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Screening Wild Species of Rice (*Oryza* spp.) for Resistance to Rice Tungro Disease

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Abstract

Tungro is one of the most serious rice diseases in South and Southeast Asia. Production of tungro-resistant cultivars is a major breeding objective. Wild species of rice (*Oryza* spp.) and African cultivated rice, *O. glaberrima*, were evaluated as possible sources of resistance to rice tungro disease. Two hundred and ten accessions were tested for resistance to RTBV and RTSV infection. Of these, 52 accessions were not infected with RTSV and 15 accessions were not infected with RTBV when inoculated with viruliferous green leafhoppers. Three accessions of *O. rufipogon* (IRGC Acc. no. 105908, 105909 and 105910), 3 accessions of *O. officinalis* (IRGC Acc. no. 105100, 105365 and 105376) and 1 accession of *O. redleyi* (IRGC Acc. no. 100821) showed a degree of resistance to RTBV infection independent of vector resistance. The level of resistance to rice tungro disease shown in these 7 accessions of 3 wild species was higher than that found in cultivated rice. These wild rice accessions could be useful in developing rice cultivars with a high resistance to tungro.

Discipline: Plant disease
Additional key words: antibiosis, ELISA, green leafhopper, Nephotettix

Introduction

Tungro is one of the most serious diseases of rice in South and Southeast Asia. The composite disease is caused by 2 kinds of viruses; rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). These viruses are known to be transmitted by 6 leafhopper species, of which the green leafhopper (GLH; *Nephotettix virescens* Distant) is the principal vector[3].

Use of resistant cultivars is the most important approach for the control of tungro. Although some varieties with resistance to GLH have been identified, resistance to the vector usually breaks down a few years after intensive cultivation of a variety[4]. More than 40,000 cultivated rice accessions have been screened for tungro resistance. Some accessions showed a low overall infection with RTBV and RTSV, while others showed a low or absence of infection with RTSV or tolerance to RTBV. However, it was observed that no variety was

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completely resistant to RTBV which is the main causal agent of the tungro symptoms[5,10].

Wild relatives of crop plants are an important reservoir of genes for resistance to diseases[2]. However, evaluation for tungro resistance in wild rice species has been limited[6]. We evaluated wild species of rice (*Oryza* spp.) and the African cultivated rice, *O. glaberrima* Steud. to identify possible sources of resistance to rice tungro disease. Classification of the *Oryza* species in this study follows the taxonomy proposed by Vaughan[11]. The present paper is a summary of our previous studies[8,9].

Test for resistance to infection with rice tungro viruses using *N. virescens*

A total of 210 accessions, representing the genetic diversity and range of distribution of all available species in the genus *Oryza*, were

Table 1. Number of accessions of *Oryza* spp. not infected with RTBV and RTSV in screening test using *Nephotettix virescens*

Species	Genome	No. of accessions		
		Tested	No infection with	
			RTBV	RTSV
O. sativa complex				
O. nivara	AA	56	0	5
O. rufipogon	AA	20	3	10
Natural hybrids	AA	35	0	6
O. glaberrima	AA	4	0	0
O. barthii	AA	9	0	3
O. meridionalis	AA	2	0	0
O. longistaminata	AA	8	0	2
Subtotal		134	3	26
O. officinalis complex				
O. officinalis	CC	15	4	6
O. rhizomatis	CC	6	1	1
O. eichingeri	CC	5	0	2
O. malampuzhaensis	BBCC	3	0	1
O. minuta	BBCC	13	0	6
O. punctata	BB	4	0	2
O. punctata	BBCC	3	0	0
O. latifolia	CCDD	5	1	1
O. alta	CCDD	3	2	0
O. grandiglumis	CCDD	2	0	0
O. australiensis	EE	4	0	2
Subtotal		63	8	21
O. ridleyi complex				
O. longiglumis	Tetraploid	3	1	0
O. ridleyi	Tetraploid	5	2	0
Subtotal		8	3	0
Not in any complex				
O. brachyantha	FF	5	1	5
Total		210	15	52

tested for tungro resistance (Table 1). Of these, 202 accessions were tested at the seedling stage. Seedlings of each accession were raised in clay pots. When seedlings were 14 days old, they were inoculated with 10 viruliferous GLH (*N. virescens*) adults per plant for 4 hr. Leaves were individually sampled 2–3 weeks after inoculation to diagnose RTBV and RTSV infection by enzyme-linked immunosorbent assay (ELISA)[1]. Cuttings from 8 accessions of *O. longistaminata* A. Chev. *et* Roehr. were tested instead of seedlings. Taichung Native 1 (TN1) and Utri Merah (International Rice Germplasm Center Acc. no. 16680) were used as susceptible and resistant checks, respectively.

Among the 210 accessions of wild species evaluated for resistance to infection with tungro viruses, 52 (24.7%) were not infected with RTSV, and 15 (7.1%) were not infected with RTBV (Table 1). Of the 15 accessions representing 8 species resistant to RTBV infection, 3 accessions of *O. rufipogon* Griff. (IRGC Acc. no. 105908, 105909 and 105910), 2 of *O. officinalis* Wall *ex* Watt (IRGC Acc. no. 105100 and 105365) and one each of *O. rhizomatis* Vaughan (IRGC Acc. no. 103421) and *O. brachyantha* A. Chev. *et* Roehr. (IRGC Acc. no. 100115) were resistant to infection with RTSV as well (Table 2).

Resistance to leafhopper vectors

The resistance to RTBV of 15 wild accessions (Table 2) may depend on the resistance to the vector leafhopper. To determine whether the resistance to tungro virus of the 15 accessions that were resistant to RTBV infection is dependent on vector resistance, the level of antibiosis to leafhoppers (*Nephotettix* spp.) which is a major factor of resistance to the vector, was evaluated.

The level of antibiosis of each accession was evaluated as high, moderate or low, based on the results of the nymph survival test[9].

One accession of *O. rufipogon* (IRGC Acc. no. 105909) showed a low level of antibiosis to *N. virescens* (Table 3). The other 2 accessions of *O. rufipogon* (IRGC Acc. no. 105908 and 105910) showed a moderate level of antibiosis. The other 12 accessions of wild rice

Table 2. Accessions of wild *Oryza* spp. not infected with RTBV in screening test using *Nephotettix virescens*

Genome	Species	IRGC Acc. no.	Origin	Plants tested (no.)	Infection rate (%) with	
					RTBV	RTSV
AA	*O. rufipogon*	105908	Thailand	20	0	0
		105909	Thailand	23	0	0
		105910	Thailand	23	0	0
CC	*O. officinalis*	104672	Malaysia	20	0	5
		105100	Brunei	28	0	0
		105365	Thailand	41	0	0
		105376	Thailand	29	0	3
CC	*O. rhizomatis*	103421	Sri Lanka	18	0	0
CCDD	*O. latifolia*	105139	Guatemala	25	0	4
CCDD	*O. alta*	100967	Surinam	19	0	10
		105685	Brazil	29	0	11
Tetraploid	*O. longiglumis*	105146	Indonesia	24	0	33
Tetraploid	*O. ridleyi*	100821	Thailand	11	0	9
		101453	Malaysia	30	0	3
FF	*O. brachyantha*	100115	Guinea	29	0	0

Table 3. Level of antibiosis to leafhoppers (*Nephotettix* spp.) of wild species of rice (*Oryza* spp.)

Species	IRGC Acc. no.	Level of antibiosis to[a)]	
		N. virescens	*N. nigropictus*
O. rufipogon	105908	M	-
	105909	L	-
	105910	M	-
O. officinalis	104672	H	M
	105100	H	L
	105365	H	L
	105376	H	L
O. rhizomatis	103421	H	L
O. latifolia	105139	H	H
O. alta	100967	H	H
	105685	H	H
O. longiglumis	105146	H	H
O. ridleyi	100821	H	M
	101453	H	M
O. brachyantha	100115	H	-
O. sativa (Check varieties)			
TN1		L	L
IR64		H	-
PTB2	6215	-	L
ARC11554	21473	-	L

a): H; High, M; Moderate, L; Low, -: Not tested.

species showing a low nymph survival were considered to display a high level of antibiosis to *N. virescens*.

To confirm the virus resistance of the 12 accessions which were resistant to *N. virescens*, an alternative vector *N. nigropictus* Stål was employed for further evaluation.

As a result of the nymph survival test[9)], 3 accessions of *O. officinalis* (IRGC Acc. no. 105100, 105365 and 105376) and 1 of *O. rhizomatis* (IRGC Acc. no. 103421) were found to show a low level of antibiosis to *N. nigropictus* (Table 3). On the other hand, the level of antibiosis of 1 accession of *O. longiglumis* Jansen (IRGC Acc. no. 105146), 1 of *O. latifolia* Desv (IRGC Acc. no. 105139) and 2 of *O. alta* Swallen (IRGC Acc. no. 100967 and 105685) was high. One accession of *O. officinalis* (IRGC Acc. no. 104672) and 2 of *O. ridleyi* Hook. f. (IRGC Acc. no. 100821 and 101453) showed a moderate level of antibiosis to *N. nigropictus*.

Test for resistance to infection with rice tungro viruses using *N. nigropictus*

Wild *Oryza* accessions which showed a high level of antibiosis to *N. virescens* (Table 3) were inoculated with rice tungro viruses using *N. nigropictus* as a vector, to confirm the resistance to the viruses of these accessions.

Twelve wild *Oryza* accessions were grown in a seedling box and infested with viruliferous *N. nigropictus* (mass-inoculation test) (Table 4). Two weeks after the inoculation, each plant was indexed by ELISA.

Of the 12 wild accessions, 6 accessions — 3 of *O. officinalis* (IRGC Acc. no. 105100, 105365 and 105376), 1 each of *O. rhizomatis* (IRGC Acc. no. 103421), *O. ridleyi* (IRGC Acc. no. 100821) and *O. brachyantha* (IRGC Acc. no. 100115) — were not infected with RTBV (Table 4). Of these, 4 accessions (IRGC Acc. no. 105365, 105376, 103421 and 100115) did not become infected with either RTBV or RTSV, although data were obtained from a small number of plants.

Four accessions of *O. officinalis* and 1 of *O. rhizomatis*, which showed a low or moderate level of antibiosis to *N. nigropictus* (Table 3), were tested for resistance to inoculation of tungro viruses according to the same procedure as that described in the previous section using *N. virescens*.

Table 4. Infection rate with tungro viruses of wild rices in mass-inoculation test[a)] using *Nephotettix nigropictus*

Species	IRGC Acc. no.	No. of plants tested	Infection rate (%) with		
			RTBV	RTSV	None
O. officinalis	104672	21	10	19	81
	105100	16	0	6	94
	105365	9	0	0	100
	105376	2	0	0	100
O. rhizomatis	103421	6	0	0	100
O. latifolia	105139	30	7	17	80
O. alta	100967	27	7	15	81
	105685	29	7	0	93
O. longiglumis	105146	7	14	100	0
O. ridleyi	100821	28	0	7	93
	101453	28	4	7	93
O. brachyantha	100115	4	0	0	100
O. sativa (Check varieties)					
TN1		13	69	46	23
ARC11554	21473	27	15	0	85
Utri Merah	16680	21	24	0	76

a): See text.

Table 5. Infection rate with tungro viruses of wild species of rice (*Oryza* spp.) by *Nephotettix nigropictus*

Species	IRGC Acc. no.	No. of plants tested	Infection rate (%) with		
			RTBV	RTSV	None
O. officinalis	104672	28	7	0	93
	105100	28	4	0	96
	105365	23	4	0	96
	105376	26	0	0	100
O. rhizomatis	103421	30	30	3	70
O. sativa (Check varieties)					
TN1		30	37	17	63
Utri Merah	16680	30	0	0	100

As a result, 1 accession of *O. officinalis* (IRGC Acc. no. 105376) was not infected with either RTBV or RTSV (Table 5). Only a few plants in the other 3 accessions of *O. officinalis* (IRGC Acc. no. 104672, 105100 and 105365) were infected with RTBV.

Discussion

Resistance to RTBV infection is considered to be more important and more effective in tungro disease control since RTBV is the main cause of the tungro symptoms. Among 210 accessions of wild species tested for tungro resistance using *N. virescens* as a vector, 15 were not infected with RTBV.

Of these 15 accessions, 3 accessions of *O. rufipogon* (IRGC Acc. no. 105908, 105909 and 105910) were not infected with both tungro viruses and showed a low or moderate level of antibiosis to the major vector *N. virescens*. The results obtained suggest that these accessions are resistant to infection with tungro viruses, regardless of vector resistance. These accessions of *O. rufipogon* from Thailand may thus be useful donors for breeding tungro-resistant rice cultivars because *O. rufipogon* has the same genome constitution (AA) as cultivated rice and can be readily crossed to the cultigen.

The level of antibiosis to *N. nigropictus* of 3 accessions of *O. officinalis* (IRGC Acc. no. 105100, 105365 and 105376) was low. Few plants of these *O. officinalis* accessions were infected with RTBV by *N. nigropictus*. Of these, 1 accession (IRGC Acc. no. 105376) was not infected with RTBV in any of the inoculation tests. Thus, these 3 accessions of *O. officinalis* appear to be resistant to RTBV regardless of vector resistance although the resistance of these 2 accessions was not complete.

One accession of *O. ridleyi* (IRGC Acc. no. 100821) showed a moderate level of antibiosis to *N. nigropictus* and was not infected with RTBV by this leafhopper species in the mass-inoculation trial. The resistance to RTBV infection of this accession may be independent of vector resistance.

The level of resistance to rice tungro disease displayed by these 7 wild *Oryza* accessions was higher than that found in cultivated rice. Although a number of barriers limit the production of hybrids between related species, significant advances have been made to overcome these barriers and alien gene transfers have been achieved[7]. The various sources of resistance reported in this paper are potential donors of resistance to tungro and are considered to be very useful for the control of tungro disease.

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