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## Short Communication

# Segregation Ratios of Tungro Virus Resistance on F<sub>2</sub> Progenies Derived from Utri Merah and ARC12596

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## Abstract

**Background and Objective:** Rice tungro disease remains as one of the major threat to rice production in Indonesia and other countries. One of the proven methods in preventing the occurrence of tungro virus is through the use of resistant varieties. The development of resistance of glutinous rice (*Oryza sativa glutinosa*) to tungro virus is expected to be achieved through hybridization between glutinous rice with tungro resistance rice. The aim of this study was to estimate number of gene controlling tungro resistance and obtain rice progenies that resistant to the virus. **Materials and Methods:** Around 230 F<sub>2</sub> progenies of Ketonggo × Utri Merah as well as Ketonggo × ARC12596 were evaluated. Forced-tube inoculation method was used to inoculate tungro disease and standard evaluation system for rice from IRRI was applied to assess the tungro infection symptoms. Scoring was performed at 2, 4 and 10 weeks after inoculation (wai). **Results:** The distinguished progenies with recovery abilities and stable have been obtained. The inheritance of tungro resistance was supposed to be controlled by recessive genes with different patterns, for Ketonggo × Utri Merah was susceptible 3: 1 resistant (complete dominance) and susceptible 9: 7 resistant (duplicate recessive epistasis) for Ketonggo × ARC12596. **Conclusion:** Recessive genes are supposed to control the tungro virus resistance but the inheritance pattern on both crosses is slightly different, indicating donor parents have dissimilar resistant genes.

**Key words:** *Oryza sativa glutinosa*, resistance, tungro, Utri Merah, ARC12596

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Rice tungro disease (RTD) is one of the factors that inhibit rice productivity in Indonesia and other countries. In 2011, the Directorate of Food Crop Protection, Ministry of Agriculture, Indonesia reported that 13,868 ha of rice fields in Indonesia have been attacked by tungro<sup>1</sup>. Data from the Ministry of Agriculture of Indonesia<sup>2</sup> showed that tungro is still an important disease in rice with an extensive attack in 2015, 2016 and 2017 which were reaching an area of 4.803, 3.518 and 5.159 ha, respectively. The disease is caused by infection with 2 viruses, which are the Rice Tungro Bacilliform Virus (RTBV) and the Rice Tungro Spherical Virus (RTSV). Both viruses are effectively transmitted by semi-persistent green leafhopper (GLH) *Nephotettix virescens* (Distant)<sup>3</sup>.

Control of tungro virus can be done by managing the crop environment. Jefferson and Chancellor<sup>4</sup> stated that the eradication of virus sources and reducing vector population density in the field can be reached through the disease management system. The use of resistant varieties to tungro is efficient controlling method to be applied by the farmer both in the technical irrigated rice fields and in the rainfed lowland rice fields<sup>5</sup>. In Indonesia, tungro-resistant varieties are used to suppress the spread of disease. Improving the resistance of varieties to tungro disease is done through plant breeding program using tungro resistant genetic sources<sup>5</sup>. However those rice lines have not been released yet and resistant rice lines produced are *Oryza sativa* rice, not *Oryza sativa* var. *glutinosa*, hence, the tungro-resistant glutinous rice has not been obtained so far. It is therefore that the aim of the research was to evaluate segregation ratios of tungro virus resistance in  $F_2$  progenies at the 2 crosses and obtain tungro resistant genotypes from 2 sources of resistant varieties, i.e., Utri Merah and ARC12596. Thus it is expected that we obtain the tungro-resistant glutinous rice genotypes that would suppress the spread of tungro.

## MATERIALS AND METHODS

The experiment was conducted from February-May, 2018 in the Indonesian Center for Rice Research (ICRR) greenhouse and experimental station of the Faculty of Agriculture, Padjadjaran University.  $F_2$  progenies were planted as many as 230 genotypes for both crosses: Ketonggo  $\times$  Utri Merah and Ketonggo  $\times$  ARC12596. Five check varieties (3 parents, one resistant variety and one susceptible variety) were grown also. Artificial hybridization was carried out in 2014 at the Indonesia Tungro Disease Research Station, Lanrang, south Sulawesi. Tungro isolates were obtained from Subang, West Java in

2017, which maintained in a greenhouse. Inoculation was done according to Cabauatan *et al.*<sup>6</sup>. Observation of visual symptoms was conducted 2 and 4 weeks after inoculation (wai hereafter) and in the field at 10 wai. Observations were made based on the standard evaluation system (SES) for 1-9 scale rice<sup>7</sup>. Test of segregation ratios was performed by Chi-square.

## RESULTS

**Evaluation of symptoms:** The  $F_2$  progenies obtained from the 2 crosses were evaluated for tungro resistance at the age of 7 days after germination. The progenies of  $F_2$  derived from the Ketonggo  $\times$  Utri Merah were classified as susceptible due to the appearance of their symptoms i.e., discoloration leaves from yellow to orange and stunted (Fig. 1a,b). The similar appearance was also found on the progenies of Ketonggo  $\times$  ARC12596 (Fig. 1c,d). Meanwhile, another symptom of the tungro infection was also found, such as rolled out of leave, like a spiral. They were categorized as susceptible (Fig. 2a-c).

Progenies from both crosses showed various symptoms at different time of the observation (Fig. 3). In the second and 4th wai as many as 181  $F_2$  progenies of the Ketonggo  $\times$  Utri Merah did not show any symptoms of tungro virus infection but at the 10 wai, there were only 49 progenies that did not show any symptoms of tungro infection. From Ketonggo  $\times$  ARC12596, 126 progenies did not show symptoms in the second wai but the number of the progenies without symptoms decreased in the 4th and 10 wai i.e., 115 and 31 progenies, respectively (Fig. 3).

In particular genotypes from both crosses, symptoms were seen at 2 and 4 wai but those symptoms disappeared at the end of the vegetative phase. Eight progenies from Ketonggo  $\times$  Utri Merah and 12 progenies from Ketonggo  $\times$  ARC12596 which show resistance reaction at the 10 wai were fruitfully obtained (Table 1). These genotypes had recovery abilities and did not show symptoms of discoloration of leaves until the end of the vegetative phase.

**Segregation pattern analysis:** The Chi square test was performed to estimate the number of genes controlling the tungro resistance. The grouping was made based on the scores of tungro symptoms as seen in the field. The Mendelian ratio and this modification were classified into groups of classes (2, 3 and 4 classes, Table 2 and 3).

Chi-square test showed 2 segregation ratios that fit the Mendelian ratio i.e., 3:1 for Ketonggo  $\times$  Utri Merah (Table 2)



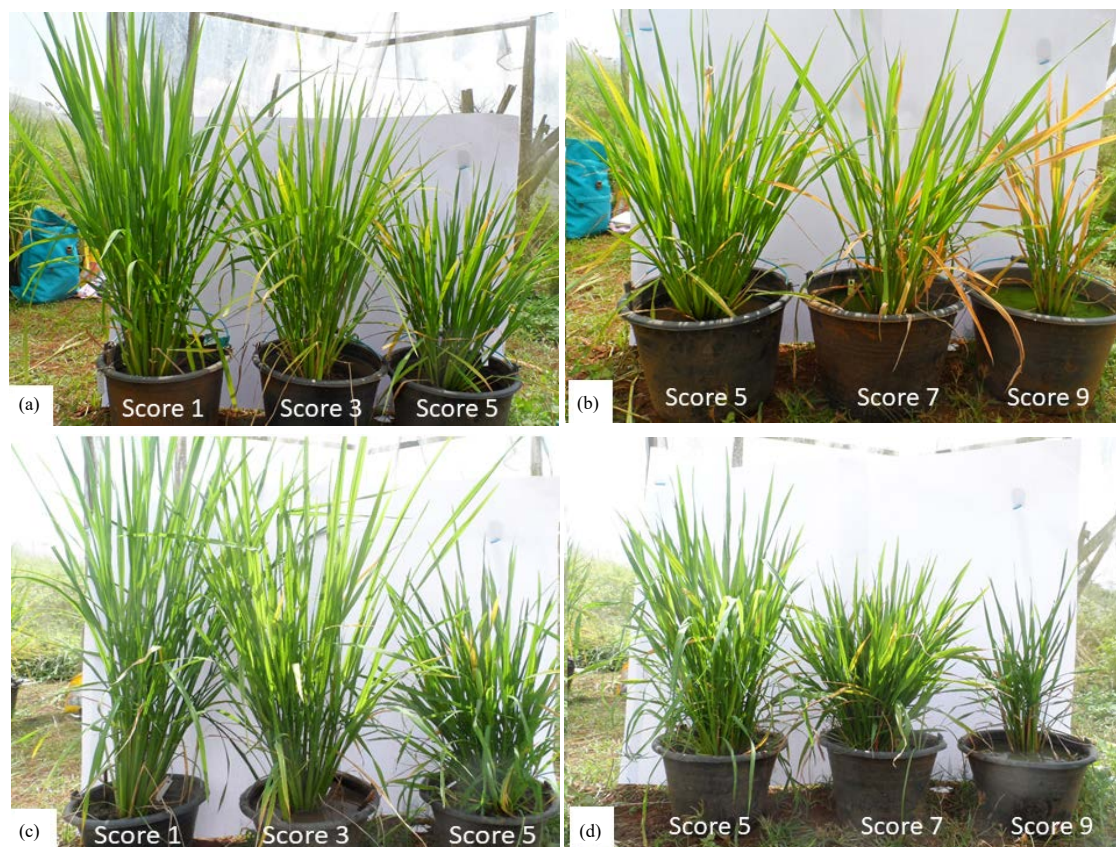


Fig. 1(a-d): Comparison of tungro virus resistance score in both crosses (a,b) Ketonggo×Utri Merah and (c, d) Ketonggo×ARC12596

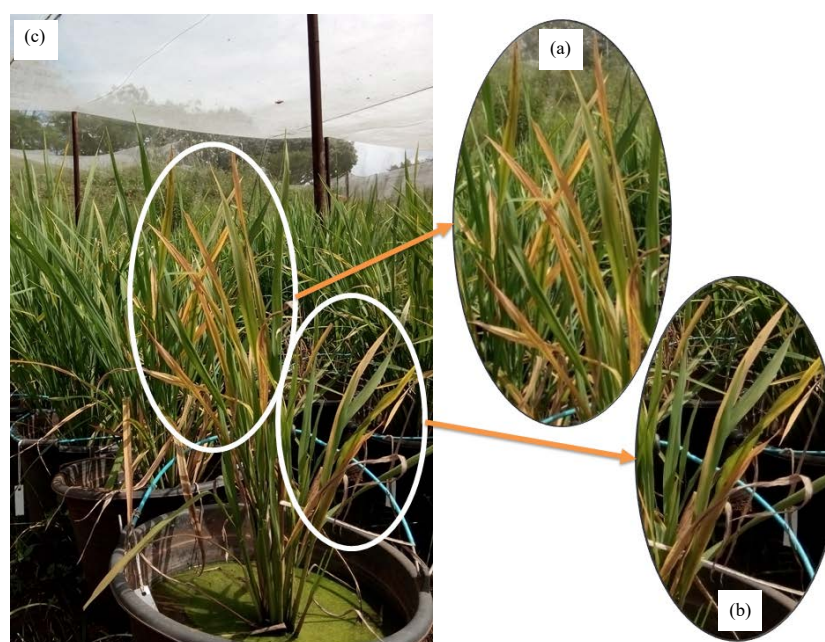


Fig. 2(a-c): Symptoms of tungro infected plants, (a) Rice genotypes expressing typical tungro symptoms, (b) Twisted leaf symptoms and (c) Leaf discoloration from yellow to orange

Table 1: Score recovery genotypes from Ketonggo × Utri Merah and Ketonggo × ARC12596 at 2, 4 and 10 wai

Ketonggo × Utri Merah				Ketonggo × ARC12596			
Genotypes	2 wai	4 wai	10 wai	Genotypes	2 wai	4 wai	10 wai
C.1.6.3	7	7	1	D.1.6.5	9	9	1
C.2.3.2	7	7	3	D.1.7.9	7	7	3
C.3.1.4	9	7	3	D.2.2.10	7	7	3
C.3.7.5	9	9	3	D.2.3.10	7	7	1
C.4.1.10	1	9	3	D.2.6.1	1	7	3
C.4.3.6	1	7	3	D.3.4.3	7	7	3
C.4.4.6	9	1	3	D.3.4.4	1	7	1
C.4.4.9	9	9	1	D.3.6.6	7	7	1
				D.3.7.8	1	7	1
				D.4.1.2	7	7	1
				D.4.2.7	7	7	3
				D.4.5.10	7	1	1

wai: Week after inoculation

Table 2: Inheritance of resistance to tungro disease in F2 Ketonggo × Utri Merah

Mendelian ratio and modification	Observed	$\chi^2$	p-value
<b>Two grouping</b>			
3:1	159:71	3.92 <sup>ns</sup>	0.025-0.050
9:7	159:71	14.99*	<0.001
13:3	159:71	21.39*	<0.001
15:1	159:71	233.74*	<0.001
<b>Three grouping</b>			
1:2:1	123:36:71	1045.71*	<0.001
9:3:4	123:36:71	4.66 <sup>ns</sup>	0.050-0.100
9:6:1	123:36:71	252.64*	<0.001
12:3:1	123:36:71	238.43*	<0.001
<b>Four grouping</b>			
9:3:3:1	49:58:47:76	269.66*	<0.001
6:3:3:4	49:58:47:76	7.48*	<0.001

ns: Not significant at  $\alpha = 1\%$ , \*Significant at  $\alpha = 1\%$ 

Table 3: Inheritance of resistance to tungro disease in the F2 Ketonggo × ARC12596

Mendelian ratio and modification	Observed	$\chi^2$	p-value
<b>Two grouping</b>			
3:1	146:84	15.68*	<0.001
9:7	146:84	4.59 <sup>ns</sup>	0.025-0.050
13:3	146:84	46.52*	<0.001
15:1	146:84	354.56*	<0.001
<b>Three grouping</b>			
1:2:1	84:89:57	220.60*	<0.001
9:3:4	84:89:57	64.72*	<0.001
9:6:1	84:89:57	142.39*	<0.001
12:3:1	84:89:57	220.60*	<0.001
<b>Four grouping</b>			
9:3:3:1	31:142:49:8	52.43*	<0.001
6:3:3:4	31:142:49:8	270.11*	<0.001

ns: Not significant at  $\alpha = 1\%$ , \*Significant at  $\alpha = 1\%$ 

and susceptible 9:7 resistant (duplicate recessive epistasis) for Ketonggo × ARC12596 (Table 3).

## DISCUSSION

Estimating number of genes controlling the resistance to tungro virus from 2 resistant parents is greatly challenging and the effort is also important in order to determine suitable

selection method that will be applied for next successive generation. The gene controlling the trait in rice is not known yet so far. Therefore we are interested in exploring the resistant genotypes i.e., Utri Merah and ARC12596 as donor parents through phenotypic assessment of tungro virus infection.

Chancellor *et al.*<sup>8</sup> stated that the initial symptom of virus infection in susceptible rice found that rice plants are very

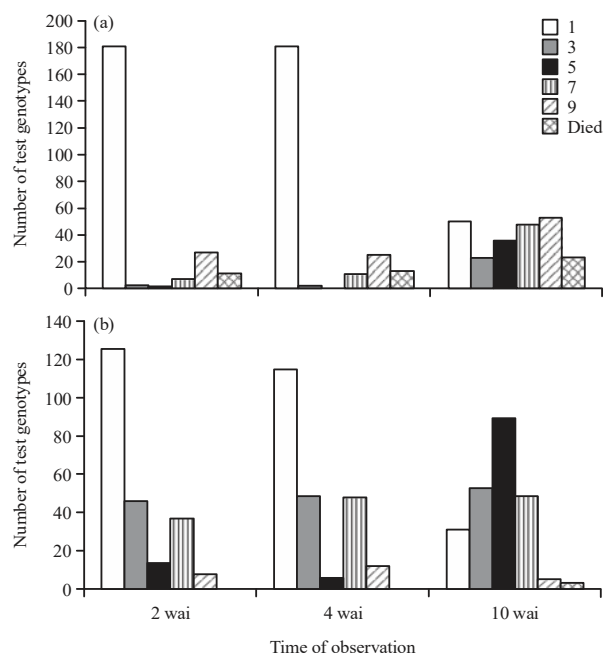


Fig. 3(a-b): Changes in the number of test genotype shown tungro symptoms at different time of observation, (a) Ketonggo × Utri Merah and (b) Ketonggo × ARC12596

stunted, low number of tillers and changes in leaf color, from yellow to orange. In addition, Hibino *et al.*<sup>3</sup>, rice plant infected RTBV dan RTSV showed stunted and yellowing leaf symptoms. Tungro symptoms in rice plants, according to Azzam and Chancellor<sup>4</sup>, were very dependent on the age of the plant, the rice variety and the strain of the virus. The leaves of rice plants infected with the tungro virus Ling<sup>9</sup> found to have phenotypic appearance such as, slim and rolled out and spiral.

Tungro infection leads to chlorophyll reduction in a plant, therefore leaf colour changes to yellow. Jabeen *et al.*<sup>10</sup> reported that reduction in chlorophyll A and B content was high in susceptible plant, followed by moderate and the resistant cultivars that showed less reduction. However the colour changes can also be caused by the inhibited water transportation process, nutrition and photosynthesis product. Cruz *et al.*<sup>11</sup> found that RTBV occurred both in the xylem and phloem, whereas RTSV directly to the xylem where the virus multiplies. Phloem and xylem damage affect the inhibition of plant growth may cause rice plant stunting and tiller number decrease.

The study demonstrated the presence of consistent resistant genotypes at 2, 4 and 10 wai. The results of this research showed the genotypes that having the similarity of resistant reaction from 2-10 wai were stable resistant genotypes. From the crossing of Ketonggo × Utri Merah,

47 stable resistant genotypes were obtained, while the Ketonggo × ARC12596 was 32 genotypes.

Genotypes that had recovery ability are potentially be used to control the spreading of tungro disease. Khatun *et al.*<sup>12</sup> argued that the most effective method for controlling tungro disease was by using resistant varieties or varieties that have recovery ability when it compared to the method of destroying vectors using insecticide. However, Khatun *et al.*<sup>12</sup> pointed out that susceptible varieties which did not have recovery ability would be much suffering from the rice tungro infection and had a potential of 100% yield losses.

Chi-square test showed 2 segregation ratios that fit the Mendelian ratio i.e., 3:1 (Ketonggo × Utri Merah). Segregation pattern with a ratio of 3:1 has a smaller p-value 0.025. (Table 2). This implies that resistance to tungro in Utri Merah (acc. #16680) is controlled by one pair gene with complete dominance (3 susceptible: 1 resistant). This finding is different with Azzam *et al.*<sup>13</sup>, who found segregation ratio 9:7.

A segregation ratio of 9 susceptible: 7 resistant with p-value 0.025-0.05 (Table 3) fitted for Ketonggo × ARC12596. This ratio indicates that the resistance gene in ARC12596 is controlled by 2 pairs of recessive genes or duplicate recessive epistasis. It implies a recessive allele at one locus and a recessive at other interact to produce expressed phenotype. Griffiths *et al.*<sup>14</sup> defined complementary epistasis as a gene interaction where the gene functions would be needed by other genes in metabolism. Mendelian ratio from both crosses, showed that each resistant parent has a different genetic composition. Based on our data, it is assumed that genetic composition of rice donor plant plays significant role in the response to the virus infection.

## CONCLUSION

Tungro virus resistance is controlled by simple genic, one and 2 pairs of recessive genes are supposed to control the trait.

Two recessive genes are supposed to be controlling the tungro virus resistance but the inheritance pattern on both crosses is slightly different, indicating donor parents have dissimilar resistant genes. The resistant genotypes are highly valuable for developing resistant glutinous rice to tungro virus.

The resistant genotypes are highly valuable for developing resistant glutinous rice to tungro virus.

## SIGNIFICANCE STATEMENT

The study discovered F<sub>2</sub> progenies of glutinous rice lines that resistant to tungro virus with different symptoms and

inheritance patterns thus will open the possibility to develop more resistant rice lines and to release new resistant variety for *Oryza sativa glutinosa*. This study will help researchers, most particularly plant breeders to uncover the critical area of inheritance patterns of tungro resistance that many researchers were not able to explore. Thus a new approach on breeding *Oryza sativa glutinosa* with tungro resistance may be arrived at.

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