Molecular detection of tungro virus on mekongga and inpari 4 rice varieties in West Papua

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Abstract. Tungro disease is one of the important diseases in rice plants. One of the efforts to reduce the spread of tungro virus is to ensure the presence of the virus in the field. This is the first step to prevent the spread and control of tungro disease, especially in West Papua. One detection technique that can be done is molecular detection through PCR techniques. Rice samples detected were Mekongga and Inpari varieties taken from rice plants in West Papua. Total DNA of RTBV, one of the viruses that cause tungro disease, was extracted and amplified using DAF primers (5-GGAATTCCGGCCCTCAAA AACCTAGAAG-3) and DAR (5-GGGGGTACCCCCCTC CGATTTCCCATGTATG-3). The PCR RTBV results showed that the positive samples were infected with Rice tungro bacilliform virus (RTBV). This is indicated by amplification of DNA measuring ± 1400 bp which is the target size of the DAF and DAR primers. The results of this study are preliminary information that can be used as a basis for tungro control and recommendations for future cropping.

1. Introduction

Rice is one of the most important food products in Indonesia. Efforts to increase rice production are always being carried out, one of which is by managing pests and plant diseases in the field. Tungro is the fifth important pest in rice cultivation in Indonesia. The yield potential of a rice variety will not be achieved if the infected plant is infected with tungro disease, even the plant will not produce grain if the infection occurs since the early vegetative phase or in the nursery stage [1].

Tungro is caused by two different types of viruses, namely the stem form virus Rice tungro bacilliform virus (RTBV) and the round shape virus Rice tungro spherical virus (RTSV), which is transmitted by the green leafhopper vector. RTBV is a virus that plays a role in the appearance of symptoms, while RTSV plays a role in transmission [2]. If the plants are only infected with RTBV, the symptoms are milder, namely yellow leaves, whereas if the plants are only infected with RTSV, the plants will not show symptoms of disease [3]. Green planthopper can transmit RTSV and RTBV simultaneously from an inoculum source containing both types of viruses. RTBV transmission only occurs when the vector has first inhaled RTSV, whereas RTSV transmission can occur without RTBV assistance [4]. Besides infecting rice plants, the tungro virus is also reported to infect several types of weeds. Weeds infected with the tungro virus can become inoculums if there are no rice plants in the field [5].

The existence of tungro disease in Indonesia was first reported in 1983-1984 [6]. The main distribution areas for tungro are in the provinces of West Java, East Java, Central Java, Bali, South Sulawesi, Lampung, Banten, Central Sulawesi, North Sumatra, South Kalimantan, and Irian Jaya [7]. The tungro in Papua was first confirmed in 1999 and was found in Nabire and Jayapura [8]. There are no reports regarding the existence of tungro in West Papua until 2020

The presence of tungro disease in the field cannot be ensured if it is only based on observation of symptoms. It is because other biotic and abiotic factors can also cause the symptoms that arise due to tungro disease. Therefore, the detection method using the PCR technique is one of the most widely used methods because of its accuracy and sensitivity in detecting viruses. According to Takahashi et al. [9], PCR technique has a sensitivity 10^4 times better than the enzyme-linked immunosorbent assay (ELISA).

The results of this study are expected to provide information related to tungro virus infection in rice cultivation in West Papua, especially in the Mekongga and Inpari varieties using PCR detection techniques so that it can be the first step in efforts to prevent the spread and control of tungro disease in Papua

2. Methodology

2.1. Sampling

Samples of the Mekongga and Inpari 4 varieties of rice plants showing symptoms of tungro disease were taken randomly from the rice plantations of West Papua BPTP in November 2017. The samples were detected molecularly at the Plant Disease Laboratory, Indonesian Rice Tungro Research Center, Sidrap, South Sulawesi.

2.2. Detection by PCR technique

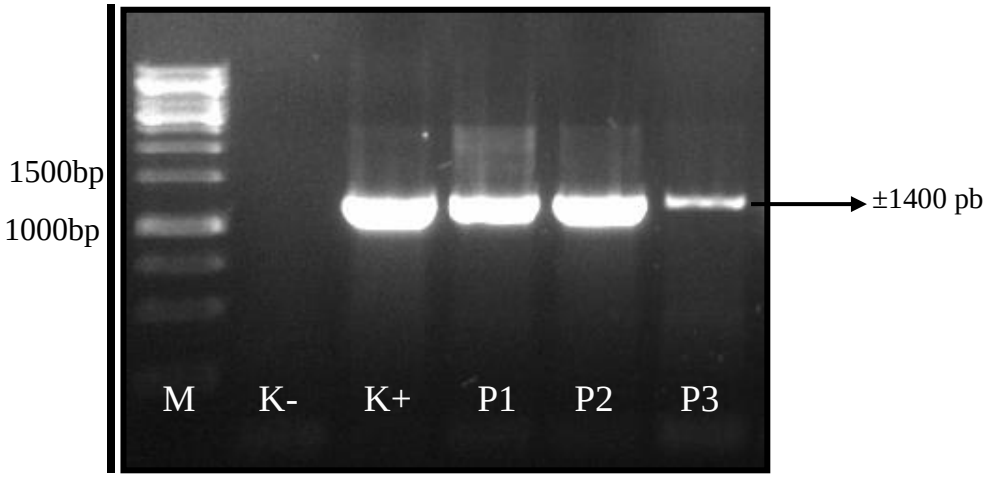
Total DNA extraction. Total DNA extraction. Total DNA was isolated from 0.1 g of leaves suspected of being infected with tungro disease by the Cethyl Trimethyl Ammonium Bromide (CTAB) method [10]. As much as 1 g of rice leaves crushed with liquid nitrogen in a sterile mortar then added 750 µL of extraction buffer. The scour was put into a 2 mL microtube and incubated at 65^{o}C for 60 minutes. During the incubation period, the tube was reversed every 10 minutes to aid in the lysis process. After that, 500 µL of chloroform: isoamyl alcohol (24:1) was added and vortexed for 1 minute. The solution was then centrifuged at a speed of 12,000 rpm for 15 minutes. The supernatant in the top layer was transferred into a 1.5 mL microtube and 10% sodium acetate was added to the volume of the supernatant and isopropanol 2/3x the volume of the supernatant + sodium acetate. The solution was incubated at -20^{o}C for 16 hours. The solution is pelleted by centrifugation at a speed of 12,000 rpm for 10 minutes. Then 750 µL of 70% ethanol was added, the tube was reversed twice, and the ethanol was carefully removed. Total DNA was dried and dissolved in 50 µL of TE buffer.

2.2.1. PCR. Amplification was carried out with a total volume of 25 µL PCR premix consisting of 2 µL DNA, 1 µL 10 µM forward primer, 1 µL10 µM reverse primer, 12.5 µL Go Taq Green (Thermo Scientific), 0.5 µL $MgCl_2$, and 8 µL nuclease-free water. The DNA used was the DNA from 1:10 dilution of the total DNA extraction. RTBV amplification used a specific primer pair of protein coat genes, namely DAF primers (5'-GGAATTCCGGCCCTCAAAAACCTAGAAG-3') and DAR (5'GGGGGT ACCCCCCTCCGATTTCCCATGTATG-3'), with a target amplification product of 1.400 bp. The amplification process consists of initial denaturation for 5 minutes at 94^{o}C, followed by 34 amplification cycles, including denaturation of 1 minute at 94^{o}C, annealing for 1 minute at 62.2^{o}C, synthesis for 2 minutes at 72 ^{o}C, then time to the final synthesis step is 10 minutes at 72^{o}C

2.2.2. Visualization of PCR results. PCR results were electrophoresed on 1% agarose gel in 0.5x TBE and given 0.5% peqGreen dye. A total of 5 µL of 1 kb DNA marker (Thermo Scientific) and 5 µL of PCR DNA were each inserted into the gel well and electrophoresed for 50 minutes at a voltage of 50V. The results of electrophoresis were then viewed using geldoc.

3. Results and discussion

The results of RTBV DNA amplification which is one of the viruses that cause tungro can be seen in figure 1. A total of 3 plant samples from West Papua which were sent to the Indonesian Rice Tungro Research Center showed the appearance of DNA bands measuring ± 1400 bp. DNA bands measuring ± 1400 were the targets of the DAF and DAR primers. This result follows the planting conditions in the field that the symptoms of rice plants being infected by RTBV are yellow to orange leaves. Yellow or orange-yellow discoloration of leaves, stunting of plant growth, reduction in the number of effective tillers, shortness of panicle length, and often sterile or partial filled grains are the characteristics of tungro disease in rice [11]



M = Marker, DNA Marker 1 kb
K- = Negative control
K + = Positive control (diseased plants)
P1 = Mekongga variety, Aiwasi location, Yatin Farmers
P2 = Mekongga variety, Aiwasi location, Lasno Farmers
P3 = Inpari variety, Bowi Subur location, Lasno Farmers

Figure 1. RT PCR result of RTBV on mekongga dan inpari 4.

Rice tungro disease is a disease caused by the infection of two different viruses, namely RTBV and RTSV [12]. RTSV functions as a helper virus in this case and RTBV cannot be transmitted if it is not present [13]. The viral complexes of both viruses cause poor outcomes when infection occurs [14]. As reported by Hibino et al [15], found two types of virus particles in rice plants affected by tungro disease in Indonesia. Plants showing severe symptoms have both types, but plants with moderate symptoms only have RTBV particles

Several factors, namely influence tungro disease epidemic; 1) plants (variety resistance level, genetic uniformity of varieties in an area, plant type and stage), 2) tungro virus (availability of inoculum sources, variation and virulence of tungro virus strains), 3) green leafhoppers as vectors (fluctuations in green leafhoppers population, density infective vector population, biotype variation, and efficiency of tungro virus transmission by green planter), 4) environmental conditions (climate, temperature and humidity) and 5) cultivation practices. The interaction of these factors largely determines the occurrence of an epidemic of tungro disease in an area. Management of tungro disease based on the epidemiological concept is carried out by managing the factors causing the epidemic which are arranged in a theoretical and practical framework, a protocol for predicting and monitoring tungro disease, and a control strategy [16].

Varieties have an important role in the tungro disease epidemic. The rice varieties detected in this study were mekongga and inpari 4. Farmers often use this variety because it is easy to obtain and sometimes given free from the government. If planting this variety is carried out extensively and followed by cultivation that supports the development of tungro disease, it can cause the incidence and severity of tungro disease to be higher. Therefore, it is important to plant tungo-resistant varieties suitable for the environmental conditions for growing rice in West Papua. The recommended tungro-resistant rice varieties in West Papua are Inpari 36, Inpari 7, Inpari 8. The resistant cultivars infected with the virus showed delayed flowering, while the sensitive rice plants did not produce flowers [17]. Transmission of the rice tungro viruses by *N. virescens* depends on the adaptability of the green leafhopper to cultivars containing resistance genes to the insect vector [18].

4. Conclutions

RTBV infection has occurred in rice plantations in West Papua as indicated by amplified DNA measuring 1400bp in mekongga and inpari varieties. Planting tungro resistant varieties is highly recommended to reduce the spread of tungro disease in West Papua,.

References

[1] Hasanuddin A 2002 *Pengendalian Penyakit Tungro Terpadu: Strategi dan Implementasi* (Jakarta: Badan Litbang Pertanian Jakarta)
[2] Dahal G, Hibino H and Saxena R C 1990 Association of leafhopper feeding behavior with transmission of rice tungro to susceptible and resistant rice cultivar *Phytopathol.* **80** 371-377
[3] Cabauatan P Q and Hibino H 1988 Isolation, purification, and serology of Rice tungro bacilliform and Rice tungro spherical viruses *Plant Dis.* **72** 526–528
[4] Hibino H, Saleh N and Roechan M 1977 Transmission of two kinds of rice tungro associated viruses by insect vectors *Phytopathol.* **69** 1266-8
[5] Ladja F T, Hidayat S H, Damayanti T A and Aunu Rauf 2016 Deteksi virus tungro pada gulma padi sawah menggunakan teknik PCR *Penelitian Pertanian Tanaman Pangan* **35** 39-44
[6] Azzam O and Chancellor T C B 2002 The biology, epidemiology and management of rice tungro disease in Asia *Plant Dis.* **86** 88-100
[7] Raga I N 2008 Perkembangan dan penyebaran penyakit tungro di Indonesia 2008 *Prosiding Seminar Nasional Strategi Pengendalian Penyakit Tungro Mendukung Peningkatan Produksi Beras* (Makassar: Pusat Penelitian dan Pengembangan Tanaman Pangan, Badan Penelitian dan Pengembangan Pertanian)
[8] Davis R I, Parry J N, Geering A D W, Thomar J E and Rahamma R 2000 Confirmation of the presence of Rice tungro bacilliform virus in Papua (formerly Irian Jaya), Indonesia *Austral. Plant Path.* **29** 223
[9] Takahashi Y, Tiongco F R, Cabauatan P Q, Koganezawa H, Hibino H and Omura T 1993 Detection of Rice tungro bacilliform virus by Polymerase Chain Reaction for assessing mild infection of plants and viruliferous vector leafhoppers *Phytopathol.* **83** 655-659
[10] Doyle J J and Doyle J L 1990 Isolation of plant DNA from fresh tissue *Focus* **12** 13-15
[11] Khatun M T, Latif M A , Rahman M A, Hossain M, Ansari T H, Nessa B, Khan M A I , Ali M A and M M Hanafi M M 2017 Recovering ability of upland and rainfed lowland rice varieties against rice tungro disease *Bangladesh Rice J*. **21** 91-100
[12] Hull R 1996 Molecular biology of rice tungro viruses *Ann. Rev. Phytopathol*. **34** 275-297
[13] Latif M A, Rahman M M, Ali M E, Askani S and Rafii M Y 2013 Inheritance studies of SSR and ISSR molecular markers and phylogenetic relationship of rice genotypes resistant to tungro virus *C. R. Biol.* **336** 125-133
[14] Hibino H. 1983. Relations of rice tungro bacilliform and tungro spherical viruses with their vector *Nephotettix virescens Ann.* Phytopathol. *Soc. Japan* **49** 545-553
[15] Hibino H, Roechan M and Sudarisman S 1978 Association of two types of virus particles with penyakit habang (tungro disease) of rice in Indonesia *Phytopathol.* **68** 1412-1416

[16] Praptana H R and Yasin M 2008 Epidemiologi dan Strategi Pengendalian Penyakit Tungro *Iptek Tanaman Pangan* **3** 184-204
[17] Bunawan H, Dusik L, Bunawan S Nand Amin N M 2014 Rice Tungro Disease: From Identification to Disease Control *World Appl. Sci. J.* **31** 1221-1226
[18] Rosida N, Kuswinanti T, Amin N and Nasruddin A 2020 Epidemiological study on the current status of rice tungro disease in South Sulawesi, Indonesia *OnLine J. Biol. Sci.* **20** 221-231