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Genetic analysis of resistance to rice tungro spherical virus in several rice varieties

(Analisis genetik kerintangan beberapa varieti padi terhadap virus sfera penyakit merah padi)

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Key words: *Oryza sativa*, rice tungro spherical virus, resistance, genetics

Abstrak

Pewarisan kerintangan lapan varieti padi terhadap satu isolat virus sfera penyakit merah padi (RTSV) dari Malaysia telah dikaji. Analisis jangkitan virus dilaksanakan melalui kaedah cerakinan imunopenyerap rangkaian enzim (ELISA). Kerintangan varieti padi Utri Merah (IRGC 16682) terhadap jangkitan RTSV dikawal oleh tindakan satu gen resesif tunggal. Gen resesif tunggal yang wujud dalam varieti Basmati 370, Habiganj DW8, Kataribhog, MR 81, Pankhari 203 (IRGC 5999), Utri Rajapan (IRGC 16684) dan Y 1036 didapati alel kepada gen tunggal dalam Utri Merah.

Abstract

The inheritance of resistance to a Malaysian isolate of rice tungro spherical virus (RTSV) was studied in eight varieties of rice. Analysis of RTSV infection was done by the enzyme-linked immunosorbent assay (ELISA) method. The resistance of rice variety Utri Merah (IRGC 16682) is controlled by a single recessive gene. A single recessive gene present in Basmati 370, Habiganj DW8, Kataribhog, MR 81, Pankhari 203 (IRGC 5999), Utri Rajapan (IRGC 16684) and Y 1036 was found to be allelic to the one in Utri Merah.

Introduction

Tungro is an important viral disease of rice in the South and South-East Asian countries (Hibino 1987). The disease is transmitted by the rice green leafhopper (GLH), *Nephotettix virescens* (Homoptera: Cicadellidae), in a semi-persistent manner. GLH is commonly controlled by insecticides, but more importantly by using vector-resistant varieties (Khush 1977). These indirectly reduce the virus disease incidence and severity. At least, eight different genes for GLH resistance were identified (Khush and Brar 1991) from which many improved varieties were released. However, vector resistance has its limitation due to the development of new GLH biotypes capable of overcoming plant resistance (Takita and Habibuddin 1986). Therefore, breeding varieties directly resistant to tungro viruses is believed to be another good approach in managing the disease problem.

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Tungro disease is caused by a complex of two viruses, namely the rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). RTSV is transmitted independently by GLH, but the transmission of RTBV is dependent on the previous or simultaneous acquisition of a hypothetical 'helper factor' from RTSV-infected plant (Hibino 1983). Therefore, the presence of RTSV-infected plants is important in tungro epidemiology, and the use of RTSV-resistant varieties may help in reducing the chances of disease epidemic. Several varieties have been reported to be resistant to RTSV in the Philippines (Hibino et al. 1990). Rice varieties Basmati 370, Habiganj DW8, Kataribhog, MR 81, Pankhari 203 (IRGC 5999), Utri Merah (IRGC 16682), Utri Rajapan (IRGC 16684) and Y 1036 were also found to be resistant to infection against a Malaysian isolate of RTSV (Habibuddin et al. 1994). Y 1036 is an improved variety resulting from a cross involving Pankhari 203 (Habibuddin et al. 1991). The objective of this study was to investigate the mode of inheritance to RTSV resistance in the varieties and their allelic relationship as well as to identify the possible additional resistant genes among them.

Breeding and selection for RTSV resistance based on symptoms have several limitations. While susceptible plants infected with RTBV alone or doubly with RTBV + RTSV showed obvious symptoms, the plants infected with RTSV alone were symptomless (Hibino et al. 1978; Hassanuddin and Hibino 1989). Hence, RTSV-resistant plants are difficult to be identified based solely on symptoms and might be confused with the healthy plants. Recently, serological techniques such as the enzyme-linked immunosorbent assay (ELISA) made it possible to index tungro virus resistance (Hibino et al. 1990; Dahal et al. 1992; Imbe et al. 1993). A low percentage of or no RTSV infection especially of GLH-susceptible varieties is generally considered due to their resistance to RTSV infection. By using ELISA, ShahJahan et al. (1991) identified resistance genes in Utri Merah, Kataribhog and Pankhari 203. Resistance of Utri Merah was controlled by a single recessive gene while three complementary recessive genes were reportedly controlled resistance in Kataribhog and Pankhari 203. Resistance of Kataribhog and Pankhari 203 is reported to be allelic while the single recessive gene in Utri Merah is non-allelic to the genes in both varieties.

Materials and methods

Materials used, tungro inoculation and ELISA

Crosses for inheritance studies were made between RTSV-resistant varieties Basmati 370, Habiganj DW8, Kataribhog, MR 81, Pankhari 203 (IRGC 5999), Utri Merah (IRGC 16682), Utri Rajapan (IRGC 16684) and Y 1036 with a susceptible tester variety Taichung Native 1 (TN1). Reciprocal crosses were also made for Basmati 370/TN1 and MR 81/TN1 pairs. The hybrids (F_1) of Utri Merah/TN1 and Utri Rajapan/TN1 were backcrossed to their parents to produce BC_1 (i.e. F_1/P_1) and BC_2 (F_1/P_2) populations. The F_2 and F_3 populations were established by selfing. Crosses for allelic studies were conducted between RTSV-resistant varieties and Utri Merah or Kataribhog; their F_1, F_2 and F_3 populations were prepared similarly.

Seedlings were raised in seedling trays, by sowing individual seeds at a distance of 3 cm x 3 cm. Seedlings at 10 days after sowing (DAS) were then inoculated individually using three viruliferous GLH within a cylinder cage for a 24-h inoculation access feeding period. Unless specified, the GLH used was from a colony which had continuously been bred on TN1 (TN1-colony). For crosses involving Pankhari 203 and Y 1036, a GLH colony bred on Pankhari 203 (P203-colony) was used as the vectors (Habibuddin et al. 1994). The viruliferous vectors were prepared by giving them a 48 h acquisition on tungro-infected TN1 plants. ELISA for viral infection was carried out 3 weeks after inoculation,

following the methods described by Clark and Adams (1977), and Habibuddin et al. (1994). Absorbance values of samples were measured by an ELISA reader (EASY READER 400, SLT Labinstrument, Austria) at 405 nm. Any plant with ELISA value twice the value of average absorbance of healthy sap was classified as infected (Sutula et al. 1986; Hibino et al. 1990; Dahal et al. 1992).

Inheritance of RTSV resistance

Preliminary tests were conducted to assess the resistance of F_1 populations of all the crosses between resistant (P_1) and susceptible (P_2) parents. Fifteen seeds each of the P_1, P_2, F_1, BC_1 and BC_2 populations were sown, inoculated and assayed for RTSV infection by ELISA.

Thirty seeds each of P_1 and P_2, and about 400 F_2 seeds from each cross were further tested against RTSV infection. Individual F_2 seedlings were classified as either uninfected or positively infected. The Chi-square (χ^2) test was used to analyse the fitness of segregation ratios of infected/non-infected seedlings to the estimated Mendelian ratio. About 100–400 F_3 lines from the respective crosses were further evaluated for their segregation for resistance. Eight plants of each F_3 line were inoculated individually, crushed together and tested for infection by ELISA. Therefore, each line was classified as either homogenously uninfected or positively infected. Those infected lines included both segregated and homogenously infected lines (ShahJahan et al. 1991). The F_3 ratio thus obtained was used to confirm the finding of their F_2 populations.

Allelic studies

The F_1 and F_2 populations, and F_3 lines of crosses among resistant varieties with Utri Merah and Kataribhog were assessed for RTSV infection. Their P_1 and P_2 populations were included as check cultivars. Tests for allelism were done according to the principle: Should all F_1, F_2 and F_3 progenies of any cross between the two resistant varieties were resistant, it is most likely that the RTSV-resistant genes involved in the two respective parents were closely linked or allelic to each other.

Results

Inheritance of RTSV resistance

All seedlings of the F_1 generations of every cross evaluated were infected, suggesting their susceptibility to infection, an indication of the presence of recessive gene(s) for RTSV resistance *(Table 1)*. These included the F_1 of reciprocal crosses of Basmati 370/TN1 and MR 81/TN1. Results from these two pairs of reciprocal crosses suggested the absence of maternal cytoplasmic effect. Sixty per cent of BC_1 individuals of Utri Merah/TN1 cross were uninfected and 40% were positively infected, which fit into the ratio of 1 resistant: 1 susceptible (p = 0.25–0.50). All individual BC_2 plants of this cross were infected, suggesting their susceptibility to RTSV infection. Analysis on the Utri Rajapan/TN1 cross showed that the segregation of its BC_1 population also fit into the ratio of 1 resistant: 1 susceptible (p = 0.75–0.90). The nature of segregation of the BC_1 and the susceptibility of all the BC_2 plants are evidence on the presence of a recessive gene action for RTSV resistance in the two resistant varieties.

All the F_2 populations of the tested crosses, except Pankhari 203/TN1, segregated in the ratio of one uninfected or resistant to three infected or susceptible to RTSV infection *(Table 2).* These segregations were confirmed from reactions of F_3 lines of those crosses. For example, the F_2 of Basmati 370/TN1 segregated into 109 uninfected: 291 infected, which fit into the ratio of 1 resistant: 3 susceptible (p = 0.25–0.50), a condition of a single recessive gene for resistance. The F_3 lines of that cross segregated into 34 uninfected and 116 infected. These infected lines included both the segregated and homogenously infected lines. The 1 resistant: 3 susceptible ratio

Table 1. Resistance against RTSV infection among parents, the hybrid and backcrosses to both parents for crosses between RTSV-resistant varieties and susceptible tester TN1

Cross	P_1	P_2	F_1	BC_1	BC_2
Basmati 370/TN1	R	S	S	–	–
TN1/Basmati 370[a]	S	R	S	–	–
MR 81/TN1	R	S	S	–	–
TN1/MR 81[a]	S	R	S	–	–
Habiganj DW8/TN1	R	S	S	–	–
Kataribhog/TN1	R	S	S	–	–
Pankhari 203/TN1[b]	R	S	S	–	–
Utri Merah/TN1	R	S	S	60% R	100% S
Utri Rajapan/TN1	R	S	S	47% R	100% S
Y 1036/TN1[b]	R	S	S	–	–

P_1 and P_2 = parents
$BC_1 = F_1/P_1$
$BC_2 = F_1/P_2$
R = resistant
S = susceptible

[a]Reciprocal cross
[b]Inoculated by green leafhoppers of P203-colony

Table 2. Number of RTSV-infected and uninfected seedlings of F_2 and F_3 generations from crosses between RTSV-resistant varieties with susceptible tester parent TN1

Cross	F_2 generation		P-value 1R: 3S	F_3 generation		P-value 1R: 3S[a]
	R	S		R	S	
Basmati 370/ TN1	109[b]	291	0.25–0.50	34	116	0.50–0.75
MR 81/ TN1	95	305	0.50–0.75	106	294	0.25–0.50
Habiganj DW8/ TN1	62[b]	138	0.75–0.90	29	71	0.25–0.50
Kataribhog/ TN1	80	200	0.10–0.25	69	208	0.90–0.95
Pankhari 203/ TN1[c]	121[b]	279	0.75–0.90	32	88	0.50–0.75
Pankhari 203/ TN1	153	36	<0.001	na	–	–
Utri Merah/ TN1	116[b]	284	0.50–0.75	47	132	0.50–0.75
Utri Rajapan/ TN1	107	293	0.25–0.50	21	79	0.25–0.50
Y 1036/ TN1[c]	98	302	0.75–0.90	21	79	0.75–0.90

R = resistant
S = susceptible
na = not available

[a]Susceptible including segregating and homozygous susceptible families
[b]Expected values for χ^2 test are adjusted from the observed values of both parents based on the formulae of Washio et al. (1968)
[c]Inoculated by green leafhoppers of P203-colony

confirms that the resistance of Basmati 370 to RTSV is governed by a single recessive gene.

Inoculation with P203-colony on the F_2 plants of both Pankhari 203/TN1 and Y 1036/TN1 crosses produced a segregation ratio of 1R: 3S *(p* = 0.75–0.90, *Table 2)*. Their F_3 lines segregated into similar ratios. These ratios suggested a single recessive gene action operating for RTSV resistance in both varieties. However, when the same F_2 population of Pankhari 203/TN1 was inoculated by the TN1-colony, its individuals segregated into 153 uninfected: 36 infected, which did not fit into the ratio of 1R: 3S (p <0.001).

Table 3. Number of RTSV-infected and uninfected seedlings of F_2 and F_3 generations from crosses between RTSV-resistant varieties with Utri Merah or Kataribhog

Cross	F_2 generation		P-value 7R: 9S	F_3 generation		P-value 7R: 9S[a]
	S	R		R	S	
Crosses with Utri Merah						
Basmati 370/Utri Merah	373	27	<0.001	98	2	<0.001
MR 81/Utri Merah	394	6	<0.001	98	2	<0.001
Habiganj DW8/Utri Merah	383	17	<0.001	80	20	<0.001
Kataribhog/Utri Merah	395	5	<0.001	99	1	<0.001
Pankhari 203/Utri Merah[b]	234	6	<0.001	96	4	<0.001
Utri Rajapan/Utri Merah	381	19	<0.001	94	6	<0.001
Y 1036/Utri Merah[b]	367	33	<0.001	94	6	<0.001
Crosses with Kataribhog						
Basmati 370/Kataribhog	274	6	<0.001	118	2	<0.001
MR 81/Kataribhog	400	0	<0.001	103	7	<0.001
Habiganj DW8/Kataribhog	343	57	<0.001	98	2	<0.001
Pankhari 203/Kataribhog[b]	399	1	<0.001	160	0	<0.001
Utri Rajapan/Kataribhog	310	90	<0.001	86	14	<0.001
Y 1036/Kataribhog[b]	373	27	<0.001	82	18	<0.001

R = resistant
S = susceptible
[a]Susceptible including segregating and homozygous susceptible families
[b]Inoculated by green leafhopper of P203-colony

Allelic studies

Data for the allelic studies was obtained from crosses involving resistant varieties with Utri Merah (IRGC 16682) and Kataribhog used as the female parents. In crosses involving Utri Merah, all F_1 populations were not infected, which suggested that resistant genes in all the varieties might be closely linked or allelic to the one in Utri Merah. The F_2 plants of Kataribhog/Utri Merah segregated into 395 uninfected and 5 infected *(Table 3)*, significantly deviated (p <0.001) from the expected ratio of 7 resistant: 9 susceptible (7R: 9S = 3 aaB_, 3 A_bb and 1 aabb resistant: 9 A_B_ susceptible). This result proved the absence of segregation for two independent recessive genes. Crosses involving Basmati 370, Habiganj DW8, MR 81, Pankhari 203, Utri Rajapan or Y 1036 with Utri Merah also showed significant deviation from 7R: 9S. Likewise, all F_2 plants from crosses involving Basmati 370, Habiganj DW8, MR 81, Pankhari 203, Utri Rajapan or Y 1036 with Kataribhog were significantly deviated from the 7R: 9S ratio *(Table 3)*. The resistance of the F_1 and the significant deviation from the 7R: 9S ratio of the F_2 populations suggested that the gene governing RTSV resistance in these tested cultivars was closely linked or allelic. These results were confirmed by the segregation of their F_3 families.

Discussion

For resistance to RTSV infection, maternal cytoplasmic influence was not evident. Segregations in the F_2 populations and F_3 lines from the cross between Utri Merah (IRGC 16682) with TN1 were explained by the presence of a single recessive gene. Thus, this study confirmed the earlier report of a single recessive gene controlling RTSV resistance in Utri Merah (ShahJahan et al. 1991). Resistance in Pankhari 203 and Kataribhog was also found to be controlled by a single recessive gene, allelic to the one in Utri Merah and Utri Rajapan (IRGC 16684). This conclusion is in agreement with the result of Ebron et al. (1994). This

single recessive gene was recently assigned as *tsv1* (Kinoshita 1995).

Our study also indicated that Basmati 370, Habiganj DW8, MR 81 and Y 1036 also possess a single recessive gene for RTSV resistance. Similar allelic reactions were also observed from crosses of these varieties with Utri Merah. Since all the tested varieties are controlled by a single recessive allelic gene, then it is suggested that MR 81 and Y 1036 could possibly be better donors for RTSV resistance because they are improved variety and line respectively which are agronomically more superior to the other varieties studied and more suited to the Malaysian environment (Hadzim et al. 1988; Habibuddin et al. 1991).

The segregation ratio for RTSV infection in the F_2 of Pankhari 203/TN1 was found affected by the types of GLH colonies used in the inoculation. The ratio of 1R: 3S was obtained by using the P203-colony which overcame the effect of vector resistance to GLH in Pankhari 203 progenies (Habibuddin et al. 1994), and allowed RTSV resistance to be fully expressed. This ratio indicated that RTSV resistance in Pankhari 203 was controlled by a single recessive gene. On the other hand, the segregation did not fit into the 1R: 3S ratio when the TN1-colony was used as the vector. The larger number of uninfected seedlings when using TN1-colony was partly due to the compounded effect of their vector resistance which reduced successful infection. GLH resistance was reported to have an influence in reducing the number of tungro-infected plants (Heinrichs and Rapusas 1983; Hibino et al. 1987).

This study indicated that sources of resistance to RTSV are available, and breeding for RTSV resistance is possible. However, the results also inevitably demonstrated a critical scenario in managing RTSV resistance. Screening of more than 40 000 rice accessions at the International Rice Research Institue (IRRI) in the Philippines indicated that promising sources of resistance to tungro viruses are scarce (Hibino et al. 1990). Presently, only a limited number of sources with known genes in action are known. The other known RTSV-resistant gene is *tsv2*, reportedly present in another accession of Utri Merah, IRGC 16680 (Ebron et al. 1994; Kinoshita 1995). Hence, the attempt to search for new genes and their allelic relationship should be continued.

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