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Research Article

Molecular Detections and Resistance Response of Six Rice Varieties to Tungroviruses from South Sulawesi *

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ABSTRACT

The suspected rice virus is found in the field, namely the tungrovirus which is transmitted by green leafhoppers (*Nephotettix virescens*). The study aimed to detect the tungrovirus molecularly and examine the resistance response of six rice varieties from the transmission of tungrovirus samples from South Sulawesi on a greenhouse scale. Based on the results of molecular detection with RTSV PCR of the double infected sample with DNA bands 1115 bp and RTBV of around 430 bp, Sidrap, and Maros samples were infected by 430 bp size RTBV, while Wajo sample was not detected by both viruses. The results of RTBV sequence analysis showed that the grouping of Sidrap was still one group with Maros and Pinrang samples and different from the group of samples from Malaysia, Thailand, and Philippines. While the grouping of RTSV shows that Pinrang samples are still one group with samples from Bali, Subang, and different from those of the Philippines, India, and Malaysia. The results of transmission in the greenhouse on six rice varieties (TN1, Ciherang, Mekongga, Tukad Unda, Inpari 36, Inpari 37) showed different plant resistance responses such as susceptible, moderately resistant, and resistant reactions based on the amount of disease intensity caused. Varieties that are classified as susceptible are TN1 and Ciherang varieties, moderately resistant, namely Mekongga and Tukad Unda varieties, and resistant varieties namely Inpari 36 and Inpari 37 varieties.

Keywords: resistance responses; South Sulawesi; tungrovirus detections; varieties

INTRODUCTION

South Sulawesi is the fourth highest rice production center in Indonesia of 5.74 million tons (BPS, 2018). One of the difficulties in rice production is the tungrovirus. Tungro disease has been reported in Sidrap, South Sulawesi, in 2018 covering an area of 6 ha, and now there are many symptoms similar to tungro disease. The most common symptoms of tungro are dwarf, yellowish leaves, stunted growth, and the inability to produce panicle. Tungro disease can reduce rice production and even cause *puso* (harvest failure) if the infection occurs since the beginning of the vegetative phase or at the nursery stage (Hasanuddin, 2002).

Tungro is caused by two different types of viruses: stem-shaped virus, *Rice tungro bacilliform virus* (RTBV) with DNA type genome; and spherical virus, *Rice tungro spherical virus* (RTSV) with RNA type. RTBV has a diameter of 35 × 150–350 nm with a length of 100.300 nm while the RTSV has a diameter of 30 nm (Hibino *et al*., 1978; Omura *et al*., 1983). Both types of viruses do not have the same serological kinship but can infect plants together without causing cross-protection between the viruses (Mukhopadhyay, 1995). The tungrovirus is only transmitted by green leafhoppers in a semi-persistently (Hibino & Cabunagan, 1986).

Molecular detection with *Polymerase Chain Reaction* (PCR) techniques to detect viruses with DNA genome and *Reverse Transcription* (RT)-PCR for viruses with RNA genome is very sensitive and accurate compared to other methods such as serology and nucleic acid hybridization. (Takahashi *et al*., 1993). The PCR technique is very advantageous in detecting the presence of rice viruses because it is easier and faster than other techniques such as in South Sulawesi, one of the largest rice production centers in Indonesia, which currently has many tungro disease symptoms. The symptoms of the outbreak are varied and the intensity is getting higher hence

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apart from being based on the symptoms it is necessary to further identify the distribution and cause so that it can be used as a basis for developing effective and environmental-friendly control strategies.

Many methods are used to control tungro disease such as the use of insecticides to control planthopper. However, this method is considered less effective and harms the environment. One ofthe environmental-friendly control alternatives is using varieties resistant to the tungrovirus and green leafhopper as vector insects (Sama, 1985 *cit*. Praptana & Muliadi, 2005).According to Suprihatno (1985) *cit.* Praptana *et al*. (2005), known and utilized sources of tungro disease resistance genes are Latisail, CR-94-13, Gam-Pai 15, and resistant varieties which are crossed breeding from those parents. Varieties with vertical resistance have always been a mainstay in reducing the planthopper. The use of resistant varieties is constrained by the adaptability of green leafhoppers by forming new biotypes so that the varieties that are released shortly afterward was broken their resistance. Tungro disease infection in resistant varieties causes no symptoms in the form of a slightly yellowish leaf that disappears as the plant ages (Choi *et al*., 2009). Tungro symptoms would begin to appear when the plants aged 10–15 days after the inoculation of the virus, whereas in fields, symptoms would appear when the plants are 21–30 days after planting (Raga *et al*., 2004). This study aimed to detect the presence of the tungrovirus molecularly in South Sulawesi and determine the response of the resistance of some rice varieties to the tungrovirus.

MATERIALS AND METHODS

The survey was conducted in several districts of rice production centers in South Sulawesi: Maros, Sidrap, Pinrang, and Wajo. Laboratory research was conducted at the Laboratory of Plant Virology, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta. Materials and types of equipment used are tungro symptomatic rice leaf samples collected from various locations, RNA extraction kit Mini plant kit (Geneaid), DNA extraction kit Mini plant kit (Geneaid), Kit for making cDNA (Toyobo), PCR Mix Ready to Use (MyTaq HS Mix), agarose, PCR machine (Bio-Rad T100TM Thermal Cycler), a set of equipment for electrophoresis, and ultraviolet (UV) transilluminator. Research in the greenhouse was conducted at the Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, and in the greenhouse of the Tungro Disease Research Station (Lollittungro), Sidrap, South Sulawesi. Tungro symptomatic rice plants were collected from several locations in South Sulawesi. The rice varieties used in the disease resistance test were Ciherang, TN1, Mekongga, Tukad Unda, Inpari 36, and Inpari 37.

RTSV Detection Using RT-PCR Technique

The detection phase of RTSV began with the extraction of total RNA from rice leaf samples, the stage of total RNA extraction followed the protocol of the commercial kit (mini RNA kit plant, Geneaid). The total RNA extracted was used as a template in the reverse transcription reaction to produce cDNA (complementary DNA). The making of cDNA was performed by the RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) method with a total volume of 10 µl containing 2 µl total RNA of, 3.5 µl RNase Free H_2O, 0.5 µl RNase Inhibitor, 0.5 µl ReverTraAcc, 2 µl 5x RT Buffer, 1 µl dNTP Mixture, and 0.5 µl oligo primer (dt) 20. The reverse transcription reaction was carried out at 42ºC for 20 minutes, followed at 99ºC for 5 minutes, and at last 4ºC. The result of cDNA was used as a DNA template in the amplification reaction.

Amplification was conducted using a specific pair of RTSV-F primers (AAACGGTCATTGTGG GGAGGT) and RTSV-R (CAGGCCCAGCAACG ACATAA) with a target of 1115 bp (Shen*et al*., 1993). The reaction for PCR was made with a total volume of 10 µl containing 0.5 µl RTSV-F primers, 0.5 µl RTSV-R primers, 3 µl DH_2O, 1 µl cDNA Samples, and 5 µl MyTaqTM HS RedMix PCR Mix. The amplification process was preceded by initial denaturation temperature of 95ºC for 2 minutes, denaturation temperature of 95ºC for 30 seconds, the annealing temperature of 57ºC for 30 seconds, extension temperature of 72ºC for 30 seconds, the final temperature of extension of 72ºC for 7 minutes, and the hold temperature of 4ºC ∞, over35 cycles. The amplification results were electrophoresed at 100 V for 30 minutes and colored with ethidium bromide (0.5 g/ml) for 15 minutes. The results of DNA visualization on the UV transilluminator were then documented with a digital camera.

RTBV Detection Using PCR Technique

RTBV detection began with the extraction of total DNA from each rice leaf sample. Total DNA extraction followed the protocol of a commercial kit (DNA Mini kit plant, Geneaid). The total DNA was used as a DNA template in the PCR reaction using a pair of RTBV-B2F specific primers (GCAGAA CAGAACTCTAAGGC) and RTBV-B2R (GTCTAA GGCTCATGCTGGAT) with product target of 430 bp (Cabauatan *et al*., 1999). The PCR reaction was made with a total volume of 10 µl containing 1 µl DNA template, 0.5 µl RTBV-B2F primer, 0.5 µl RTBV-B2R primer, 3 µl DH_2O, and 5 µl MyTaqTM HS RedMix PCR Mix. The amplification process was performed over 35 cycles preceded by initial denaturation temperature of 95ºC for 2 minutes, denaturation temperature of 95ºC for 30 seconds, the annealing temperature of 53ºC for 30 seconds, temperature extension 72ºC for 30 seconds, final extension temperature of 72ºC for 7 minutes, and the hold temperature at 4ºC ∞. The amplification results were electrophoresed at 100 V for 30 minutes and colored with ethidium bromide (0.5 g/ml) for 15 minutes. Visualization of the amplified DNA results was similaras described previously.

Tungrovirus Transmission Test in Six Rice Varieties

Samples of rice contained both types of tungrovirus (RTSV and RTBV) based on the results of detection by PCR then used as a source of inoculum. The acquisition of the virus was carried out by inoculating 200 green leafhoppers into confinement containing rice plants that were positively infected by the two tungroviruses over 24 hours for the acquisition feeding period. The inoculation feeding by green leafhoppers was conducted for 24 hours on healthy rice seeds of each variety aged 1 week using the test tube method (tube test). Each test tube (in a total of 10 tubes) containing 1 plant and 1 green leafhopper with 3 replications. Inoculated seedlings were then transplanted in pots containing planting media. Observation of symptoms was done after the plants aged 2 weeks and scoring based on the Standard Evaluation System for Rice (IRRI, 1996). The formula for Disease Incidence (I) and Disease Intensity (DI) (Zadoks & Schein, 1979) are as follows:

$$I = \frac{n}{N} \times 100\%$$

Remarks: I = disease incidence, n = number of plants affected by tungro, N = number of plants observed

$$DI = \frac{n(1)+n(3)+n(5)+n(7)+n(9)}{tn}$$

Remarks: DI= disease index, n = number of plants affected by tungro with a certain score, tn = total unhealthy plants according to the attack category, Z = highest disease symptom score, N = number of plants observed.

Percentage of table grouping disease severity based on plant disease symptoms

DIt (%)	Reaction
0–30	Resistant/tolerance
31–50	Slightly resistant/moderate
>51	Susceptible

Furthermore, the calculation results of the disease severity were used to classify the response of plant resistance to disease using IRRI classification (1996) (Table 1 and 2).

Table 1. Score assessment of tungro disease symptoms of rice plants in the greenhouse based Standard Evaluation System for Rice (IRRI, 1996)

Score	Criteria
1	Asymptomatic
3	1–10 % decrease of plant height, without leaf discoloration
5	10–30 % decrease of plant height, without leaf discoloration
7	31–50 % decrease of plant height, with leaf discoloration (yellow to orange)
9	50 % decrease of plant height, with leaf discoloration from yellow to orange

Table 2. Score assessment of tungro disease symptoms of rice in the field (IRRI, 1996)

Score	Category	Symptoms Type
0	Healthy	Asymptomatic
1	Mild	Slightly stunted and yellow leaves
2	Moderate	Stunted, withered or yellow leaves, tillers appear normal
3	Heavy	Stunted, yellow leaves, increased thetillers numbers
4	Crop failure	Very stunted, dry and yellow leaves, increased the tillers numbers

RESULTS AND DISCUSSION

Observation tungro disease symptoms on rice were carried out on the rice fields owned by farmers in several districts in South Sulawesi consisting of 19 observation locations and 4 districts (Wajo, Maros, Pinrang, and Sidrap). Several symptoms of tungro disease were found, i.e. yellowish leaf, twisting, stunted, and increasedtillers, with the disease incidence ranged of 10–55% (Table 3). It was suspected that the virus was transmitted by green leafhoppers in the previous planting season and then survived on weeds and the rest of the rice plants that have been harvested (*ratun*) around the rice fields. The disease intensity in the fields in Kabrinang was 5–10%, Maros Regency was 5-10%, Sidrap was 5–15%, and Wajowas 5–12%. Insect populationvector such as green leafhoppers also contributed to the growth of rice plants in the field, while observations on fields in several districts in South Sulawesi showed that the tungrovirus attack had a different disease intensity and insect population vector. The higher the number of insect populations vector, the higher the disease incidence caused, as in Sidrap has the highest disease incidence of 55% with a population of 10 insect vectors (Table 3).

The most likely symptom detected in the field was leaf discoloration and differences in the plant height (uneven growth) from visual observation. Tungro and stunted symptomatic plants were found clustered in one plot and there were uneven spots and plant growths seen on the rice fields. Vectors play an important role in the transmission and spread of the viruses. The highest population of green leafhopper obtained from Sidrap and Maros, ranged from 10–14 individuals, with disease incidence reached 50–55% (Table 3) showed that the higher the vector population density, the higher the disease incidence (Hibino & Cabunagan, 1986).

Tungrovirus Detection by PCR

PCR analysis results indicated that the presence of rice tungrovirus had been detected, namely RTBV and RTSV on plant samples obtained in several districts in South Sulawesi. Observation of disease incidence in the field was found with severe symptoms of tungro

Table 3.Disease incidence and insect population vector in several districts of rice production centers in South Sulawesi

Location	Variety	Age (DAP)	Planthopper Population				I (%)	Symptoms variation
			bp	gl	wp	zl		
Wajo Region								
Tonralipue -Tanah.Sitolo	Ciherang	30	0	5	0	1	10	ss,ys
Assorajang - T.Sitolo	Inpari 64	60	2	3	52	2	25	ys,ss,tl
Buloe - Maniangpajo	Mekongga	21	1	1	0	2	32	ss,ys
Maros Region								
Semangki - Simbang	Mekongga	25	1	0	0	0	15	ss
Jenetesa - Simbang	Mekongga	21	0	2	1	0	16	ss
Kalabirang - Bantimuriung	Inpari 7	30	0	6	0	0	18	ys
Alatengae - Bantimurung	Inpari 4	20	0	2	0	0	16	ys
Minasabaji - Bantimurung	Ciherang	14	5	4	0	19	10	ys
Leangleang - Bantimurung	Ciherang	15	12	4	0	2	18	ys
Borribelaya - Turikale	Inpari 3	60	7	14	0	0	50	ys,ss
Pinrang Region								
Paleteang - Paleteang	Inpari 32	60	1	1	1	0	15	ys,ss
Toe - Tiroang	Ciherang	40	1	3	1	2	25	ys
Sallo - Matirrosompe	Inpari 8	40	1	0	3	0	10	y
Sidrap Region								
Panreng - Baranti	St bagendit	80	3	7	0	16	15	ss,bs
Tangkoli - Baranti	Inpari 7	70	2	0	5	0	10	ys
Tonrongerijang - Baranti	Inpari 4	70	4	9	21	1	15	ys,ss
Kedidi - Pancarijang	Ciherang	14	0	9	0	7	16	ys,ss
Tanete - Maritengae	Ciherang	10	7	0	0	30	15	ys,ss
Carawali - Watangpulu	Inpari 4	60	2	10	5	0	55	ys,ss

Remarks: DAP: Days After Planting, I: Disease Incidence, bp: brown planthopper, gl: rice green leafhopper, wp: white-backed planthopper, zl: zigzag leafhopper, ss: stunted symptoms, ys: yellow symptoms, tl: twisted leaf, bs: brown spots on the leaves (Primer data sources in the field)

Figure 1.Variations of suspected disease symptoms of rice tungrovirus in Sidrap and Pinrang Regencies; (A) mild symptoms, (B) severe symptoms (Table 7)

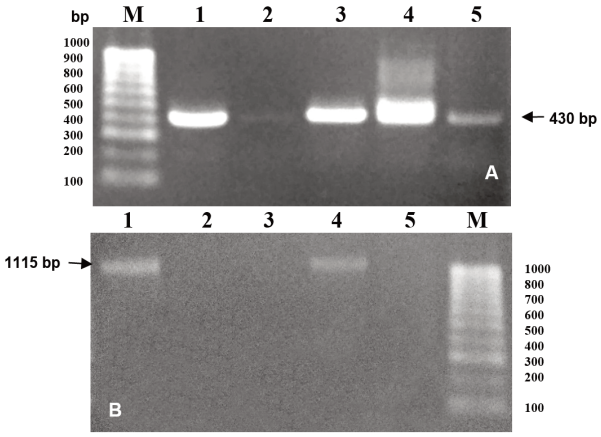

Figure 2. Visual detection of rice tungrovirus using primary pair RTSV-F/R (A) and RTBV-B2F/B2R (B) using PCR from several locations in South Sulawesi, (1) Control positive, (2): Wajo, (3) Sidrap, (4) Pinrang, (5) Maros, (M) Marker DNA Ladder 100 bp

disease in Pinrang District (Figure 1), this was proven by laboratory test results using PCR techniques showed that the Pinrang sample had been infected by RTSV with DNA band size of 1115 bp and RTBV of 430 bp (Figure 2). Other samples such as Sidrap and Maros isolates positively infected by RTBV, based on observations in the field with mild symptoms and also found a green leafhopper vector. Detection results indicated that the tungrovirus infection had transmitted by green leafhopper.

Sequencing Analysis

Homology analysis of RTBV showed that the first subgroup (Sidrap, Maros, and Pinrang) had a kinship of 97%. Meanwhile, the second subgroup (Philippines IC/G1) had a kinship of 94% and the third subgroup (Chainat-Thailand, Seberang Perai-Malaysia, and Serdang-Malaysia) of 92% (Table 4). The results of the dendrogram analysis showed that the sample from Sidrap had a very close relationship and belonged to one group with the sample from Maros and Pinrang, but had a close or different group from the Philippines, Malaysia and Thailand samples (Figure 3).

RTSV homology analysis showed that the RTSV nucleotide sequences of Pinrang had similarities between nucleotide bases and Subang (92.49%), Bali (92.69%), India (82.96%), Malaysia (84.38%), and Philippines (85.80%)(Table 5). This finding indicated that the RTSV sample in Indonesia had a close relationship of 92% compared to samples from other Asian regions, such as India, Malaysia, and the Philippines ranging from 82–85% (Table 5). This was similar to King *et al*. (2012) that a virus has a close kinship if it has a homology of nucleotide sequences >89%. Dendrogram of the genetic relationship between RTSV samples based on the nucleotide base sequence of polyprotein gene sequences showed that the Pinrang sample was in one group with AF113827–Subang and AF113823–Bali samples. (Figure 4). The variations in the nucleotide bases that make up the DNA and amino acid sequences produce the genetic diversity of RTSV samples. This is because the diversity of the nucleotide structure and virulence rate of the tungrovirus in Southeast Asia differ from the tungrovirus gene in South Asia (Azzam & Chancellor, 2002).

The Response of Six Rice Varieties to Tungrovirus

Based on theresistance test of six rice varieties showed that there were symptoms and different plant heights were observed, namely yellowish of leaves from the tips to the base (Figure 5). Disease Incidence (I) ranged from 40–100% with the highest Icame from TN1 variety (100%) and the lowest was Inpari 36 (40%). While the highest Disease Intensity (DI) was TN1 variety (60.66%, susceptible) and the lowest was Inpari 36 (22.21%, resistant). The average incidence of tungro disease from transmission in each variety ranged from 40–100%, with an average incubation period of 8–17 days, while the intensity of tungro disease ranged from 22–60% (Table 7).

All varieties inoculated with tungrovirus showed symptoms of the tungro diseasse. TN1 and Ciherang varieties showed the most severe symptoms until the plant became stunted and had a yellowish color.

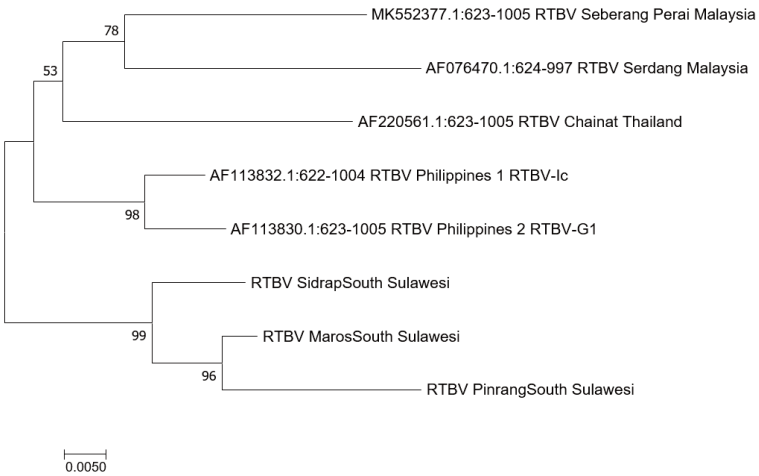

Figure 3. Phylogenic three of RTBV molecular from Maros, Sidrap, dan Pinrang with other various RTBV samples that had been published by Genbank data base NCBI

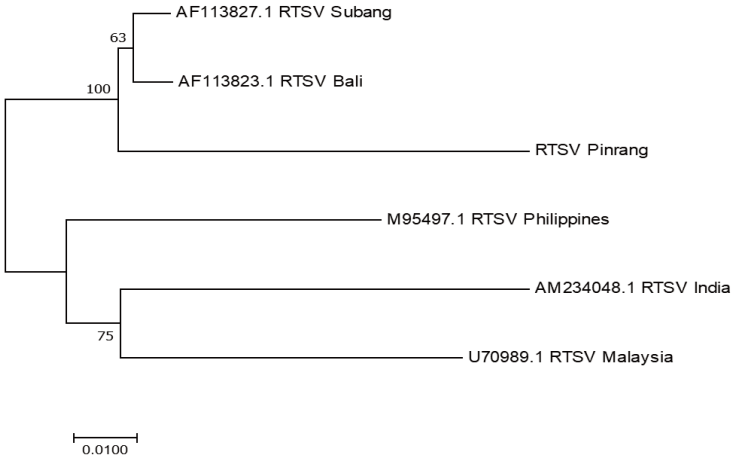

Figure 4. Phylogenic three of RTSV molecular from Maros, Sidrap, dan Pinrang with other various RTSV samples that had been published by Genbank data base NCBI

Table 4. Homology level of RTBV P4 gene nucleotide sequences on Maros, Pinrang, Sidrap, and other RTBV samples obtained from Genbank NCBI

No.	Sample Origin	Accession Code	Homology (%)						
			1	2	3	4	5	6	7
1.	RTBV Maros-South Sulawesi		ID						
2.	RTBV Pinrang- South Sulawesi		97.15	ID					
3.	RTBV Sidrap- South Sulawesi		97.72	95.44	ID				
4.	RTBV Philippines 1 RTBV- Ic	AF113832.1	94.58	92.87	94.58	ID			
5.	RTBV Philippines 2 RTBV- G1	AF113830.1	94.58	92.30	94.01	98.29	ID		
6.	RTBV Chainat - Thailand	AF220561.1	92.30	90.59	92.87	94.30	93.73	ID	
7.	RTBV Seberang Perai - Malaysia	MK552377.1	92.30	90.59	93.16	93.73	93.73	92.59	ID
8.	RTBV Serdang - Malaysia	AF076470.1	92.02	89.74	92.02	92.87	92.87	92.30	93.44

Table 5. Homology level of RTSV polyprotein gene nucleotide sequences on Pinrang samples and other RTSV samples obtained from Genbank NCBI

No.	Sample Origin	Accession Code	Homology (%)				
			1	2	3	4	5
1.	RTSV Pinrang- South Sulawesi		ID				
2.	RTSV Subang- West Java	AF113827.1	92.49493	ID			
3.	RTSV Bali	AF113823.1	92.69777	98.78296	ID		
4.	RTSV India	AM234048.1	82.96146	89.04665	89.24949	ID	
5.	RTSV Malaysia	U70989.1	84.38134	90.26369	89.85801	88.03245	ID
6.	RTSV Philippines	M95497.1	85.80122	91.48073	91.07505	87.62677	88.64097

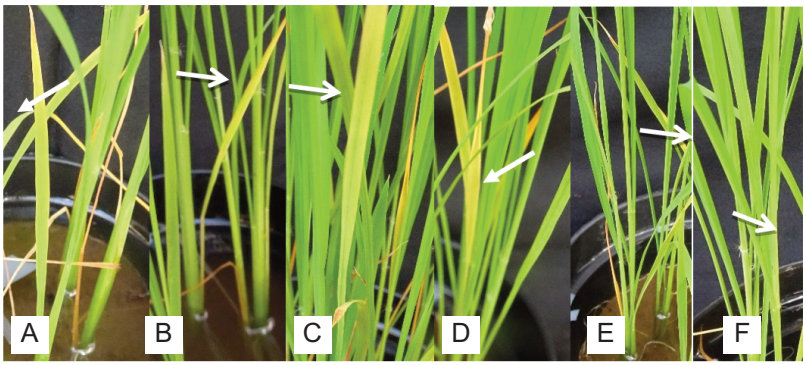

Figure 5. Variation of tungro disease symptoms on each variety aged 4 WAI (weeks after inoculation by RTSV and RTBV simultaneously); (A) TN1, (B) Ciherang, (C) Mekongga, (D) Tukad Unda, (E) Inpari 36, (F) Inpari 37

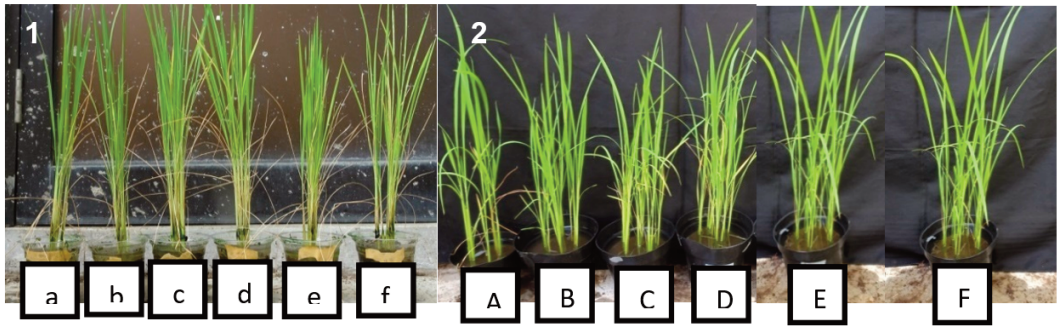

Figure 6. Plant aged 4 WAI (weeks after inoculation by RTSV and RTBV simultaneously); (1) control plant (without treatment), (2) plant with tungrovirus inoculation; (A, a) TN1, (B, b) Ciherang, (C, c) Mekongga, (D, d) Tukad Unda, (E, e) Inpari 36, (F, f) Inpari 37

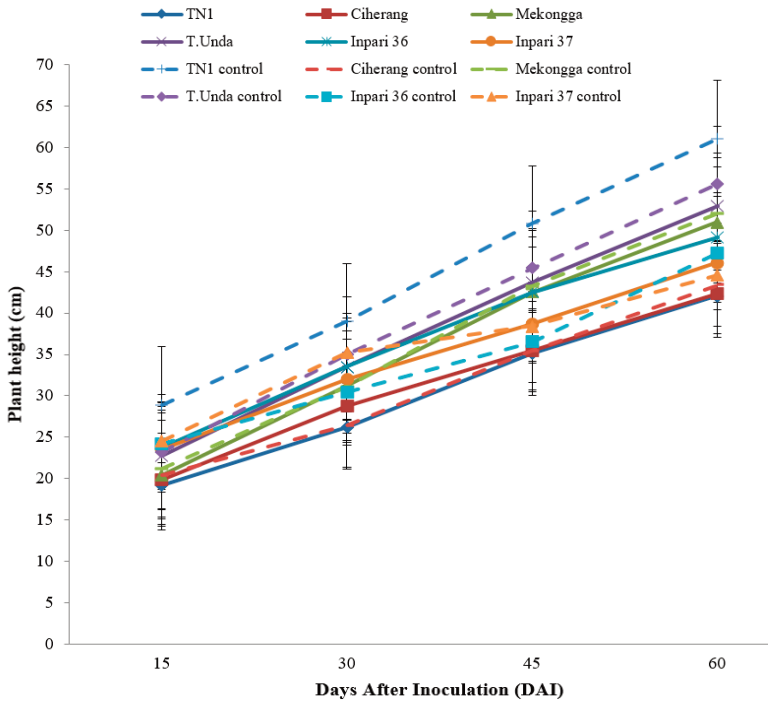

Figure 7. The plant height after inoculated by tungrovirus on several varieties in the greenhouse

Table 6. Scoring of tungro disease symptoms from inoculation in the greenhouse

No.	Variety	Scoring					Σ Sample
		1	3	5	7	9	
1	TN1	0	5	14	10	1	30
2	Ciherang	1	8	16	3	2	30
3	Mekongga	1	14	10	4	1	30
4	Tukad Unda	7	14	7	2	0	30
5	Inpari 36	18	9	3	0	0	30
6	Inpari 37	14	11	3	2	0	30

Table 7.The disease incidence and disease intensity of tungro

No.	Variety	IP (Days)	I (%)	DI (%)	Reaction
1	TN1	8.20d	100a	60.66a	susceptible
2	Ciherang	8.40d	96.66a	53.32ab	susceptible
3	Mekongga	10.90c	96.66a	48.14b	slightly resistant
4	TukadUnda	14.40b	76.66	36.29c	slightly resistant
5	Inpari 36	17.30a	40c	22.21d	resistant
6	Inpari 37	15.10b	53.33c	28.14d	resistant

Remarks: Means followed by the same letter in the same column were not significantlydifferentaccording to DMRT (P = 0.05). IP: Incubation Period, I:Disease Incidence, DI: Disease Intensity

Meanwhile, Mekongga, Tukad Unda, Inpari 36, and Inpari 37 varieties showed mild symptoms that were characterized by leaf discoloration, yellowish. The TN1 and Ciherang varieties showed more susceptible reactions compared to Mekongga, Tukad Unda, Inpari 36, and Inpari 37 varieties which is more resistant. The change in the resistance reaction with the inoculation test from susceptible to slightly resistant or resistant might be because the resistance of the varieties was specific to the strain of the virus at a particular region but not to the strain of the virus in other areas. According to Praptana *et al*. (2005), some varieties that are initially known to be resistant in one location would show a susceptible reaction in other locations, and previously susceptible varieties became resistance in other locations. The resistance of rice varieties to green leafhopper vectors is also determined by other factors, i.e. biochemical factors such as nutrient content and biophysical factors (plant tissue thickness or the interaction of these two factors on reproductive cells) hence it affects the number and hatching rate of green leafhopper eggs (Pakki, 2011).

Several varieties of rice tested showed that not all plants could be infected by the tungrovirus. The severity score of disease symptoms per plant was mostly 1, 3, and 5, and only a few plants were valued 7 and 9 (Table 6). Furthermore, there were differences in disease intensity between resistant, slightly resistant, and susceptible varieties (Table 7). Tungro disease symptoms were characterized by leaf discoloration from green to yellow-orange and stunted growth about 1–10% compared that to the control plants of each variety. Each variety could potentially be infected by the tungrovirus. The intensity of the disease with the same or different values depends on the variety planted. This finding showed that there were different varieties in responding to the tungrovirus infection by green leafhoppers.

CONCLUSION

Viruses detected using the PCR technique was *Rice tungro bacilliform virus* (RTBV) DNA band size of 430 bp from Maros, Sidrap, and Pinrang samples; and *Rice tungro spherical virus* (RTSV) DNA band size of 1115 bp from Pinrang sample. Transmission of the tungrovirus in the greenhouse showed that the response of resistance to six different rice varieties in terms of disease intensity, disease incidence, and incubation period. The highest percentage of tungro disease intensity was from TN1 variety, while the slightly resistant rice varieties were Mekongga and Tukad Unda, and resistantvarieties were Inpari 36 and Inpari 37.

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LITERATURE CITED

Azzam, O. & T.C.B Chancellor. 2002. The Biology, Epidemiology, and Management of Rice Tungro Disease in Asia. *Plant Disease* 86: 88–100.

[BPS] Badan Pusat Statistik. 2018. *Luas Panen dan Produksi Padi di Indonesia*.No. 83/10/Th. XXI, 24 Oktober 2018. Badan Pusat Statistik, Jakarta. https://www.bps.go.id/pressrelease.html, modified 10/10/ 2019.

Cabauatan, P.Q., Melcher, U., Ishikawa, K., Omura, T., Hibino, H., Koganezawa, H. &Azzam, O. (1999).Sequence changes in six variants of *Rice tungro bacilliform virus* and their phylogenetic relationships. *Journal of General Virology* 80, 2229–2237.

Choi, I.R., P.Q. Cabauatan, & R.C. Cabunagan. 2009. Rice Tungro Disease. Rice Fact Sheet, IRRI, Sep. 2009: 1–4.

Hasanuddin, A. 2002.*Pengendalian Penyakit Tungro Terpadu: Strategi dan Implementasi*. Orasi Pengukuhan Ahli Peneliti Utama. Badan Litbang Pertanian, Jakarta.

Hibino, H., M. Roechan, & S. Sudarisman. 1978. Association of Two Types of Virus Particles with Penyakit Habang (Tungro Disease) of Rice in Indonesia. *Phytopathology* 68: 1412–1416.

Hibino, H. & R.C. Cabunagan. 1986. Rice Tungro Associated Viruses and their Relation to Host Plants and Vector Leafhopper. *Tropical Agriculture Research*19: 173−182.

[IRRI] International Rice Research Institute. 1996. *Standard Evaluation System for Rice*. 4th Edition July 1996 INGER Genetic Resources Center, Manila,The Philippines. 52 p.

King, A.M.Q., M.J. Adam, E.B. Carstens, & E.J. Lefkowitz. 2012. *Virus Taxonomy Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses.* Elsevier Academic Press.Birmingham US.1327 p.

Mukhopadhyay, A.N. 1995. Rice Tungro. p. 429–444. *In* U.S. Sing, A.N. Mukhopadhyay, J. Kumar, H.S. Chaube (eds.), *Plant Disease of International Importance.Vol. 1.Disease of Cereals and Pulse.* Prentice May, New Jersey.

Omura, T., Y. Saito, T. Usugi, & H. Hibino. 1983. Purification and Serology of Rice Tungro Spherical and Rice Tungro Bacilliform Viruses. *The Phytopathological Society of Japan* 49: 73–76.

Pakki, S. 2011.Variabilitas Penyakit Tungro pada Beberapa Varietas Unggul Padi Inbrida di Wilayah Endemis, p. 1–8. *Seminar dan Pertemuan Tahunan XXI PEI, PFI Komda Sulawesi Selatan dan Dinas Perkebunan Pemerintah Provinsi Sulawesi Selatan.* Hotel Singgasana, Makassar, June 7, 2011.

Praptana, R.H. & A. Muliadi. 2005. Ketahanan Sepuluh Varietas Padi Lokal Nusa Tenggara Barat (NTB) terhadapTungro. p. 85–88. *In Prosiding Seminar Ilmiah dan Pertemuan Tahunan PBJ dan PFJ XVJ Komda Sulawesi Selatan.* Loka Penelitian Penyakit Tungro. Sulawesi Selatan, May 15–20, 2005.

Praptana, R.H., A. Bastian, & M. Yasin. 2005. Penyakit Tungro dan Pengendaliannya, p. 53–56. *In Prosiding Seminar Ilmiah dan Pertemuan Tahunan PBJ dan PFJ XVJ Komda Sulawesi Selatan.* Loka Penelitian Penyakit Tungro. Sulawesi Selatan, May 15–20, 2005.

Raga, I.N., W. Murdita, M.P.L. Tri, S.W. Edi, & Oman. 2004. Sistem Surveillance Antisipasi Ledakan Penyakit Tungro di Indonesia, p. 49–59. *In* A. Hasanuddin, I.N. Widiarta & Sunihardi (eds.), *Strategi Pengendalian Penyakit Tungro: Status dan Program, Prosiding Seminar Nasional Status Program Penelitian Tungro Mendukung Keberlanjutan Produksi Padi Nasional.* Makassar, September 7–8, 2004.

Shen, P., M. Kaniewska, C. Smith, & R.N. Beachy. 1993. Nucleotide Sequence and Genomic Organisation of *Rice tungro spherical virus. Virology* 193: 621–630.

Takahashi, Y., E.R. Tiongco, P.Q. Cabauatan, H. Koganezawa, H. Hibino, & T. Omura. 1993. Detection of *Rice tungrobacilliform virus* by Polymerase Chain Reaction for Assessing Mild Infection of Plants and Viruliferous Vector Leafhoppers. *Phytopathology* 83: 655–655.

Widiarta, I.N. 2005. Wereng Hijau (*Nephotettix virescens* Distant.): Dinamika Populasi dan Strategi Pengendaliannya sebagai Vektor Penyakit Tungro. *Jurnal Litbang Pertanian* 24: 85–92.

Zadoks, C.J. & R.D. Schein. 1979. *Epidemiology and Plant Disease Management*. Oxford University Press. New York. 427 p.