fujian_RRSV      GATGGTTGAGTGGCCCGCTG
RRSV_Yogya      GATGGTTGAGTGGCCCGCA-
RRSV_Bali       GATGGTTGAGTGGCCCGCA-
RRSV_KLATEN     GATGGTTGAGTGGCCCGCA-
Guandong_RRSV  GATGGTTGAGTGGCCCGCTG
Chanting_RRSV  GATGGTTGAGTGGCCCGCTG
Thailand_RRSV   GATGGTTGAGTGGCCCGCTG
Philippines_RRSV GATGGTTGAGTGGCCCGCTG
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Fig. 3. Nucleotide Multiple sequence alignment of the sample isolates from Java and Bali compared with the RRSV isolate from GeneBank.

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CLUSTAL O(1.2.4) multiple sequence alignment

RRSV_GB          TWVLPSPDPEDSSIEVATPSHETFFDINTMIYIIMCCGSITNPMIQLRNGIVTRYNTTN
RRSV_RK          TWVLPSPDPEDSSIEVATPSHETFFDINTMIYIIMCCGSITNPMIQLRNGIVTRYNTTN
RRSV_AY          TWVLPSPDPEDSSIEVATPSHETFFDINTMIYIIMCCGSITNPMIQLRNGIVTRYNTTN
RRSV_Chanting_China TWVLPSPDPEDSSIEVATPSHETFFDINTMIYIIMCCGSITNPMIQLRNGIVTRYNTTN
RRSV_Philippines TWVLPSPDPEDSSIEVATPSHETFFDINTMIYIIMCCGSITNPMIQLRNGIVTRYNTTN
RRSV_Thailand    TWVLPSPDPEDSSIEVATPSHETFFDINTMIYIIMCCGSITNPMIQLRNGIVTRYNTTN
RRSV_Thailand_Protease TWVLPSPDPEDSSIEVATPSHETFFDINTMIYIIMCCGSITNPMIQLRNGIVTRYNTTN
RRSV_Xinyi_Guandong_China TWVLPSPDPEDSSIEVATPSHETFFDINTMIYIIMCCGSITNPMIQLRNGIVTRYNTTN
*****

RRSV_GB          YVVSYPDTDDGRKKALAEARAVITVDGKYYKCYNDIKADTKRRILNPAVIKEVMISLRHY
RRSV_RK          YVVSYPDTDDGRKKALAEARAVITVDGKYYKCYNDIKADTKRRILNPAVIKEVMISLRHY
RRSV_AY          YVVSYPDTDDGRKKALAEARAVITVDGKYYKCYNDIKADTKRRILNPAVIKEVMISLRHY
RRSV_Chanting_China YVVSYPDTDDGRKKALAEARAVITVDGKYYKCYNDIKADTKRRILNPAVIKEVMISLRHY
RRSV_Philippines YVVSYPDTDDGRKKALAEARAVITVDGKYYKCYNDIKADTKRRILNPAVIKEVMISLRHY
RRSV_Thailand    YVVSYPDTDDGRKKALAEARAVITVDGKYYKCYNDIKADTKRRILNPAVIKEVMISLRHY
RRSV_Thailand_Protease YVVSYPDTDDGRKKALAEARAVITVDGKYYKCYNDIKADTKRRILNPAVIKEVMISLRHY
RRSV_Xinyi_Guandong_China YVVSYPDTDDGRKKALAEARAVITVDGKYYKCYNDIKADTKRRILNPAVIKEVMISLRHY
*****

RRSV_GB          CGSVIHYRERMEATHISQVFCLLMGICYGGLDTKKIRCRWFEPWA
RRSV_RK          CGSVIHYRERMEATHISQVFCLLMGICYGGLDTKKIRCRWFEPWA
RRSV_AY          CGSVIHYRERMEATHISQVFCLLMGICYGGLDTKKIRCRWFEPWA
RRSV_Chanting_China CGSVIHYRERMEATHISQVFCLLMGICYGGLDTKKIRCRWFEPWA
RRSV_Philippines CGSVIHYRERMEATHISQVFCLLMGICYGGLDTKKIRCRWFEPWA
RRSV_Thailand    CGSVIHYRERMEATHISQVFCLLMGICYGGLDTKKIRCRWFEPWA
RRSV_Thailand_Protease CGSVIHYRERMEATHISQVFCLLMGICYGGLDTKKIRCRWFEPWA
RRSV_Xinyi_Guandong_China CGSVIHYRERMEATHISQVFCLLMGICYGGLDTKKIRCRWFEPWA
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Fig. 4. Amino acid multiple sequence alignment of sample isolates from Java and Bali compared with the RRSV isolate from GeneBank.

3.2.2 Analysis of Amino Acid Sequence

Amino acid sequence after BLAST with NCBI GeneBank database showed that RRSV from Indonesia were highly identical (similarity percentage more than 99 %) with isolates from Thailand (Acc. No. NP_955625.1). With lower similarity, there are Philippine isolate (Acc. No. AAL96663), Thailand isolate (Acc. No. NP_620528), Chanting-China isolate (Acc. No. AEC32888), Xinyi China isolate (Acc. No. AEC32898) and isolate which had the lowest similarity (between 98 % to 99 %) with Shaxian China (Acc. No. AEC32908) (Table 2).

Comparison of multiple sequence alignment analysis of test samples from Indonesia (AY, RK & GB) with RRSV isolate from Thailand, Chanting-China, Xinyi-China, Shaxian-China & Philippines showed that the difference found in nucleotide alignment, did not give any effect for translation because there is a high similarity between amino acid sequence of test samples and RRSV from GeneBank database (Fig. 3, Fig. 4).

3.3. Phylogeny

Neighbor-Joining (NJ) method was used to

construct phylogenetic trees using nucleotide and amino acid sequences of RRSV from test samples and RRSV isolate from GeneBank. The dendrogram of molecular affinity of RRSV based on nucleotide sequence showed that RRSV isolate from Bali (RRSV-GB) belongs to the same group with isolate from Klaten (RRSV-RK) and Special Region of Yogyakarta (RRSV-AY). Isolate from Java and Bali also belongs to the same group with isolate from Chanting-China, Thailand, Philippines and Xinyi-China, while Shaxian-China isolates were more distant members and belongs to the separate group (Fig. 2A).

The dendrogram of molecular affinity of RRSV based on amino acid sequence shows that all RRSV isolate from Java and Bali (RRSV-RK, RRSV-AY, RRSV-GB), belongs to the same group with isolate from Chanting-China, Thailand, Philippines and Xinyi-China. Shaxian-China isolate was a more distant member and was in a different group (Fig. 2B).

4. DISCUSSION

BPH attack on 2010 became an international concern and took a lot of attention from the government and researchers because the population density of this

insect increased dramatically and was prevalent in some rice-production countries in South East Asia, South Asia, and part of Central Asia. BPH is vector of some of rice viruses, especially RRSV and RGSV. In Indonesia, a high level of mixed and RRSV individual infections were observed and known to become more dominant in the field [9].

Reverse Transcriptase PCR (RT-PCR) and nucleotide sequencing are the current and reliable methods for the RRSV detection due to its high sensitivity, specificity, and rapidity [13]. Based on an RT-PCR test it was known that RRSV was widespread in Java and Bali, and there is a possibility that the virus might spread to Sumatra and Sulawesi considering that BPH has already attacked on that regions [2]. RRSV can spread from an endemic area to another area because of its vector's ability in making a long-distance migration [12].

Molecular diversity based on BLAST and Multiple Sequence Alignment from nucleotide and amino acid sequence of RRSV samples from Yogyakarta Specific Region (RRSV-AY), Klaten Central Java (RRSV-RK) & Bali (RRSV-GB) have a low-diversity. The same result also showed on previous study. Comparison on RRSV BLAST from NCBI GeneBank showed that the RRSV isolates from Java and Bali had high percentages of similarity (more than 99 %) with those from China (HM125546.1 & HM125566.1), Thailand (L46682.1) and Philippines (AF486811.1) with the highest percentage on Chanting-China and Thailand isolates. Based on dendrogram phylogenetic tree (Fig. 2A & 2B) isolate from Java and Bali could be classified into one group with Chanting-China, Xinyi-China, Thailand and Philippines isolate and have the closest affinity with Chanting-China isolate (HM125556.1). The research result ease the presumption of RRSV new strain emergence. But further research is still needed to compare complete sequence from the whole virus segment.

5. CONCLUSIONS

The sequence of nucleotide and amino acid of all RRSV isolates from Java and Bali exhibited a high similarity to the isolates from China (HM125546.1) and Thailand (L46682.1). Molecular diversity of RRSV from Java and Bali has close affinity with

the isolate HM125546.1 from Chanting, Fujian, China.

6. ACKNOWLEDGEMENTS

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