Green leafhopper (*Nephotettix virescens* Distant) biotype and their ability to transfer tungro disease in South Sulawesi, Indonesia

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Abstract. The study was conducted to determine the variation of the green leafhopper biotype in South Sulawesi and their ability to transmit the tungro virus. GLH colonies were collected from several districts in South Sulawesi (Sidrap, Pinrang, Maros and Gowa). Green leafhopper colonies were selected for their population growth and their ability to transfer tungro virus. All GLH colonies from Pinrang, Sidrap, Gowa, and Maros have a high level of virulence in all resistant varieties, where the percentage of nymphs becomes the second instar and the number of green leafhopper populations in one life cycle (first-generation / F1) in all resistant varieties were not significantly different from TN1 (no gene for resistance). Among resistant varieties, IR 38 (Glh 6) was the most adaptive variety to the four colonies of green leafhoppers (Pinrang, Sidrap, Gowa, and Maros) with the highest average population. All GLH colonies are able to transfer tungro virus to all resistant varieties. The percentage of tungro virus infection ranged from 50% to 100% in all resistant varieties transmitted by each GLH. The ability of GLH colonies to transfer viruses ranked from high to low, Sidrap, Pinrang, Gowa, and Maros. Of all GLH resistant varieties tested, no more varieties were resistant to GLH colonies from Sidrap. There are three variants colonies of GLH were successfully identified, namely biotypes 1650, 1654, and 1604.

1. Introduction

Tungro disease is the most destructive virus disease on rice industry in Indonesia. The disease is caused by double infections of Rice tungro Bacilliform Virus (RTBV) and Rice tungro Spherical Virus (RTSV) and those kinds of viruses are transmitted by only green leafhopper with a semi-persistent manner [1,2]. *Nephotettix virescens* (Distant) is one of the green leafhopper species found in Indonesia [3,4]. Apart from *N. virescens*, *N. nigropictus* is also sometimes found in South Sulawesi [5]. *N. virescens* is the most dangerous green leafhopper species because it develops faster and more efficiently transmits the tungro virus than other species such as *Rosilia dorsalis* and *Nephotettix nigropictus* [6].

Green Leafhopper (GLH) is usually controlled with an insecticide. Farmers are encouraged to use high levels of nitrogen fertilization and insecticide applications [7]. But nowadays awareness about the impact of pest control with chemicals is becoming more open. In recent decades, research on
resistance rice varieties is the main alternative in controlling planthopper and green leafhopper through molecular breeding [8, 9]. There are 14 genes of rice resistance to green leafhoppers that have been found, i.e Glh1, Glh2, Glh3 [10], Glh5 [11], glh4 recessive genes [11], Glh6 and Glh7 [12], glh8 recessive genes [13], Glh9 [14], glh10 recessive genes, Glh11 [15], Glh12, Glh13 [16] and Glh14 [17]. Among the 14 resistance genes, there are four resistance genes that have been used in Indonesia [18]. About 28 years ago in South Sulawesi tungro disease was successfully controlled, by combining the right planting time and rotation of green leafhopper resistant varieties. Rotation of resistant varieties is distinguished based on the parent resistance gene against *N. virescens* into four groups: T0 = group of varieties that do not have resistant parent; T1 = group of varieties that have parents who are resistant to the Glh1 gene; T2 = group of varieties that has resistant parents with the Glh6 gene; T3 = a group of varieties that have parents who are resistant to the Glh5 gene; T4 = variety group that has resistant parents with the glh 4 gene [19]. At present, rotation patterns of green leafhopper resistant varieties are no longer found in South Sulawesi. The tendency of farmers to plant certain varieties that have high yields and the taste of rice according to their preferences, causing farmers not to choose to plant varieties that are resistant to green leafhoppers [20]. Resistant varieties will select green leafhopper populations for their ability to adapt to resistant varieties. The level of adaptation of green leafhopper to resistant varieties can also be seen from its ability to transmit the tungro virus. Based on the transmission, differences in physiological characteristics between populations (colony variants) can be identified. Biotypes have been used to express differences in physiological abilities between brown plant hopper colonies resulting from the selection in a greenhouse [21]. Biotype characterization is carried out based on its ability to survive and reproduce and transmit the virus to varieties containing certain resistant genes [22, 4]. In this study, we tested the ability of green leafhoppers to survive and reproduce and transmit the tungro virus in green leafhopper resistant varieties to get a picture of changes in the green leafhopper biotype in South Sulawesi.

### 2. Methodology

The study was carried out at the Greenhouse of Indonesian Tungro Disease Research Station, Lanrang, Sidenreng Rappang from August until December 2018. GLH colonies were collected from rice fields with a sweep net. Ten pairs of GLH from each area were maintained and developed in TN1 varieties (without resistant genes). The pairs of insects were left laying eggs for a week in oviposition cages, then transferred to another cage for next egg-laying for several generations. In this way, uniform insects can be found in one cage. The rice varieties used in this study are listed in table 1.

| Varietas | Group | Donor parent for *N. virescens* resistance | Resistance gene | Reference |
|----------|-------|-------------------------------------------|-----------------|-----------|
| TN1      | T0    | -                                         | No resistant gene | -         |
| IR 46    | T1    | Pankhari 203                              | Glh 1           | [10, 19, 23] |
| IR 38    | T2    | TAPL 796                                  | Glh 6           | [12, 19, 23] |
| Ciliwung | T2    | TAPL 796                                  | Glh 6           | [12, 19, 23] |
| IR 64    | T3    | ASD 8                                     | Glh 5           | [11, 19, 23] |
| IR 48    | T3    | ASD 8                                     | Glh 5           | [11, 19, 23] |
| IR 66    | T4    | Ptb8                                      | glh 4           | [11, 19, 23] |

Glh: gen dominan; glh: gen reesif

#### 2.1. Population growth study

Population growth study was carried out with following the methods [24, 25]; which was modified. Each variety was placed in a cylindrical cage and infested with 4 pairs of 3-d-old *N. virescens* adults. All varieties were arranged in a randomized block design with 3 replications. The number of F1
generations was recorded 30 days later. If the amount of GLH in resistant varieties is equal to or greater than the amount of GLH in TN1 (no gene for resistance), then the green leafhopper colonies are virulent biotypes in resistant varieties.

2.2. Tungro virus transmission test
Each GLH colony (3-d-old N. virescens adults) was placed into an insect cage containing rice plant infected with the tungro virus to acquire the viruses for 24 hours (24- hours acquisition feeding period). After that, the viruliferous GLH was fed on 10-day old seedlings of five different resistance groups of rice varieties (table 1) for 24 hours. Two adults were transferred per hill; 10 replicates per variety arranged in a completely randomized design. The percentage of Tungro infection was determined using the formulation method [25]:

\[
I = \frac{\text{Number of plant infected}}{\text{Number of plant inoculated}} \times 100\%
\]

The average of infected percentage was undertaken to evaluate the reaction as follows: 0-30% (resistant); 31-60% (intermediate) and 61%-100% (susceptible).

2.3. Statistical analysis
Generation 1 population (F1) and the percentage of nymphs developing to second instar were analyzed using ANOVA and the Tukey method after arcsine data transformation. The percentage of tungro incidents was analyzed using Anova if there were differences between treatments (variety groups) followed by the DMRT at 0.05 using the SPSS program ver.21.

2.4. Naming the GLH colony variant
The GLH colony variant was classified based on its level of adaptation to the resistant genes of the variety groups tested following the method used by [18]. The naming of variations of GLH colony uses four numbers derived from resistant genes. In the group of GLH varieties that have been adaptive, the resistant gene numbers are included; on the contrary, GLH groups that have not yet adapted are numbered 0. With this procedure, the GLH biotype which has been adapted to four resistant varieties is called colony 1654, while GLH colonies that have not adapted to the four resistant varieties are named colony 0000. In this way, a combination of possible adaptations GLH can be coded with four digits according to its ability to survive, develop and transmit the tungro virus in four groups of resistant varieties.

3. Result and discussion
The virulence test of green leafhopper colonies against several resistant varieties was carried out by counting the population of the first generation of green leafhopper colonies (F1) and the percentage of development of nymphs becoming second instars (figure 1). All green leafhopper colonies from Pinrang, Sidrap, Gowa, and Maros have high virulence levels in all differential varieties (IR 46, IR 38, Ciliwung, IR 64, IR 48 and IR 66), where the population green leafhoppers in one life cycle (first-generation / F1) in all resistant varieties were not significantly different from TN1 varieties. Green leafhopper with biotype 170 in Bali and West Nusa Tenggara (NTB) has adapted to all GLH resistant varieties (T1, T2, T3 and T4 / IR 26, Ciliwung, IR64, Barumun) [21]. In this study, GLH colonies from Pinrang, Sidrap, Gowa, and Maros can grow and reproduce in resistant varieties controlled by GLH resistant genes (Glh 1, Glh 5, Glh 6 and glh 4). These results indicate that all GLH colonies have deadly genetic variations in resistant varieties. Unbalanced genetic variation in the population will change the virulence of GLH [26]. In addition, the resistance of resistant varieties can be broken by virulent GLH from South Sulawesi (Pinrang, Sidrap, Gowa, and Maros). N. virescens that is maintained continuously on resistant varieties will remain virulent in resistant varieties. But quickly loses their virulence when maintained in susceptible varieties [27].
Among resistant varieties, IR 38 (Glh 6) was the most adaptive variety to the four colonies of green leafhoppers (Pinrang, Sidrap, Gowa, and Maros) with the highest average population. In contrast to the report of Trisnaningsih et al (1999) that, Ciliwung (Glh 6) is still resistant to N.virescens colonies in South Sulawesi with a survival rate of 10% [28].

![Graphs of population growth test columns and vertical bars show the means and standard errors of the population of GLH 30 day after infestation (DAI). For each line, columns with the same letter are not significantly different in virulence among the rice varieties at 0.05 Tukey’s test.](image)

**Figure 1.** Virulence of selected colony of *N.virescens* among rice varieties in the population growth test columns and vertical bars show the means and standard errors of the population of GLH 30 day after infestation (DAI). For each line, columns with the same letter are not significantly different in virulence among the rice varieties at 0.05 Tukey’s test.

The percentage of tungro virus infection ranged from 50% to 100% in all resistant varieties transmitted by each GLH Pinrang, Sidrap, Gowa, and Maros (table 2). The percentage of tungro virus infection in all resistant varieties (IR 46, IR 38, Ciliwung, IR 64, IR 48 and IR 66) was not significantly different from TN1 variety. The percentage of tungro virus infections in IR 66 varieties transmitted by Pinrang GLH colony is 60% with intermediate resistant category. Likewise, the percentage of tungro infections in IR 48 varieties transmitted by colonies of GLH from Gowa and Maros were 60% and 50% respectively with the category of intermediate resistance. Although the vector population is the same in every treatment, it has a different ability to transfer tungro virus. The high viral virulence can cause a faster infection process [29]. The difference in the level of virus transmission by GLH is one of the bases for controlling the tungro virus through rotation of varieties [19]. The system of cropping patterns that are not synchronous in Bali illustrates that varieties with different sources of resistance genes have different levels of presence of tungro in each of these varieties are also different [30].
Table 2. Response of differential varieties plants to the tungro virus transmitted by green leafhoppers \textit{(N.virescens)} from several districts in South Sulawesi

| No | GLH population | Rice variety/Resistance gene | Percentage of infections (%) | Reaction |
|----|----------------|------------------------------|----------------------------|----------|
| 1  | Pinrang        | TN1 (-) (kontrol)           | 90.0 a                     | susceptible |
|    |                | IR 46 (Glh 1)               | 80.0 ab                    | susceptible |
|    |                | IR 38 (Glh 6)               | 80.0 ab                    | susceptible |
|    |                | Ciliwung (Glh 6)            | 100.0 b                    | susceptible |
|    |                | IR 64 (Glh 5)               | 100.0 b                    | susceptible |
|    |                | IR 48 (Glh 5)               | 80.0 ab                    | susceptible |
|    |                | IR 66 (glh 4)               | 60.0 ab                    | intermediate |
| 2  | Sidrap         | TN1 (-) (Kontrol)           | 80.0 a                     | susceptible |
|    |                | IR 46 (Glh 1)               | 100.0 a                    | susceptible |
|    |                | IR 38 (Glh 6)               | 100.0 a                    | susceptible |
|    |                | Ciliwung (Glh 6)            | 90.0 a                     | susceptible |
|    |                | IR 64 (Glh 5)               | 90.0 a                     | susceptible |
|    |                | IR 48 (Glh 5)               | 100.0 a                    | susceptible |
|    |                | IR 66 (glh 4)               | 100.0 a                    | susceptible |
| 3  | Gowa           | TN1 (-) (kontrol)           | 100.0 b                    | susceptible |
|    |                | IR 46 (Glh 1)               | 100.0 b                    | susceptible |
|    |                | IR 38 (Glh 6)               | 100.0 b                    | susceptible |
|    |                | Ciliwung (Glh 6)            | 100.0 b                    | susceptible |
|    |                | IR 64 (Glh 5)               | 80.0 ab                    | susceptible |
|    |                | IR 48 (Glh 5)               | 60.0 a                     | intermediate |
|    |                | IR 66 (glh 4)               | 80.0 ab                    | susceptible |
| 4  | Maros          | TN1 (-) (kontrol)           | 100.0 ab                   | susceptible |
|    |                | IR 46 (Glh 1)               | 100.0 ab                   | susceptible |
|    |                | IR 38 (Glh 6)               | 100.0 ab                   | susceptible |
|    |                | Ciliwung (Glh 6)            | 100.0 ab                   | susceptible |
|    |                | IR 64 (Glh 5)               | 80.0 ab                    | susceptible |
|    |                | IR 48 (Glh 5)               | 50.0 a                     | intermediate |
|    |                | IR 66 (glh 4)               | 80.0 ab                    | susceptible |

Means followed by the same letter are not significantly different among rice varieties based on 0.05 Tukey test.

In contrast to the results of the study of Widiarta et al. (2015) who recommended planting a resistant group of rice varieties (T1) in South Sulawesi because they were still resistant to GLH [4], whereas in this study the T1 (Glh1) & T2 (Glh6) group varieties was infected with a virus transmitted by GLH colonies from Pinrang, Sidrap, Gowa and Maros. IR 48 (T3) varieties are intermediate resistant to GLH Gowa and Maro's colonies. IR 66 (T4) varieties are intermediate resistant to GLH colonies from Pinrang but are not resistant to GLH colonies from Sidrap, Gowa and Maros. Of all GLH resistant varieties tested, no more varieties were resistant to GLH colonies from Sidrap. To control tungro disease, it is necessary to assemble resistant varieties with new sources of green leafhopper resistant genes, for example, Glh 7- Glh 14 from Moddai Karupan varieties that are known to be still resistant [28, 9] or use strains with genes pyramid resistant [31, 32] or using tungro virus resistant elders [33].

All GLH colonies are able to transfer tungro virus to all resistant varieties. The ability of GLH colonies to transfer virus ranked from high to low, Sidrap, Pinrang, Gowa, and Maros (table 2). Three variants of green leafhopper biotypes were identified, namely colonies 1650, 1654, and 1604. Biotypes 1650 from Pinrang have adapted to almost all resistant varieties except with resistant varieties with the resistance gene glh4 (IR 66). Biotype 1654 has adapted to all resistant varieties in Sidrap. Biotype
1604 found in Gowa and Maros has adapted to almost all resistant varieties except with resistant varieties with the resistance gene Glh 5 (IR 48).

4. Conclusion

Based on population development test and transmission efficiency of tungro virus, it is known that: 1) GLH colonies from Pinrang, Sidrap, Gowa and Maros are able to develop (virulent) in all GLH resistant varieties (IR 46, IR 38, Ciliwung, IR 64, IR 48 and IR 66); 2) All GLH colonies are able to transfer tungro virus to all resistant varieties. The ability of GLH colonies to transfer virus ranked from high to low, Sidrap, Pinrang, Gowa, and Maros. Almost all GLH colonies from all regions have high adaptability to all resistant varieties. There are three variant colonies of GLH that were successfully identified, namely biotypes 1650, 1654, and 1604. Biotype 1654 in Sidrap has been adaptive in all varieties of GLH resistance.

Acknowledgments

The work was funded by the Indonesian Agency Agricultural Research and Development Ministry of Agriculture. Thank is also extended to Mr. Yusran Arifin for his assistance in collecting experimental data.

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