

VARIATION IN RICE TUNGRO VIRUS TRANSMISSION ABILITY BY GREEN LEAFHOPPER, *Nephotettix virescens* DISTANT (HOMOPTERA: CICADELLIDAE) ON RICE RESISTANT VARIETIES

Variasi Efisiensi Penularan Virus Tungro oleh Wereng Hijau, Nephotettix virescens Distant (Homoptera: Cicadellidae) pada Beberapa Varietas Padi Tahan

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ABSTRACT

Green leafhopper (GLH), *Nephotettix virescens*, is the most efficient vector of rice tungro virus disease. The disease is endemic in some provinces of Indonesia and commonly controlled using resistant varieties. Resistance of rice varieties to tungro could be classified into resistance to a virus and a vector. The history of GLH resistant varieties adoption affected the GLH adaptation in an area. The study was conducted in the period of 2009-2011 to evaluate the resistance status of five GLH resistant rice variety groups (T0-T4) using survival and transmission test. The GLH populations were collected from 15 tungro endemic provinces in Indonesia. The GLH was then reared in the greenhouse before used for the test. The degree of resistance to tungro viruses was calculated by adding the value of survival (weight x score of survival rate) and virus transmission rate (weight x score of transmission rate). The weights for survival and transmission rate were set to 40 and 60, respectively. The results showed that the rank of resistant variety groups in decreasing order of resistance were T4, T1, T2 and T3. Five variations in GLH transmission efficiency were identified, i.e. 170, 070, 050, 030 and 010. GLH populations from Bali and West Nusa Tenggara were the most efficient vector for rice tungro virus. We concluded that there were diversities in the degree of resistance among GLH resistant varieties. Variation in virus transmission efficiency (biotype) among GLH populations collected from various tungro endemic areas closely related to the history of adoption of rice varieties.

[**Keywords:** Rice, green leafhopper, *Nephotettix virescens*, resistant variety, rice tungro virus, virus transmission efficiency]

ABSTRAK

Wereng hijau *Nephotettix virescens* merupakan penular penyakit virus tungro padi yang paling efisien. Penyakit tungro bersifat endemis di beberapa provinsi di Indonesia dan umumnya dikendalikan dengan menggunakan varietas tahan. Ketahanan

varietas padi terhadap tungro dapat dikelompokkan menjadi ketahanan terhadap virus dan wereng hijau. Riwayat adopsi varietas tahan memengaruhi perkembangan dan penularan virus. Penelitian ini dilakukan pada tahun 2009-2011 untuk menguji status ketahanan lima kelompok varietas padi tahan wereng hijau (T0-T4) dilihat dari survival dan efisiensinya dalam menularkan virus tungro. Wereng hijau dikoleksi dari 15 provinsi endemis tungro, lalu diperbanyak di rumah kaca dan diuji survival dan kemampuannya menularkan virus tungro pada lima golongan ketahanan varietas padi (T0-T4). Efisiensi penularan virus atau tingkat ketahanan varietas dihitung dengan menambahkan nilai survival (bobot x skor survival) dan efisiensi penularan (bobot x efisiensi penularan). Bobot untuk survival dan efisiensi penularan masing-masing adalah 40 dan 60. Hasil pengujian menunjukkan bahwa urutan tingkat ketahanan varietas semakin menurun dari T4, T1, T2, dan T3. Penelitian berhasil mengidentifikasi lima variasi efisiensi penularan wereng hijau, yaitu 170, 070, 050, 030, dan 010. Populasi wereng hijau dari Bali dan Nusa Tenggara Barat paling efisien menularkan virus tungro. Dari hasil penelitian ini dapat disimpulkan adanya perbedaan ketahanan golongan varietas padi dan variasi efisiensi penularan (biotipe) pada 15 populasi wereng hijau terhadap empat golongan varietas tahan dan ini berhubungan dengan riwayat adopsi varietas tersebut.

[**Kata kunci:** Padi, wereng hijau, *Nephotettix virescens*, varietas tahan, virus tungro, efisiensi penularan virus]

INTRODUCTION

Tungro infection on rice plant causes youngest leaf yellowing from the tip, dwarf and reduced tiller number (Hibino *et al.* 1978). Leaf yellowing reduces the rate of photosynthesis so the number of un-filled grains increases. Yield losses of rice due to tungro were mainly caused by reduction of productive tillers and filled grains (Shahjahan *et al.* 1990).

Rice tungro disease (RTD) is caused by two kinds of viruses, i.e. rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV). Both viruses are effectively transmitted by green leafhopper (GLH) (Hibino *et al.* 1978; Froissart *et al.* 2002). Among others, *Nephotettix virescens* Distant is the most efficient vector of tungro virus with transmission ability of 80% (Hibino and Cabunagan 1986).

Tungro disease was found in almost all provinces of rice growing areas in Indonesia (Hasanuddin *et al.* 1997; Widiarta *et al.* 1997; Raga 2008). The disease has been controlled using resistant varieties, however it still occurs sporadically in some rice growing areas. The GLH resistant rice varieties released in Indonesia could be differentiated into four groups, namely T1, T2, T3 and T4 (Sama *et al.* 1991). The T1 included IR20, IR30, IR26, IR 6, Citarum and Serayu varieties which bear the *Glh 1* resistant gene. The T2 composed of IR32, IR38, IR36, IR47, Semeru, Asahan, Ciliwung, Krueng Aceh and Bengawan Solo which have the *Glh 6* resistant gene. The T3 consisted of IR50, IR48, IR54, IR52 and IR64 which their parents bear *Glh 5* resistant gene, while IR50, IR48, IR54, IR52, IR68, Barumun and Klara were grouped into T4 which their parents have *glh 4* resistant gene.

A recent report indicated that tungro virus heavily infected rice crops in Java, Bali, West Nusa Tenggara, East Nusa Tenggara, South Sulawesi, West Sumatra and Bengkulu (Budiyanto *et al.* 2011) although resistant varieties have been introduced. The irrigated rice areas infected by tungro virus increased from 8,893 ha in 2005-2009 to 14,201 ha in 2011; in which 333 ha of them were completely damaged and caused losses amounted to IDR77.6 million.

The Intergovernmental Panel on Climate Change (IPCC 2007) projected that global air temperature will increase 1.1-6.4°C during the 21st century. Increase in global temperature by 2°C will significantly affect arthropod development (Kiritani 1997), including GLH, the vector of rice tungro viruses. By assuming the air temperature will increase 2°C, the number of *N. virescens* generations will double. Because the control of tungro disease was using resistant varieties (Imbe 1991; Azzam and Chancellor 2002), the doubling of vector populations may increase selection stress to varietal resistance (Holt 1996) and change survival and transmission ability of the viruses. The GLH populations in tungro endemic areas, for example, may have adapted to the introduced GLH resistant varieties. In this study, we test the resistance status of GLH resistant varieties and identify the ability of *N. virescens* populations (biotypes) in transmitting rice tungro viruses.

MATERIALS AND METHODS

Green Leafhopper Populations

Adults of *N. virescens* were collected using a sweeping net of 35 cm diameter from 15 tungro endemic areas in the three series of collections during 2009-2011. In the first series, the adults of *N. virescens* were collected from West Java, Central Java, West Nusa Tenggara, Bali and South Sulawesi. In the second series, *N. virescens* were collected from East Java, Lampung, West Sulawesi, Central Sulawesi and Papua. In the third series, *N. virescens* were collected from South-East Sulawesi, North Sulawesi, Yogyakarta, Banten and South Kalimantan.

Five to ten pairs of adults were temporary reared in a test tube before finally reared in the screen house of aluminum-framed cages (37 cm x 25 cm x 36 cm) at the Indonesian Center for Rice Research (ICRR), Sukamandi, Subang, West Java. Twenty-one day old seedlings of IR64 rice variety were supplied in the cages as food for the insects. New emerged adults were collected and used as a vector in the transmission experiment, whereas the first instar nymphs were used in the antibiosis test. Tungro infected plants collected from Subang, West Java, were planted in plastic bucket of 15 cm diameter and 13 cm height and used as a source of tungro virus inocula.

Transmission and Survival Tests

Three-day old virus-free adults emerged from the cages were collected and placed for two days in rice plant to acquire the viruses (2-day acquisition feeding period). Thereafter, the infected adults were fed on 14-day old seedlings of five different resistance groups of rice varieties (Table 1). Two adults were transferred per hill; 20 replicates per variety. The feeding period of the insect was 2 days. Tungro

Table 1. Genetic resistant characters of rice varieties to tungro virus.

Group of resistance	Resistant gene	Name of varieties
T0	No resistant gene (susceptible)	Lusi
T1	<i>Glh1</i>	IR26
T2	<i>Glh6</i>	Ciliwung
T3	<i>Glh5</i>	IR64
T4	<i>glh4</i>	Barumun

Glh = dominant gene; *glh* = recessive gene

disease symptoms were assessed once at 3 weeks after inoculation.

The survival rate of the first instar nymphs of GLH collected from 15 endemic areas was tested on five different resistance groups of rice varieties (Table 1). One GLH nymph was reared on 14-day old rice seedlings. The seedlings were re-newed every other day and the survival rate to adult was recorded. The experiment was replicated 20 times

Resistance Category and Biotype Identification

Rice variety resistance to tungro virus was scored based on its transmission efficiency and survival rate followed Hiraie *et al.* (2007) and Standard Evaluation System (SES) for rice (IRRI 1996) as shown in Table 2. Resistance value was calculated as weight multiplied by score of survival rate plus weight multiplied by score of transmission efficiency. Identity of variance to tungro infection was assessed based on the method of Mogi *et al.* (1992).

In decreasing order of resistance (from the most resistant to susceptible), rice variety groups were given a code number consecutively of 100, 040, 020 and 010. Biotype is a sum of the code number in which GLH populations are adaptive to the four groups of varietal resistance.

Table 2. Categorization of tungro resistance of GLH (*N. virescens*) collected from 15 endemic populations in Indonesia.

Score	Survival rate/ transmission efficiency (%)	Resistance value	Resistance criteria
1	Up to 20	Up to 200	Resistant (R)
3	> 20-40	> 200-400	
5	> 40-60	> 400-600	Moderately resistant (MR)
7	> 60-80	> 600-800	Susceptible (S)
9	> 80	> 800	

RESULTS AND DISCUSSION

Varietal Resistance to GLH Populations

Among the four groups of GLH resistant varieties, Barumun which bears *glh 4* gene was the most resistant variety (indicator variety T4) against tungro virus transmitted by 15 endemic GLH populations, followed by IR26 (T1), Ciliwung (T2) and IR64 (T3) (Table 3). The most resistant group of rice varieties to tungro was the indicator variety T4, whereas the most susceptible one was the T3. This result is similar to that reported by Widiarta (2006). This means that tungro viruses observed in the study were similar to those reported five years ago. In 2006, 23.6% or 12 million ha of rice fields in the country were cultivated with IR64 (T3) due to its good eating quality and resistance to brown planthopper biotype 3 (Sembiring and Widiarta 2008; Baehaki and Munawar 2009). These conditions caused the variety experiencing heavy selection stress. Similar reason was obtained on Ciliwung (T2), where 3.25% of rice fields in the country, especially in South Sulawesi were cultivated with Ciliwung. In 2012, adoption of IR64 and Ciliwung nationwide occupied 11.82% and 5.23%, respectively. Barumun and IR26 were less adopted by farmers due to their inferior eating quality. However, highly adapted GLH populations from 15 tungro endemic areas to IR64 and Ciliwung were not only caused by selection stress, but also by increasing GLH generations due to the increasing air temperature (Widiarta 2009).

Variation in Transmission Efficiency

Evaluation on transmission efficiency of GLH from 15 tungro endemic areas using the method of Mogi *et al.* (1992) identified five biotypes of GLH, i.e. biotype 010, 030, 050, 070 and 170 (Table 4-6). The higher the biotype code number of GLH populations, the higher the survival and transmission ability of GLH on resistant varieties. Those imply the limiting options

Table 3. Rank of group of rice variety resistance to tungro viruses transmitted by 15 endemic green leafhopper populations.

Varietal group/ resistant gene	Test result			Rank of resistance
	Susceptible	Moderate	Resistant	
T1-IR26 (<i>Glh 1</i>)	9	6	0	2
T2-Ciliwung (<i>Glh 6</i>)	12	3	0	3
T3-IR64 (<i>Glh 5</i>)	15	0	0	4
T4-Barumun (<i>glh 4</i>)	2	5	8	1

Table 4. Variations in transmission efficiency of green leafhopper (GLH) populations collected from five tungro endemic areas in Indonesia in the first series.

Varietal group/ resistant gene	Code number	GLH population				
		West Java	Central Java	West Nusa Tenggara	Bali	South Sulawesi
T4-Barumun (<i>glh 4</i>)	100	MR	MR	S	S	MR
T1-IR26 (<i>Glh 1</i>)	040	MR	S	S	S	MR
T2-Ciliwung (<i>Glh 6</i>)	020	S	S	S	S	S
T3-IR64 (<i>Glh 5</i>)	010	S	S	S	S	S
Biotype		030	070	170	170	030

MR = moderately susceptible, S = susceptible

Table 5. Variations in transmission efficiency of green leafhopper (GLH) populations collected from five tungro endemic areas in Indonesia in the second series.

Varietal group/ resistant gene	Code number	GLH population				
		East Java	Lampung	West Sulawesi	Central Sulawesi	Papua
T4-Barumun (<i>glh 4</i>)	100	R	R	MR	R	R
T1-IR26 (<i>Glh 1</i>)	040	MR	MR	MR	MR	S
T2-Ciliwung (<i>Glh 6</i>)	020	MR	S	S	S	MR
T3-IR64 (<i>Glh 5</i>)	010	S	S	S	S	S
Biotype		010	030	030	030	050

R = resistant, MR = moderately resistant, S = susceptible

Table 6. Variations in transmission efficiency of green leafhopper (GLH) populations collected from five tungro endemic areas in Indonesia in the third series.

Varietal group/ resistant gene	Code number	GLH population				
		South-East Sulawesi	North Sulawesi	Yogyakarta	Banten	South Kalimantan
T4-Barumun (<i>glh 4</i>)	100	R	R	MR	R	R
T1-IR26 (<i>Glh 1</i>)	040	MR	MR	MR	MR	S
T2-Ciliwung (<i>Glh 6</i>)	020	MR	S	S	S	MR
T3-IR64 (<i>Glh 5</i>)	010	S	S	S	S	S
Biotype		070	070	050	070	070

R = resistant, MR = moderately resistant, S = susceptible

in varietal rotation to control tungro virus in a specific location. In the case of biotype 170 for Bali and West Nusa Tenggara populations, these populations had adapted to all of GLH resistant varieties released in Indonesia. The control option for biotype 170 is introducing or pyramiding the rice lines carrying other GLH resistant genes (Fujita *et al.* 2010) or introducing virus resistant varieties. Recent report showed that *Allium sativum* leaf agglutinin (ASAL) from garlic leaf expressing in transgenic rice exhibited significantly anti-metabolic effect toward GLH (Saha *et al.* 2006).

In this study, we found that Bali and West Nusa Tenggara GLH populations were the most efficient vectors. All of the resistance groups, T1 to T4 were

susceptible to those populations as also reported by Widiarta (2006). New GLH resistant gene must be deployed to control tungro disease in Bali and West Nusa Tenggara (Athwal *et al.* 1971). It is also recommended to adopt virus resistant varieties in those locations (Azzam and Chancellor 2002).

As shown in Table 4 to Table 6, T4 group of GLH resistant varieties was recommended for almost all of the provinces, except Bali and West Nusa Tenggara. The T3 group for rice varieties should not be planted in tungro endemic areas, while the T2 group was recommended in East Java, Yogyakarta and Papua. The T1 group was recommended for West Java, South Sulawesi, East Java, Lampung, West Sulawesi and South Sulawesi. Sama *et al.* (1991) recommended

varietal rotation between seasons among the four groups of rice varieties in direction from dry season to wet season. In the case of Bali and West Nusa Tenggara, all the groups were susceptible, therefore, varietal rotation is impossible. A new resistance management based on vector adaptation is required.

By using adaptability categories as shown in Table 7, GLH populations from Bali and West Nusa Tenggara were categorized as highly adaptive, in contrast to those from West Java, South Sulawesi, East Java, Lampung, West Sulawesi and Central Sulawesi which were identified as less adaptive. The rest seven populations, Central Java, Papua, South-East Sulawesi, North Sulawesi, Yogyakarta, Banten and South Kalimantan were categorized as adaptive. Highly adaptive populations were indicated by their adaptability to 3-4 groups of resistant varieties, while adaptive and less adaptive populations were indicated by their adaptability to 2-3 groups and one group of resistant varieties, respectively.

Previous studies on GLH biotypes were only focused on the effect of variety on the survival of the insect (Kobayashi *et al.* 1983; Takita and Habibuddin 1985; Hiraie *et al.* 2007). In this study, we have not only observed the effect of variety on the survival of the insect, but also their ability in transmitting tungro virus.

Biotype of temperate species of green leafhopper *N. cincticeps* was named based on its adaptation to a specific resistant gene (Hiraie *et al.* 2007). They proposed that biotypes virulent to *Grh 1*, *Grh 2* and *Grh 3* carrying varieties were designated biotype 1,

Table 7. Degree of adaptability of *Nephotettix virescens* populations on rice varieties.

Source of populations	Adaptability of biotype		
	Highly adaptive (>070)	Adaptive (050-070)	Less adaptive (<050)
West Java			v
Central Java		v	
West Nusa Tenggara	v		
Bali	v		
South Sulawesi			v
East Java			v
Lampung			v
West Sulawesi			v
Central Sulawesi			v
Papua		v	
South-East Sulawesi		v	
North Sulawesi		v	
Yogyakarta		v	
Banten		v	
South Kalimantan		v	

biotype 2 and biotype 3, respectively. The method is similar to biotype naming for brown planthopper. Biotypes of brown planthopper were named as one number higher than resistant gene which a population adaptive to (Saxena and Barrion 1985; Baehaki and Munawar 2009). In this study, the GLH biotype was identified using a method to identify the pathogenic race of rice blast fungus (Mogi *et al.* 1992).

CONCLUSION

Based on an adaptation test of 15 GLH populations from tungro endemic areas, the rank of the resistance group of rice varieties in decreasing order of resistance was T4-Barumun, T1-IR26, T2-Ciliwung and T3-IR64. This order was closely related to the adoption of the varieties. Five variations of GLH transmission efficiency as indicated by survival and virus transmission efficiency in decreasing order were identified, i.e. 170, 070, 050, 030 and 010. The biotype 170 in Bali and West Nusa Tenggara have adapted to all groups of GLH resistant variety, therefore it is suggested to introduce virus resistant variety or breed new GLH resistant variety by incorporated other available resistant gene as well as transgenic rice expressing ASAL.

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