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DETECTION AND TRANSMISSION OF RICE STUNT VIRUS ON CIHERANG AND SITU BAGENDIT VARIETIES

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ABSTRACT

Detection and Transmission of rice stunt virus on Ciherang and Situ Bagendit Varieties. The explosion of brown planthoppers recently has caused reduction of rice production in Indonesia. Brown planthoppers do not only act as pest, but also transmit *Rice grassy stunt virus* (RGSV) and *Rice ragged stunt virus* (RRSV). Detection of the existence of the two viruses in rice plants and vector insects is important to be done to ensure that the virus is infected with the vector. The aim of this research is to detect the existence of virus in varieties of Ciherang and Situ Bagendit as a result of transmission in the laboratory and to find out the ability of brown planthoppers to transmit stunt virus to both of the varieties. This research was compiled using Completely Randomized Design (CRD) with 4 treatments, namely healthy rice plants of Ciherang and Situ Bagendit varieties, Ciherang and Situ Bagendit varieties which were infested by brown planthoppers each with 5 repetitions. The parameters observed were incubation period, symptoms, plant height, number of leaves and incidence of disease. The data on plant height, number of leaves and incidence of disease were analyzed using ANOVA and continued with the Least Significant Difference (LSD) test at the level of 5%. The results showed that Ciherang and Situ Bagendit varieties were only positively infected by *Rice ragged stunt virus.* The results of the rice transmission showed that Ciherang variety had a faster incubation period of 10 DAI while Situ Bagendit was 14 DAI, but the two varieties showed an inhibition of growth in plant height and number of leaves compared to healthy plants with each incidence of 51.3% and 46.3%.

Key words: Rice stunt virus, RGSV, RRSV, WBC

ABSTRAK

Deteksi dan Penularan Virus Kerdil Padi pada Varietas Ciherang dan Situ Bagendit. Ledakan wereng batang cokelat akhir-akhir ini menyebabkan penurunan bagi produksi padi di Indonesia. Wereng batang cokelat tidak hanya berperan sebagai hama, tetapi dapat menularkan penyakit *Rice grassy stunt virus* dan *Rice ragged stunt virus*. Deteksi keberadaan dua virus tersebut pada tanaman padi dan serangga vektor penting dilakukan untuk memastikan virus tertular dari vektor tersebut. Penelitian ini bertujuan untuk mendeteksi keberadaan virus pada tanaman padi varietas Ciherang dan Situ Bagendit hasil penularan di laboratorium dan mengetahui kemampuan wereng batang cokelat dalam menularkan virus kerdil pada kedua varietas tersebut. Penelitian ini disusun menggunakan Rancangan Acak Lengkap (RAL) dengan 4 perlakuan yaitu tanaman padi sehat varietas Ciherang dan Situ Bagendit, tanaman padi varietas Ciherang dan Situ Bagendit yang diinfestasi wereng batang cokelat dengan masing-masing 5 ulangan. Parameter yang diamati adalah masa inkubasi, gejala, tinggi tanaman, jumlah daun dan insidensi penyakit. Data tinggi tanaman, jumlah daun dan insidensi penyakit dianalisis menggunakan ANOVA dan dilanjutkan dengan uji *Least Significant Difference* (LSD) pada taraf 5%. Hasil penelitian menunjukkan bahwa tanaman padi varietas Ciherang dan Situ Bagendit hanya positif terinfeksi *Rice ragged stunt virus*. Hasil uji penularan tanaman padi menunjukkan bahwa varietas Ciherang memiliki masa inkubasi yang lebih cepat yaitu 10 hsi sedangkan Situ Bagendit 14 hsi, namun kedua varietas menunjukkan terjadinya penghambatan pertumbuhan tinggi tanaman dan jumlah daun dibandingkan tanaman sehat dengan insidensi masing-masing 51,3% dan 46,3%.

Kata kunci: RGSV, RRSV, virus kerdil padi, WBC

INTRODUCTION

The explosion of brown plant-hoppers (BPH or *Nilaparvata lugens* Stal) recently has caused a big decline in the rice production in Indonesia. The top attack of the BPH happened in the period of 2010 and 2011 which got up to 200 Ha (Baehaki & Mejaya, 2014). BPH are sticking and sucking insects that suck phloem vessel, decrease the chlorophyll and leaf's protein content, and also obstruct the photosynthesis rate (Watanabe & Kitagawa, 2000). The collection of imago and nymph of BPH causes the plants to grow stunted, turn the leaves yellow, faded, and finally cause the symptom attack that is called as *hopperburn* (Baehaki & Mejaya, 2014). In addition, the ability of BPH which is able to adapt in many resistant varieties also becomes a very important concern. Besides of being a pest, BPH also plays role as virus disease vector, such as *Rice grassy stunt virus* (RGSV) and *Rice ragged stunt virus* (RRSV) (Hibino *et al*., 1985; Hibino, 1996; Toriyama *et al*., 1997; Chomchan *et al.,* 2003). RRSV is a member of the family of *Reoviridae* genus *Oryzavirus* with the virus particle as *icosahedral* with 50 mm diameter (Boccardo & Milne, 1984). RRSV particle consists of five main structural proteins which is very imunoreactive with the weight of molecule around 33, 39, 43, 70 and 120 kDa and has five small structural protein (49, 60, 76, 90, and 94 kDa) (Huihua *et al.,* 1988). RGSV belongs to family of *Bunyaviridae*, Genus *Tenuivirus* (Hull, 2002). Particle of RSGV organized as *pleomorphic*, can be seen as thin filament or rounded filament and oftentimes shaped in spiral configuration (Toriyama *et al.*, 1997).

Ditlin (2010) reported that the total attack of BPH was followed by virus infected in the period of 2001-2010 in Indonesia had achieved 351,748 ha which 11,354 ha of them were dried up. The virus will be multiplicity in the vector body and keep the virus inside the body even after the process of molting (change the skin) but does not inherit the virus to the next generation through eggs (Hibino, 1996; Milne & Ling, 1982). The attack of these viruses in the field has been reported, there is even double infection recorded in the field (Suprihanto *et al*., 2015; Kusuma *et al*., 2018).

The double infection that is caused by stunt virus shows serious symptom compared to the single infection (Dini *et al*., 2015; Du *et al*., 2005). The ability of brown planthoppers infecting RGSV and RRSV has been reported (Suprihanto *et al*., 2015). However, up to now the report about the ability of BPH in infecting double infection simultaneously in plant has not known yet. One of the ways to know the existence of virus in the vector insect that many be done is by using PCR and infection in the rice plant (Suprihanto *et al*., 2015; Rahmawati *et al*., 2015). This method will give information about the existence of the double infection in the vector body and its ability to infect the double infection in host plant. This information will give new opinion about how far the ability of BPH in infecting RGSV and RRSV in rice plant.

MATERIALS AND METHODS

Research Site. This research was done in February to May 2018 in Virology Laboratory, Department of Plant Pests, Faculty of Agriculture, Gadjah Mada University.

Observation and Sample Rice Plant Taking. The observation and sample taking of the plant was done in the rice field when Situ Bagendit variety was two months old after planted in the vast 3225 m^2 in Pleret village, Pleret Sub District, Bantul Regency, Daerah Istimewa Yogyakarta. Sample taking was done randomly with the method of sample taking diagonally. In the spread out area of the rice field was observed and taken the sample of the plant with double infection attack then was kept to be used as inoculum. Double infection was showed with the symptom of stunted plant, turned yellow in the tip of the leaf, there was none of panicles**,** grow upright, ragged, gall and twisted tip leaf especially in the younger part (Helina *et al*., 2019). Meanwhile, single infection reckon on the virus that attacked the rice plant. RGSV attack showed stunted symptom, excessive number of paddy tillers**,** grow upright and shorten, narrowed leaf, leaf color became pale green up to yellow and there was no panicles (Suprihanto *et al*., 2015). Besides of that, rice plant infected by RRSV experienced stunting, leaf color became darker with jagged edge or twisted tip, and leaf bone had swellings or lumps at the bottom of the leaf blade and the outer surface of the leaf midrib (Cabauatan *et al*., 2009). Before was used as inoculum, the sample was confirmed positive infected by stunt virus by using *Polymerase Chain Reaction* (PCR) method.

Propagation of Brown Planthoppers. BPH used was biotype 3 with instar 2. Planthopper used was planthoppers non viruliferus collection of the laboratory propagation. Propagation of planthoppers was done in a jar that closed with lint. Feed used was 2 weeks old rice plant.

Variety Test. Rice plant varieties Ciherang and Situ Bagendit were used to infection stunt virus test. Both varieties were dominantly planted by farmers in Bantul

regency, Yogyakarta. Plants used were 10 days old after planted and kept in plastic pot with diameter 10 cm with plant medium consisted of soil and manure with comparison 2:1. Infection test started with planthoppers instar 2 given food (acquisition) for nine days in inoculum that was gotten in the field and then moved (inoculation) for 24 hours in the rice plant of variety test. The process of moving the planthoppers was done using aspirator. Each of plant was given 2 planthoppers per clump and then closed with plastic tube for inoculation period. After inoculated, planthoppers killed to avoid infection to the healthy rice plant.

Experimentation Plan and Statistic Analitic. This research was arranged using *Completely Randomized Design* (CRD) with 4 treatments and 5 repetitions. Treatment consisted of healthy rice plant varieties Ciherang and Situ Bagendit, rice plant variety Ciherang infected with BPH, and rice plant variety Situ Bagendit infected with BPH with 5 repetition for each so that the total plant was 20 plants. Parameters observed were incubation time, symptom, disease incidence, plant's height and number of leaves. The data of plant's height, number of leaves, and disease incidence were analyzed using ANOVA and if there was any significant different, the following data was examined using *Least Significant Difference* test in 5% from the significant level.

Molecular Detection of Rice Stunt Virus in Inoculum and Brown Planthoppers. Rice leaf with symptom obtained from the field, rice leaf and BPH insect infection result were used to detect the existence of rice stunt virus. Molecular detection was done through RNA total extraction, cDNA formation, cDNA amplification and DNA visualization. The RNA total extraction of the plant was done by using *Total RNA mini kit (plant)* and RNA extraction of the insect by using *Total RNA mini kit (Tissue)* with suggested procedure as in *Geneaid*.

Complementary-DNA (cDNA) Formation. cDNA formation was done through RT-PCR *(Reverse Transcriptase-Polymerase Chain Reaction)* by using *First Strand cDNA Synthesis Kit ReverTra Ace* with the suggested procedure as in TOYOBO. The preparation was started by preparing micro tube that had given with label then was put each 4 µL 5x RT Buffer (contains 25mM Mg 2+), 1 µL) *Primer Oglio* (dT) 20 (10 pmol/µL), 2 µL dNTP *mixture* (10 mM), 1 µL *ReverTra Acc*, 1 µL RNase *Inhibitor* (10 U/µL) and 8 µL *RNase-free* H20 in the tube. After that, extraction result of RNA was put in the PCR tube as much 3 µL and was homogenized by using vortex machine. The sample then was placed in the PCR machine to be done the process of RT-PCR with incubation temperature 42 °C for 20 minutes, heat 99 °C for 5 minutes and 4 °C for 20 minutes.

DNA Amplification and Visualization. cDNA result from RT-PCR was amplificated by using specific primer RGSV (F1:5'-GGCTTATGATAGTCTGTGATTTG-3'/ R: 5'GTGTAAGATGGGGTAAAGTGCA-3') which was designed by Nam *et al.,* (2007) and RRSV (F3:5'-GACTAGGGATGTGCGTTC-3'/B3"5'-TGTAATCGACGTTCGCTC-3') which was designed by Le *et al*. (2010). Preparation was started by preparing micro tube that had given with label then was put each *free water* (ddH20) as much 4 µL, PCR *mix RedMix* 8 µL, Primer RRSV F and RRSV R with concentration 5 pmol each 2 µL with *template* 2 µL, so that the total volume achieved 20 µL. PCR was done with initial denaturation cycle 95 °C for 1 minute, denaturation 95 °C for 15 seconds, sticking for 15 seconds, elongation 72 °C for 10 seconds and finish elongation 72 °C for 5 minutes. The PCR result product was followed by DNA visualization through electrophoresis by using agarose gel 1% 15 ml TBE 1x and 0.15 g with voltage 50 V DC for 50 minutes. After the process of electrophoresis finished, it continued by coloring process *(staining)* by using *Ethium bromide* and visualization by using *transilluuminator UV* and was documented with digital camera.

RESULTS AND DISCUSSION

Observation result of stunt rice symptom in the field showed that rice plant variety Situ Bagendit was suspected getting infected by two rice stunt viruses *(double infection)* which were *Rice grassy stunt virus* (RGSV) and *Rice ragged stunt virus* (RRSV) by showing the symptoms such as stunted plant, turned yellow in the tip of the leaf, no panicles, excessive number of paddy tillers**,** grow upright, *ragged, gall,* and twisted tip leaf *(twisted)* especially in the younger part (Figure 1). The symptom of double infection that found was similar with the stunt virus attacks in some other areas such as Sukamandi (Dini *et al.,* 2015), Bali (Kusuma *et al.,* 2018) and other areas in Java such as Yogyakarta, Klaten, Magelang, and Cirebon (Suprihanto *et al.,* 2015). This double infection symptom was different with single infection where the single infection symptom was depending to the infected virus. Cabauatan *et al.* (2009) reported that rice plant that had infected with RGSV showed symptoms such as stunted, excessive number

of paddy tillers, grow upright and shorten, narrowed leaf, leaf color turned to pale green up to yellow and there was no tassel produced, meanwhile rice plant that had infected RRSV became stunted, leaf color became darker with jagged edge or twisted tip, and leaf bone had swellings or lumps at the bottom of the leaf blade and the outer surface of the leaf midrib.

Detection of Stunt Virus and Rice Plant Stunt Virus Infection in Ciherang and Situ Bagendit Varieties. Based on the detection of rice inoculum sample with RT-PCR by using primer specific RGSV F1/R and RRSV F3/B3 showed result that positively infected rice stunt virus *(double infection)* by seeing the DNA ribbon with the size ± 450 bp and ± 210 bp (Figure 2). However, the detection of rice sample and brown planthopper of the inoculation result showed it was only infected with *Rice ragged stunt virus* (Figure 2). Rice inoculum that was infected by double infection was caused by the existence of exuberate BPH in the field, while the result of inoculation only used 2 planthoppers per plant. The spread of BPH in the field, moreover with the availability of stunt virus inoculum source, became the cause of spreading the virus in the field. Nevertheless, BPH was assumed to contain only one kind of virus that is RGSV or RRSV, moreover up to now there is no report about the existence of the two viruses in single vector body yet.

Figure 1. Stunted symptom on rice plant in Pleret village, Pleret subdistrict, Bantul regency; (A) rice plant showed stunted symptom with upright breeds, (B) *gall* in the base of rice stalk, (C) twisted symptom *(twisted)* in the base and tip of the leaf

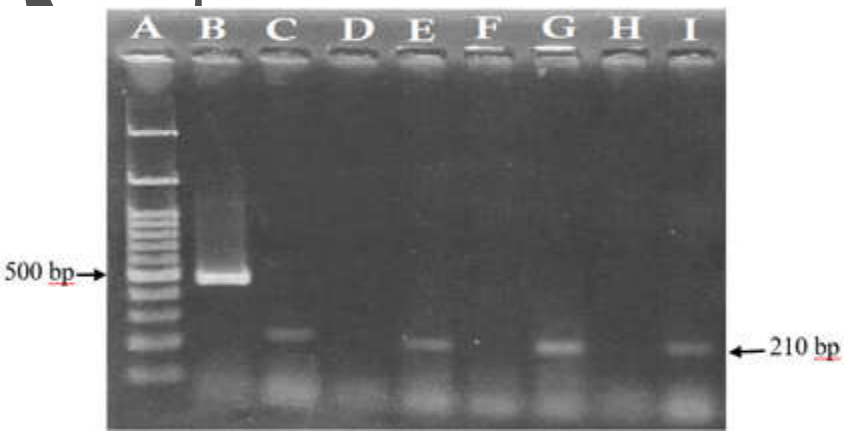

Figure 2. Visualization result RT-PCR using primer RRSV F3/B3 and RGSV F1/R in agarose gel 1,5%. A: Marker 100 bp, B: positive control RGSV Situ Bagendit, C: positive control RRSV Situ Bagendit, D: BPH inoculation result negative RGSV, E: BPH inoculation result positive RRSV, F: Ciherang rice sample inoculation result negative RGSV, G: Ciherang rice sample inoculation positive RRSV, H: Situ Bagendit rice sample inoculation result negative RGSV, I: Situ Bagendit rice sample inoculation result positive RRSV

RSGV was not detected in the sample of rice from inoculation and brown planthopper and it was assumed to be caused by some factors, such as the virus spreading route in the vector body, time of inoculation and host plant. Virus spreading route in the vector body was started by the entry of virus in the digestive duct and come to epithelium cell in *chamber filter* then anterior midgut. The virus went through basal lamina to infect muscle cell, toward saliva gland through hemolymph route (Chen *et al*., 2011; Zheng *et al.*, 2014). Besides, inoculation time became one of the factors of the limitation of virus transmission in the plant. In the infection of two stunt viruses there was possibility *"infection time"* so that it needed inoculation period longer compared to only one virus. The longer period of inoculation, then virus contains in the vector insect was rising (Suprihanto *et al.,* 2015). Host plant also had a role in spreading the virus. The ability of the plant was influenced by the surroundings and plant varieties so that it could limit or rise the spreading of virus in the plant.

The result of infection test showed the average of stunt disease incidence in Ciherang variety was 51.3% and Situ Bagendit 46.3% with similar symptom response which were pale leaf color and turned yellow and also plant's height derivation and number of leaves (Figure 3). Stunt virus that attacked Ciherang variety had incubation time faster which was 10 hsi meanwhile for Situ Bagendit variety was 14 hsi (Table 1). The difference of incubation time showed the difference of

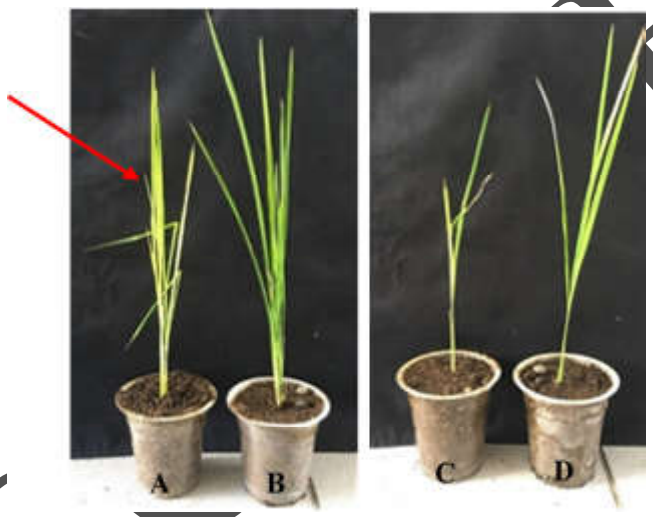

Figure 3. Symptoms of rice plant Ciherang and Situ Bagendit varieties which were 30 hsi old; (A) rice plant variety Ciherang was infected with stunt virus with symptom yellow leaf color, pale and number of leaves decreased; B Rice plant variety Ciherang was healthy; C: Rice plant variety Situ Bagendit was infected with stunt virus with symptom stunted, had pale yellow color, and few number of leaves; D: Rice plant variety Situ Bagendit was healthy

Table 1. Transmission test of stunt virus on Ciherang and Situ Bagendit varieties

Treatment	Incubation time (DAI)	Symptom	Plant's height	Number of leaves	Incidence (%)
Ciherang without inoculation	-	No Symptoms	32.7 b	6.3 a	0.0 c
Situ Bagendit without inoculation	-	No Symptoms	34.0 a	6.2 a	0.0 c
Ciherang with inoculation	10	Turned yellow and pale	24.0 c	3.7 b	51.3 a
Situ Bagendit with inoculation	14	Turned yellow and pale	24.3 c	3.6 b	46.3 b
LSD 0.05			0.89	2.10	0.21

Number that are followed by different letter in the same column showed real difference based on the Least Significant Difference test with reliance level 95%.

interaction between the plant and the virus which will be influential to the plant response appeared. This has relation to the endurance system that owned by the plant and the level of virus virulence that infecting (Subekti *et al.,* 2006). With the longer incubation time will make it possible for the plant to form endurance so that it will be able to hamper the virus development in the plant (Rahmawati *et al.,* 2015).

Both Ciherang and Situ Bagendit varieties showed growth obstruction and symptom formation showing about plant's height and leaf's total that was different with healthy plant, and also rice leaf that turned yellow after inoculated stunt virus (Table 1 & Figure 3). Virus infection caused plant metabolism system was disturbed through utilization of photosynthetic to replication and syntheses of virus particle. The effect was the plant would be lack of basic material to do vegetative growth and normal generative. Besides, stunt virus caused plant biochemist process was disturbed so that plant could not do its function well. It caused varied plant responses toward virus attack such as stunting, unproductive plant, malformation and other symptom variation (Baehaki *et al.,* 2011).

Ciherang and Situ Bagendit are the varieties have good endurance toward brown planthopper's attack and the virus it is infected (Suprihanto *et al.,* 2015; Rahmawati *et al.,* 2015). However, the endurance of the varieties was broken because of brown planthopper adaptive character and the planting of the varieties continually. According to Ling (1972), high or low of attack level is depending on the susceptible of the varieties were planted, because of the ability owned by the plant to prevent infection process or limit the virus pathogen colonization. Besides, environment factor also has a role in limiting and helping the spreading of virus infection. Environment that supports the development of stunt virus will cause damage to the plant.

Virus spreading in the field is very depending to the ability of the vector in doing the transmission. Rice plant that had infected with stunt virus gave the opportunity to the vector to spread the virus moreover if the virus intensity was very high and virus attack was varied in the field. It caused possibility of brown planthoppers did not only spreading RRSV or RSGV, but double infection could happen with the availability of various inoculum sources. Generally, the symptom that appeared in the double infected plant heavier if compared to plant that was infected with each of single virus. If some viruses are infecting plant together, it will cause infection with antagonistic or synergistic character (Hull, 2002). Synergistic infection generally happens when two viruses do not have any relation like RGSV and RRSV.

CONCLUSION

Based on the detection result with RT-PCR using specific primer RGSV F1/R and RRSV F3/B3 rice plant Ciherang and Situ Bagendit varieties and also brown planthopper were only positively infected by *Rice ragged stunt virus.* The result of infection test showed that Ciherang variety had incubation time 10 hsi and Situ Bagendit had 14 hsi, both varieties showed the growth obstruction of plant's height and number of leaves compared to healthy plant with each incidence 51.3% and 46.3%. Based on the inoculation result in virus infection test, brown planthopper could not infect two stunt viruses simultaneously.

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