

RESISTANCE OF TEN LOCAL RICE CULTIVARS FROM CENTRAL SULAWESI TO *Nephrotettix virescens* (Hemiptera: Cicadellidae)

Asrul¹⁾, Abd. Wahid¹⁾, Zulkifly¹⁾, Desi Wahyuni Arsih¹⁾

¹⁾ Faculty of Agriculture, Tadulako University

Email : asrul1203@gmail.com, wahid_lala@yahoo.com, zulkiflymachmud@gmail.com, desiwahyuni992@gmail.com

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ABSTRACT

The green leafhopper (*Nephrotettix virescens*) is the primary vector of the tungro virus, posing a significant threat to rice productivity in Indonesia. The utilization of local cultivars with natural resistance represents a sustainable strategy to mitigate such biotic constraints. This study aims to evaluate the resistance levels of ten local rice cultivars from Central Sulawesi against *N. virescens* using the International Rice Research Institute (IRRI) standard evaluation. The assessment covers parameters such as incubation period, symptom severity, and disease index (DI) to classify phenotypes and recommend priority genotypes. The research was conducted from April to September 2025 at the Plant Pest and Disease Green House, Faculty of Agriculture, Tadulako University, utilizing a Randomized Block Design (RBD) with three replications. The ten cultivars tested originated from Ampana (Gondu, Masai, Kamba Bulili, and Kalendeng), Sigi (PM and Kas), and Banggai (Habo, Sampara, Dongan, and Uva Buya). Results indicated that the symptom incubation period ranged from 5 to 7 days after infection (DAI). Based on the disease index (DI) observed from 14 to 56 DAI, significant genetic response diversity was found among the cultivars. The cultivar Kamba Bulili was classified as resistant with the lowest DI value of 3.8. In contrast, the cultivars Kas (DI 7.4) and PM (DI 7.0) were categorized as susceptible. The remaining seven cultivars exhibited a rather resistant response.

Keywords: *Nephrotettix virescens*, Local Rice Cultivars, Resistance Index, Tungro Disease, Plant Protection.

INTRODUCTION

Rice (*Oryza sativa* L.) is a globally strategic food commodity; however, its production continues to face major challenges due to pest infestations, particularly the green leafhopper

(*Nephrotettix virescens*). Globally, infestations of the green leafhopper not only cause physical damage through the extraction of plant cell sap but also act as the primary vector of tungro virus, which can result in yield losses of up to 100% under epidemic

conditions (Hutasoit *et al.*, 2025). Statistical data indicate that climate fluctuations in Southeast Asia have increased the risk of green leafhopper outbreaks, directly threatening national food sovereignty (Wang *et al.*, 2022).

The main problem that arises is farmers' dependence on chemical pesticides to control *N. virescens* populations. This practice negatively impacts the rice field ecosystem, induces pest resistance, and increases production costs, which burden smallholder farmers (Heong *et al.*, 2021). In addition, the widespread use of genetically uniform national high-yielding varieties across different regions increases crop vulnerability to pests that have adapted, thereby necessitating genetic diversification through the utilization of local genetic resources.

Central Sulawesi possesses remarkable local rice germplasm, such as cultivars from Ampana (Gondu, Masai, Kamba Bulili, Kalendeng), Sigi (PM, Kas), and Banggai (Habo, Sampara, Dongan, Uva Buya). However, the existing research gap shows that studies on local rice cultivars from Central Sulawesi remain largely limited to morphological characterization and have scarcely addressed biotic resistance specifically against *N. virescens*. In fact, local varieties often harbor unique resistance genes as they have adapted for hundreds of years to local environmental conditions.

The absence of comprehensive data regarding the resistance levels of these local cultivars has led to their genetic potential being overlooked in plant breeding programs. Without systematic evaluation, valuable cultivars from Ampana, Sigi, and Banggai are at risk of extinction due to land conversion and the dominance of modern varieties. Therefore, formal testing is required to map plant defense responses against green leafhopper infestations to ensure the sustainability of rice production at the regional level (Sattar *et al.*, 2023).

This research is crucial as it evaluates ten cultivars using the standard protocol of the International Rice Research Institute (IRRI).

The evaluation, which includes parameters such as incubation period, symptom severity, and disease index (DI), will provide accurate phenotypic classification. This approach enables precise identification of which cultivars exhibit resistant, moderately resistant, or susceptible responses to *N. virescens*.

Academically, this study contributes to enriching the database of Indonesian rice germplasm, particularly concerning insect resistance mechanisms. Practically, the findings will yield recommendations for priority genotypes that can be used by farmers as preferred varieties in leafhopper-endemic areas or by plant breeders as gene sources (donor parents) for developing new high-yielding pest-resistant varieties in the future.

RESEARCH METHODS

This research was conducted over a six-month period, from April to September 2025. All research activities, including inoculum propagation and symptom observation, were carried out at the Plant Pests and Diseases Greenhouse, Faculty of Agriculture, Tadulako University, Palu, Central Sulawesi.

Equipment and Materials

- **Equipment:** Gauze, green leafhopper breeding boxes, a smartphone camera for documentation, stationery, plastic bags (15 x 20 cm), jars, measuring tape, buckets, mylar plastic, shovels, hoes, tweezers, thread, magnifying glasses, spoons, plastic gutters, and shade netting (paranet).
- **Materials:** Planting media (a mixture of soil, organic matter, and cow manure), water, and ten local rice cultivars, categorized by origin: Ampana (Gondu, Masai, Kamba Bulili, and Kalendeng; Sigi (PM and Kas), dan Banggai (Habo, Sampara, Dongan, and Uva Buya).

Experimental Design The study employed a Randomized Block Design (RBD) with ten

local rice cultivars as the treatments. Each treatment was replicated three times, totaling 30 experimental units. Each unit consisted of five sample plants, resulting in a total of 150 observed plants.

1) Research Procedure

Source Propagation of Inoculum

The inoculum was obtained from tungro-infected Santana variety rice plants in Biromaru Village, Sigi Regency, during the vegetative phase1. These plants were maintained in 15 x 20 cm polybags covered with gauze to ensure they remained free from other pests.

Propagation of Green Leafhoppers (*Nephrotettix virescens*)

N. virescens were collected from farmers' fields in Biromaru and multiplied in gauze cages using vegetative-phase Mengkonga variety seedlings as feed. The population was maintained at temperatures between 30°C and 36°C to optimize growth and survival.

Rice Nursery

Seedlings were grown in 30 x 20 cm² plastic gutters using a 1:2 soil and cow manure medium, watered to field capacity 5. Before sowing, seeds were soaked for 24 hours to stimulate germination and then maintained for 10 days.

Rice Planting

Ten-day-old seedlings were transplanted into 15 x 20 cm polybags. Plants were watered twice daily, and weeds were manually removed to prevent nutrient competition.

Inclusion of Green Leafhoppers (*Nephrotettix virescens*) in Infected Plants

Adult leafhoppers were exposed to tungro-infected plants for 24 hours to acquire the virus. Subsequently, two leafhoppers were introduced to 14-day-old test plants for a 24-hour inoculation feeding period before being removed and destroyed.

2) Observation Parameters

Incubation Period

The incubation period was recorded as the duration from the start of inoculation until the first appearance of visual symptoms across all cultivars.

Resistance Index of Local Rice Cultivars

The resistance index of local rice cultivars against green leafhopper (*Nephrotettix virescens*) infestation was observed starting 14 days after infection, and the observation process continued for eight weeks. The resistance index was assessed for all plants, and the severity of plant symptoms was evaluated using the Standard Evaluation System for Rice (IRRI, 2014). The scoring system was as follows: 1 = 0% no symptoms of attack; 3 = 1–10% attacked, dwarf, and not yet yellow; 5 = 11–30% attacked, dwarf, and moderately yellow; 7 = 31–50% attacked, dwarf, and turned yellow; 9 = >50% attacked, dwarf, and turned orange. Based on symptom severity, the resistance index of local rice cultivars was calculated using the following formula:

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

Where DI represents the disease index, n represents the number of plants at a specific score, and tn is the total number of plants. Cultivars were categorized as Resistant (0–3), Moderately Resistant (4–6), or Susceptible (7–9).

RESULTS AND DISCUSSION

Symptoms and Disease Development

Figure 1 shows that rice plants infected with the virus via the *N. virescens* vector exhibited characteristic symptoms of leaf discoloration and growth inhibition. Initial symptoms appeared as chlorosis, with leaf

colors changing to yellow or bright orange, starting from the tips and progressing toward the base. In addition to discoloration, the sample plants showed significant stunting, reflecting physiological disturbances caused

by the green leafhopper-transmitted tungro virus infection. This phenomenon is consistent with research identifying *N. virescens* as an efficient vector that causes systemic damage to rice plant tissues (Muralidharan *et al.*, 2019).



Figure 1. Development of green leafhoppers (*N. virescens*) symptoms in sample plants

Incubation Period of Attack Symptoms

The incubation period serves as a primary indicator of a cultivar's resistance level to infection. Data presented in Table 1 reveal variations in the incubation period (Days After Inoculation, DAI) among the ten local cultivars. Gondu and Uva Buya exhibited the fastest symptom onset at 5 DAI. The cultivars PM, Masai, and Kalendeng showed symptoms at 6 DAI, while Dongan, Habo, Kamba, Sampara, and Kas recorded the longest incubation periods at 7 DAI. A prolonged incubation period, such as that observed in the Kamba and Kas cultivars, indicates an inherent initial ability of the plant to inhibit viral replication or systemic movement within the vascular tissues. This delay is critical because the later the symptoms appear, the greater the opportunity for the plant to maintain photosynthetic processes over a longer duration (Sattar *et al.*, 2023).

Table 1. The incubation period for symptoms of green leafhopper attack (*N. virescens*) in 10 types of local rice cultivars

No.	Varieties	Appearance of symptoms of n attack (DAI)
1	Gondu	5
2	PM	6
3	Dongan	7
4	Habo	7
5	Masai	6
6	Kamba	7
7	Sampara	7
8	Uva Buya	5
9	Kalendeng	6
10	Kas	7

Resistance Index (DI) and Symptom Development (14–63 DAI)

The evaluation of the resistance index in Table 2 illustrates the dynamics of plant damage percentages as the plants aged. During the initial observations (14–21 DAI), all cultivars showed no significant differences. However, from 28 DAI through 63 DAI,

significant variations in response emerged among the cultivars.

Table 2. The average resistance index of 10 species of local rice cultivars after infecting green leafhoppers (*N. virescens*) during the observation period of 14 DAI up to age 63 DAI.

Treatment	Age of Plants DAI (%)							
	14 DAI ^{tn}	21 DAI ^{tn}	28 DAI	35 DAI ^{tn}	42 DAI	49 DAI	56 DAI	63 DAI
Gondu	4,00	9,33	22,67abc	28,00	33,33abc	36,00ab	41,33ab	46,67a
PM	10,67	18,67	36,00cd	36,00	48,00e	64,00d	72,00de	78,00bc
Dongan	5,33	10,67	22,67ab	30,67	41,33cd	54,67cd	66,67cde	72,00e
Habo	4,00	9,33	28,00abc	28,00	37,33ab	45,33abc	56,00abc	62,67cd
Masai	5,33	10,67	24,00abc	29,33	41,33bc	45,33abc	53,33abc	50,67a
Kamba Bulili	4,00	10,67	14,67a	24,00	25,33a	29,33a	32,00a	40,00bc
Sampara	5,33	17,33	33,67bc	34,67	40,00bc	48,00bc	60,00abc	58,67bc
Uva Buya	4,00	14,67	29,67bc	32,00	44,00cd	54,67cd	62,67bcd	65,33de
Kalendeng	5,33	8,00	24,00abc	25,33	30,67abc	38,67abc	50,67abc	60,00cd
Kas	4,00	9,33	38,67d	38,67	48,00ed	68,00d	77,33e	80,00e

Description:

1. The number followed by the same letter in the same column is not significantly different from the Duncam test level of 5%.
2. tn = no significant effect
3. DAI = Day After Infection

The Kamba Bulili cultivar consistently exhibited the lowest disease index (the most resistant) throughout the observation period, reaching 40.00% at 63 DAI. Conversely, the Kas and PM cultivars showed the most drastic increases in symptom severity, reaching 80.00% and 78.00%, respectively, at 63 DAI. These high DI values indicate high susceptibility to green leafhopper infestation and the associated viral infection.

Phenotypic Classification and Priority Genotype Recommendations

Based on the IRRI standard criteria in Table 3, the phenotypic classification results demonstrate diverse resistance levels among the ten local cultivars from Central Sulawesi. Only the Kamba Bulili cultivar was identified as resistant, with a resistance index of 3.8. The rather resistant category included Gondu (4.6), Masai (5.267), Sampara (5.667), Uva Buya (5.667), Habo (5.933), Kalendeng (6.067), and Dongan (6.467).

Meanwhile, the PM (7.0) and Kas (7.4) cultivars were classified as susceptible.

Table 3. Resistance index of 10 local rice cultivars after infecting green leafhoppers (*N. virescens*) during the observation period of 14 DAI up to age 56 DAI

Type of phenotype	Phenotype resistat	Resistant Criteria
Gondu	4,6	Rather Resistant
PM	7	Susceptible
Dongan	6,467	Rather Resistant
Habo	5,933	Rather Resistant
Masai	5,267	Rather Resistant
Kamba Bulili	3,8	Resistant
Sampara	5,667	Rather Resistant
Uva Buya	5,667	Rather Resistant
Kalendeng	6,067	Rather Resistant
Kas	7,4	Susceptible

These findings identify Kamba Bulili as a high-priority genotype for further development or as a donor parent for resistance genes in rice breeding programs. Conversely, cultivating

Kas and PM in green leafhopper-endemic areas should be avoided without intensive pest management due to their high susceptibility. Utilizing local cultivars with natural resistance, such as Kamba Bulili, represents a sustainable strategy for reducing

Genetic Resistance Analysis of the Kamba Bulili Cultivar

The superior resistance exhibited by the Kamba Bulili cultivar compared to the other nine cultivars can be attributed to complex genetic defense mechanisms. Theoretically, plant resistance to the green leafhopper (*N. virescens*) and the tungro virus is generally controlled by specific resistance genes, either through major genes (monogenic) or the cumulative contribution of several minor genes (polygenic) (Hutasoit *et al.*, 2025). These genetic interactions determine the extent to which a plant can suppress vector population growth and viral replication within its tissues.

In the Kamba Bulili cultivar, the low Resistance Index (DI) of 3.8 indicates the presence of strong antixenosis (non-preference) or antibiosis mechanisms. Antixenosis occurs when the physical or chemical properties of the plant discourage the *N. virescens* vector from landing or feeding for extended periods. This reduces the likelihood of viral inoculation into the phloem tissue, thereby mitigating plant damage. Secondary metabolites, such as phenols, flavonoids, and volatile compounds, play a crucial role in stimulating antixenosis by exerting repellent effects or disrupting pest feeding behavior (Divekar *et al.*, 2022; Sahu *et al.*, 2023; Stec *et al.*, 2021). For instance, in rice, high concentrations of phenols and waxes in resistant varieties can inhibit pest development through antixenosis (Sahu *et al.*, 2023). Volatile compounds like sesquiterpenes also contribute to antixenosis; in rice challenged by brown planthoppers, the release of E- β -farnesene acts as a repellent (Kamolsukyeunyong *et al.*, 2021). Furthermore, crops like wheat and tomato produce volatile mixtures that reduce pest attraction through

antennal perception, thereby decreasing infestation (Borg *et al.*, 2024; Amegan *et al.*, 2024). These antixenosis mechanisms are vital for sustainable pest management as they reduce reliance on insecticides and lower the risk of vector-borne viral transmission (Divekar *et al.*, 2022; Sane *et al.*, 2025).

Meanwhile, the antibiosis mechanism in Kamba Bulili rice likely involves secondary metabolites such as the flavonoid isovitexin, which exhibits antifeedant activity against leafhoppers and planthoppers. Isovitexin and related compounds, such as schaftoside, can inhibit feeding behavior, reduce survival rates, and decrease the body weight of leafhoppers by interfering with their detoxification enzymes, including carboxylesterase, acetylcholinesterase, and glutathione S-transferase. These compounds also have the potential to induce plant defense responses by modulating secondary metabolites and plant hormones involved in pest resistance. Additionally, the combination of various flavonoids in rice extracts can synergistically inhibit the probing activity of the green leafhopper, which is essential for minimizing plant damage. Other studies have shown that leafhopper attacks can trigger the accumulation of defense compounds like flavonoids and alter plant hormonal profiles that support antibiosis. Thus, secondary metabolites like isovitexin play a key role in the antibiosis mechanism of Kamba Bulili against green leafhoppers through feeding inhibition and physiological disruption (Duan *et al.*, 2025; Zhan *et al.*, 2016; Zhao *et al.*, 2020), such as impairing growth or reproductive capacity after the ingestion of plant fluids (Heong *et al.*, 2021).

Furthermore, the resistance of Kamba Bulili to the green leafhopper is likely supported by *Grh* (Green Rice Leafhopper Resistance) genes, which are frequently found in local rice varieties that have long adapted to endemic ecosystems. These genes are typically single dominant genes that control resistance through antibiosis. They function by triggering systemic defense responses that inhibit the vector's

feeding pathway in the vascular tissues, thereby limiting the number of transmitted viral particles (Muralidharan *et al.*, 2019). The *Grh1* gene has been mapped to chromosome 5 within a region of approximately 670 kb and plays a role in inhibiting phloem sap-sucking, leading to increased pest mortality (Park *et al.*, 2013). Genome-wide association studies in Myanmar's local rice have shown that *Grh* resistance haplotypes are distributed across several regions and contribute significantly to green leafhopper resistance (Kham *et al.*, 2024). Additionally, *GRH2* and *GRH4* genes activate various plant defense responses, including the up-regulation of pathogenesis-related genes and the production of sesquiterpene compounds that bolster resistance (Asano *et al.*, 2015). This resistance mechanism involves complex transcriptome reprogramming with the activation of multiple transcription factors and systemic defense pathways (Kwon *et al.*, 2021). The discovery of the *Grh6* gene on chromosome 4 further enhances the understanding of the genetic basis of rice resistance to green leafhoppers, which can be utilized in marker-assisted breeding (Phi *et al.*, 2019). The "Resistant" phenotype of Kamba Bulili proves that this variety possesses superior genetic stability in recognizing attack signals (elicitors) from the saliva of the green leafhopper compared to susceptible cultivars such as Kas or PM. Practically, the genetic superiority of Kamba Bulili makes it an ideal candidate as a donor parent in breeding programs aimed at developing new varieties that combine high agronomic traits with robust biotic resistance.

CONCLUSION AND SUGGESTIONS

Conclusion

1. Among the ten cultivars evaluated, Kamba Bulili was the only genotype classified as resistant (Resistance Index 3.8), while Kas and PM exhibited the highest levels of susceptibility.

2. All tested plants displayed characteristic tungro symptoms, specifically chlorosis and stunting, with an incubation period ranging from 5 to 7 days.
3. The superior resistance of Kamba Bulili is attributed to the synergistic interaction between its genetic framework (major and polygenic genes) and biochemical defenses (antixenosis and antibiosis), along with stable local environmental adaptation that enables effective response to *N. virescens* attacks.

Suggestions

1. Farmers in Central Sulawesi are encouraged to prioritize Kamba Bulili cultivation to minimize yield losses caused by green leafhoppers and the tungro virus.
2. Plant breeders should utilize Kamba Bulili as a donor parent in rice improvement programs. Further molecular identification is required to precisely map its specific resistance genes.

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