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Rice tungro disease in central Sulawesi Indonesia: Severity, insect vectors and viruses

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Abstract
Rice tungro disease (RTD) is one of the important rice pests and obstacles in increasing rice production in Indonesia causing crop failure. It is caused by a combined infection between rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) and is transmitted by several species of green leafhopper (GLH). This research aims to assess the severity level of RTD, identify the GLH species, and detect the tungro disease virus molecularly in Central Sulawesi, Indonesia. The survey was conducted at the rice production center at five regencies in Central Sulawesi situated at different altitudes namely, Tolitoli, Donggala, Parigi Moutong, Sigi, and Poso district. The GLH was collected using sweep nets for species identification morphologically while the rice samples showed RTD symptoms were taken for detection of the tungro viruses molecularly. The results showed that RTD attacks the rice at five regencies observed and its severity was quite high ranging from 20 % to 90 %. There were four species of GLH recorded belonging to *Nephotettix* Matsumura genus, that is: *N. virescens, N. nigropictus, N. parvus,* and *N. malayanus*. They were distributed differently at the study sites. *N. parvus* and *N. malayanus* were first recorded on Sulawesi Island. DNA amplification by polymerase chain reaction (PCR) showed all rice plants collected from five districts in Central Sulawesi were infected by RTBV.

Keywords: Rice disease, Nephotettix, tungro virus, Sulawesi, distribution

Introduction
Rice crops in Asian countries experience significant yield losses due to rice tungro disease [1]. This disease is one of the most important diseases in rice and is a constraint for increasing rice production in Indonesia [2]. The attack area of Rice tungro disease (RTD) in the past three years is still high where the cumulative attack of this pest is 23.355 hectares in Indonesia and 376 hectares in Central Sulawesi [3]. The RTD is caused by a virus with two species of viruses, which are rice tungro bacilliform virus (RTBV) and ruce tungro spherical virus (RTSV). They can co-infect the plants or individually but visible signs of tungro infection are seen when both viruses are detected. The RTBV might play a more critical role in disease severity, with RTSV amplifying the damage [1, 4, 5]. When both RTSV and RTBV are present in a plant, the former causes no discernible symptoms, the latter causes mild stunting and mild yellowing of the leaves, and the latter results in mottled leaves, severe stunting, and yellow to orange discoloration of the leaves, which significantly reduces yield up to 100% yield loss [4, 5]. Tungro viruses are semi-persistently transmitted by the green leafhopper (GLH) that belongs to Ordo Hemiptera, Family Cicadellidae, Sub family Deltocephalinae and genus Nephotettix and Recilia. Eight species and one subspecies of the genus *Nephotettix* Matsumura, have been reported namely; *N. virescens* (Distant), *N. nigropictus* (Stal), N. cincticeps (Uhler), N. *modulatus* Melichar, N. *malayanus* Ishihara & Kawase, N. *parvus* Ishihara & Kawase, *N. afer* Ghauri, *N. sympatricus* Ghauri and *N. nigropictus* (Stal) s.sp. Yapicola (Linnavuori). Of all GLH *N. virescens* is the most dominant and efficient vector of RTD [6, 7]. The morphological characteristics of the GLH that are important to be observed in species identification are : body color, specific markings on vertex, pronotum and scutellum, shapes and structures of head, spots on forewing, body length, and presence of spines on male pygofer and aedeagus [7, 8, 9, 10] . According to the LAW of the Indonesian Republic No. 22 of 2019 on the Sustainable Agriculture Cultivation System, crop pests should be managed by using Integrated Pest Management (IPM). However, effective control of pest control cannot be developed without knowledge of pest biology and ecology, and in IPM, monitoring, and correct pest identification will help us to decide the best pest control technique to be implemented [7, 12]. Studies on the green leafhoppers and the transmitted viruses have been conducted on several islands in Indonesia including Sulawesi [2, 6, 13, 14, 15]. However, there is only one related study reported in Central Sulawesi, so far [16]. Therefore this study aims to: asses the severity level of RTD, identify the insect vectors of tungro disease as well as to detect the tungro viruses in rice plants and in Central Sulawesi, Indonesia. Therefore, this study aims to assess the severity level of RTD, identify the insect vectors of tungro disease as well as to detect the tungro viruses in rice plants and in Central Sulawesi, Indonesia. In this study, the tungro virus to be detected is Rice Tungro Bacilliform Virus (RTBV) as it has a broader regional distribution in South and Southeast Asia [17, 18]. It has been reported that Asian rice cultivars and their ancestor wild species carry RTBV, which is widely distributed throughout rice-producing regions in Asia [11, 19].

Materials and Methods
The rice crops infected by RTD were observed in five districts in Central Sulawesi which are the rice production centers, namely: Tolitoli, Donggala, Parigi Moutong, Sigi, and Poso District during January to Mei 2024. The study sites were varied in altitude and rice variety cultivated (Table 1).

Table 1: Description of study sites

Regency	Coordinate	Altitude (m. asl)	Rice Varieties
Tolitoli	0.7776 N 120.22132 E	71.1	Sri Huning, Mekongga, Cisantana
Donggala	0.49106 N 120.02634 E	71.3	Impari 36, Cimandi
Parigi Moutong	0.52398 N 120.63458 E	79.9	Pemburu, Impari 6, Super, Buriburi Mas
Sigi	-1.13856 S 120.05898 E	639.0	Tabe Tabe, Mekongga
Poso	-1.3431 S 120.33471 E	1297.7	Ciherang, Impari 36, Peluncur

In each district, five plots were selected to determine the level of RTD attacks by referring to the formula and severity score formula of the International Rice Research Institute [20]:

$$IS = \frac{n(1) + n(3) + n(5) + n(7) + n(9)}{N}$$

Where:
IP = Index of severity
N = Number of plants attacked by RTD with its score level
n = Total number of plants observed
The green leafhoppers were collected using sweep nets at each sample location and taken to the laboratory Plant Disease Laboratory, Faculty of Agriculture, Tadulako University, Palu, Central Sulawesi to be identified based on their morphological characters. Observation and measurements were using a D Electron Microscopy Science Olympus Inverted Microscope (Tipe: CT-ZM6745T-J4L) connected with a Camera with Sony Sensor (Tipe: XCAM1080PHB) and species identified by refers to published taxonomic keys [7, 8, 9, 10]. The plant samples taken were all parts of the rice plant from roots, stems, to leaves that showed symptoms of rice infected with tungro. Rice samples showing symptoms of infection were selected randomly in each regency, then the roots, stems and leaves of the rice were taken, washed with tap water and dried. The rice samples then sterilized using 70% alcohol for 30 seconds and rinsed again with distilled water. A small pieces of rice (± 5 cm) was cut and grind it until smooth using a mortar, then weighed as much of 25 gr/sample. The RTBV detected using a PCR method as described by Ladja *et al*., 2016 [21].

Data analysis
Severity level of RTD data was analyses using analysis of varians (ANOVA) then following with Tukey HSD test. PCR electrophoresis results visualized using DNA gel documentation band pattern (Uvitec Cambridge, England) were analyzed by comparing the presence or absence of DNA bands formed on the agarose gel with DNA fragment sizes between 1000-1500 base pairs (bp) from rice DNA samples in several regencies.

Results and discussion severity of RTD
The rice attacked by RTD was recorded at five regency in Central Sulawesi with various level of severity. The symptoms of RTD observed include leaves that are yelloworange and twisted, stunted plants and lack of tillers (Fig 1).

Fig 1: The rice crops showed the symptoms of RTD in the five districts in Central Sulawesi; **A**. Tolitoli Regency: Lempe Village, South Dampal District, **B**. Donggala Regency: Siboang Village, Sojol District, **C**. Parigi Moutong Regency: Tilung Village, Tomini District, **D**. Sigi Regency: Makmur Village, Palolo District, **E**. Poso Regency: Sedoa Village, North Pamona District.

The severity of RTD varies among the regency between 20 % attack rate of RTD in Tolitoli, Parigi Moutong, and Poso - 90%. The lowest attack rate was in Donggala Regency (20%) regency. However, was not significantly different (Figure 2).
While the highest was in Parigi Moutong Regency (90%). The

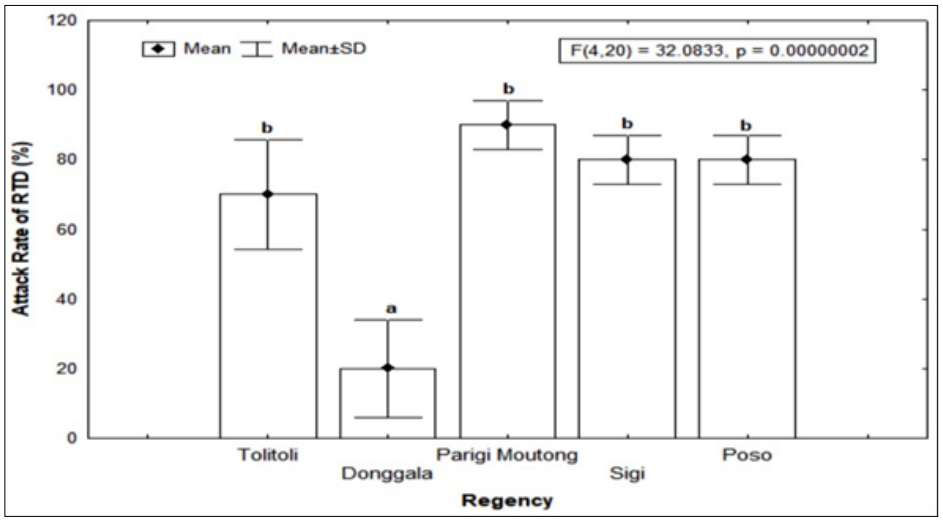

Fig 2: The attack rate of RTD in five regency in Central Sulawesi

Differences in RTD severity in the study area might be related to GLH species attacked, rice variety planted, cultivation practices, and the climatic and the weather [1, 2, 22]. As reported by several studies of all GLH, N. virescens is the most dominant and efficient vector of RTD [6, 7, 23]. Planting a certain rice variety for a long time can decrease the variety's resistance to tungro viruses due to the low biological and genetic variations of the tungro virus in that area [1].The degree and frequency of contact between green leafhoppers and these rice varieties in the field will cause the resistance of these varieties to green planthoppers to gradually decline. Meanwhile, in most cases, asynchronous planting patterns like in Sigi Regency and Poso Regency have been implemented for a long time, making them the inoculum source for other rice crops. An intensive use of pesticides is also influencing the severity level of RTD. The majority of rice farmers in Parigi Moutong and Sigi regency (more than 86%) used pesticides to control weeds, pests, and diseases. They applied pesticides eight times per planting season [24]. Pesticide use can suppress the number of eggs laid by vector insects, which decreases the risk of tungro attacks in endemic locations [25]. However, cultivation practices such as adjusting planting dates, rotation of resistant varieties to tungro virus, and strip intercropping of different rice varieties are more recommended to manage the RTD [26, 27].

The green leafhopper

The study found four species of the green leafhopper belonged to the *Nephotettix* Matsumura genera, that is: *N. virescens*, *N. nigropictus*, *N. parvus*, and *N. malayanus*. Those GLS species is distinguished mainly based on their head and pronotum characteristics (Fig 3).

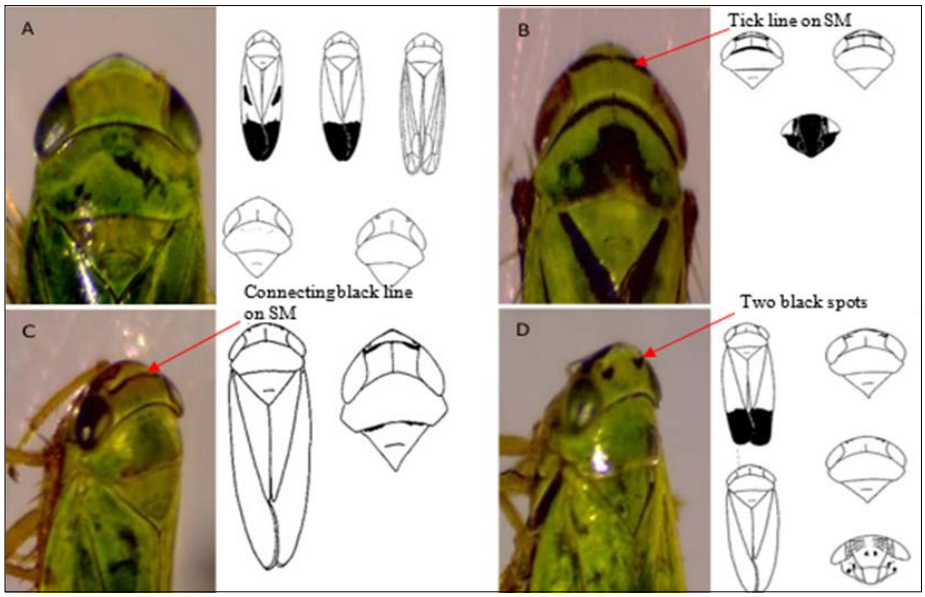

Fig 3: The head and pronotum character of green leafhoppers found in the rice fields in Central Sulawesi
A: *N. virescens.* The tip of the head is pointed (pointed vertex). No black marks on head, pronotum and scutellum,
B: *N. nigropictus*, the tip of the head is round (vertex not pointed). The thick line is on the sub marginal (SM). The anterior pronotum is thicker black.
C: *N. parvus.* Pointed vertex. In the sub marginal (SM) part there is a connecting black line,
D: *N. malayanus*. Vertex not pointed. It has two black spots on the basal fronts [7, 8]. The illustrated picture on the right side of each GLH species is adapted from Wilson and Claridge 1991[8]

Compared with the previous study conducted in Sulawesi Island, this study found quite a difference in the recorded GLH (Table 2). In South Sulawesi *N. nephotettix* is found at lowland (< 100 m ASL) and mid altitudes (300 – 600 m. Asl.) but not at high altitudes (> 1000 m asl.) while *N. nigropictus* is only found at high altitudes [23]. However, this study recorded both species at all regencies from lowland to highland altitudes. Only one species; *R. dorsalis* has not been found in Central Sulawesi and interestingly this is the first study reporting the presence of *N. parvus* and *N. malayanus* on Sulawesi Island.

Table 2: Green leafhoppers and rice tungro virus found in Sulawesi, Indonesia

GLH Species	Central Sulawesi					South Sulawesi*
	Tolitoli	Donggala	Parigi Moutong	Sigi	Poso	
1. *N. virescens*	+	+	+	+	+	+
2. *N. nigropictus*	+	+	+	+	+	+
3. *N. parvus*	+	-	+	-	+	-
4. *N. malayanus*	-	-	-	-	+	-
5. *Recilia dorsalis*	-	-	-	-	-	+

Note: +; recorded, -; not recorded, * Hutasoit *et al*. 2023 [2].

The rice tungro bacilliform virus (RTBV)

Visualization of the results of Rice Tungro Bacilliform Virus Virus (RTBV) consisting of a pair of primers (RTBV-DA F (RTBV) DNA amplification using PCR using a pair of and RTBV-DA R) can detect the presence of RTBV in the specific primers for amplification of Rice Tungro Bacilliform five regencies in Central Sulawesi (Figure 4).

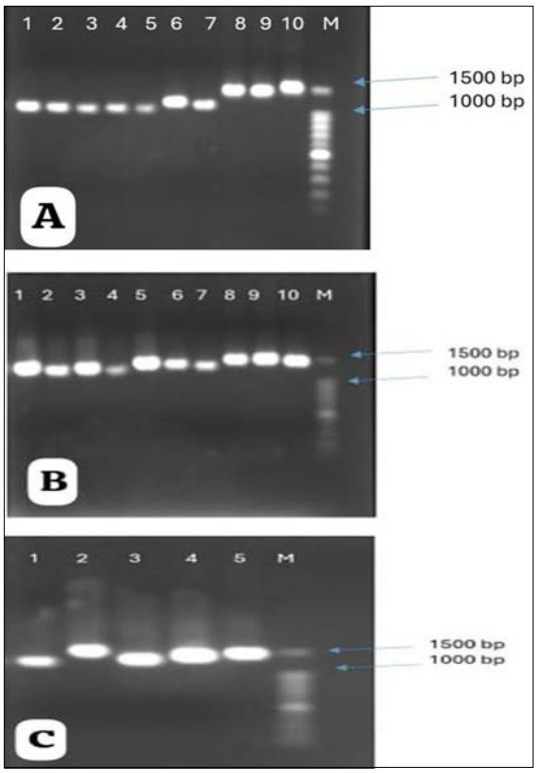

Fig 4: Visualization of DNA amplification of RTBV which causes tungro virus. **A**. Visualization of RTBV DNA amplification that causes Tungro rice virus by PCR using a pair of primers (RTBV-DA F and RTBV- DA R) with amplicons of 1000-1500 bp in Sigi and Donggala regencies (1; SG1, 2; SG2, 3; SG3, 9; DGL5, 6, M), **B**. Poso and Parigi Moutong (1; PS1, 2; PS2, 3; PS4, 5; PS5, 6, M), **C**. Tolitoli (1; TL1, 2; TL2, 3; TL3, 4; TL4, 5; TL5, M). M; Markers 1000-1500 bp. All negative samples always accompanied in each PCR although not shown in the final electrophoresis.

The Rice Tungro Bacilliform Virus which is successfully detected in this study is more widespread in South and Southeast Asia [17, 18]. The RTBV that is widely distributed in Indonesia has a high similarity of DNA sequences and amino acids particularly those isolated from Central Sulawesi, West Nusa Tenggara [16]. RTBV can infect the plants with RTSV either concurrently or separately but visible tungro infection symptoms are observed when both viruses are present in plants. Furthermore, RTBV could potentially have a greater impact on the severity of the disease, while RTSV may worsen the harm [1, 4, 5].

Conclusion

The severity of RTD in five regencies in Central Sulawesi is quite high ranging from 20% to 90 % covering from lowland to highland rice. Four species of Nephoptettix were found, namely; *N. virescens*, *N. nigropictus*, *N. parvus*, and *N. malayanus*. Those species were distributed differently according to the altitudes of the study sites. The two latter species are first recorded in Sulawesi Island. DNA amplification by polymerase chain reaction showed all rice plants collected from five regencies in Central Sulawesi were infected by Rice Tungro Bacilliform Virus.

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