Inheritance of Resistance to Grassy Stunt Virus in Rice

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ABSTRACT

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Six cross combinations of the susceptible *Oryza sativa* cultivars IR8, IR20, IR22, IR24, and IR773A-1-3 with the resistant *Oryza nivara* accession 101508 were used to determine the inheritance of resistance to the grassy stunt virus. All F_1-crossed progenies of the crosses were comparable in resistance to the selfed progenies of *O. nivara*. The F_2 populations segregated into a ratio of three resistant to one susceptible. The progenies of the first, second, and third backcrosses to the recurrent susceptible parents gave a ratio of one resistant to one susceptible. These results support the hypothesis that resistance to the grassy stunt virus in *O. nivara* is governed by a single dominant gene.

Rice grassy stunt, first observed in the International Rice Research Institute (IRRI) experimental farm in 1963, is caused by a virus transmitted by the brown planthopper *Nilaparvata lugens* (Stål) (2,3,8,10). Disease incidence has increased considerably in the Philippines, where rice is often intensively and continuously cultivated. Disease developed in epidemic proportion in the Bukidnon, Iloilo, Laguna, Nueva Ecija, and Negros Occidental areas of the Philippines, where yield losses ranging from 60 to 100% were observed. Rice plants that are artificially inoculated at an early stage of growth produce almost no yield (9). The disease has been observed in other tropical Asian countries (1).

The spread of the grassy stunt virus can be minimized by chemical control of its vector and by field sanitation immediately after harvest to reduce the source of inoculum for subsequent crops. However, the use of insecticides alone to control the insect vector is not economically feasible in some areas. An alternative approach is the breeding of resistant varieties.

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The development of varieties resistant to grassy stunt virus is a major objective of the breeding program of IRRI. About 6,723 entries of rice varieties, selections of *Oryza sativa* L. and wild species of *Oryza nivara* Shar. et Shas., have been evaluated for resistance to grassy stunt virus. Only accession 101508 (*O. nivara*), which had a consistently low percentage (an average of 6%) of infected seedlings in repeated tests (6), showed less than 30% infection. This accession was crossed with four *O. sativa* varieties and one breeding line to study the inheritance of resistance to the grassy stunt virus and to develop breeding lines with grassy stunt virus resistance and desirable agronomic characteristics.

MATERIALS AND METHODS

Four susceptible varieties—IR8, IR20, IR22, and IR24—and breeding line IR773A-1-3 or *O. sativa* were crossed with the resistant wild rice species *O. nivara* from India by the modified clip method of hybridization (4). The F_1 populations of all six crosses were backcrossed to the recurrent susceptible parents (BC_1) after their resistant reactions to the grassy stunt virus had been determined. The resistant hybrids were backcrossed to the recurrent susceptible parents three times to produce third backcross progenies (BC_3).

Seeds of six F_1 crosses and the three backcross populations were separately treated with thiram (tetramethyl-thiram desulfide, 50%) solution (1:1,000) for 5 min, rinsed three times with distilled water, germinated in petri dishes, and planted in pots.

Viruliferous insects (*N. lugens*) were allowed to feed on the F_1, F_2, and BC populations as well as on the parents of these populations at the same leaf stage (6). The plants were removed from the cages 24 hr after inoculation. The F_1 and backcross progenies were transplanted into our concrete nursery beds, and the F_2 populations were transplanted in the field.

Disease reactions of inoculated plants were evaluated 45 days after inoculation, when symptoms of grass stunt on susceptible plants were clearly visible.

The individual inoculated plants were classified as resistant when there were no symptoms and susceptible when they showed typical grassy stunt symptoms. Chi-square tests were performed to determine the goodness of fit in segregating populations.

RESULTS AND DISCUSSION

The success of artificial inoculation was measured by disease incidence, or the percentage of virus infection in susceptible varieties. This value was obtained by dividing the number of infected plants by the total number of plants inoculated with grassy stunt virus (×100). IR8, IR773A-1-3, IR24, and IR22 had 98, 96, 95, and 93% infection, respectively. IR20 had 75% infection. The high percentage of infection among susceptible plants indicated that the inoculation procedures were satisfactory.

All F_1 seedlings from six crosses among resistant *O. nivara* and the susceptible rice varieties were resistant to grassy stunt virus. The F_1 progenies of IR8 × *O. nivara* (33 seedlings), the reciprocal (six seedlings), and IR 20 × *O.*

Table 1. Reaction of F_1 and F_2 populations of *Oryza nivara* and six crosses to grassy stunt virus infection

Oryza sativa parents and hybrids	F_1		F_2					
	Infection (%)	Plants tested (no.)	Observed no. of progeny		Expected no. of progeny		χ^2 (3:1)	*P* (%)
			Resistant	Susceptible	Resistant	Susceptible		
IR8 × *O. nivara*	0	33	321	106	320.25	106.75	0.007	95–90
O. nivara × IR8	0	6	306	104	307.5	102.5	0.029	90–80
IR20 × *O. nivara*	0	16	317	97	310.5	103.5	0.544	50–40
IR22 × *O. nivara*	6.1	49	764	246	757.5	252.5	0.223	70–60
IR24 × *O. nivara*	5.7	70	691	232	692.25	230.75	0.009	95–90
IR773 × *O. nivara*	5.4	93	481	167	486	162	0.206	70–60

Table 2. Segregation in grassy stunt disease reaction of backcross 1, 2, and 3 populations of rice crosses

Oryza sativa cross combination	Observed no. of progeny		Expected no. of progeny		χ^2	*P*
	Resistant (%)	Susceptible (no.)	Resistant	Susceptible	(1:1)	(%)
			BC_1F_1			
IR8 × *O. nivara*	37	30	33.5	33.5	0.731	40–30
O. nivara × IR8	23	22	22.5	22.5	0.022	90–75
IR20 × *O. nivara*	19	12	15.5	15.5	1.581	30–20
IR22 × *O. nivara*	12	10	11	11	0.181	70–60
IR24 × *O. nivara*	33	29	31	31	0.258	70–60
IR773 × *O. nivara*	42	42	42	42	0.000	100–99
			BC_2F_1			
IR8 × *O. nivara*	113	119	116	116	0.155	70–60
O. nivara × IR8	30	36	33	33	0.545	50–30
IR20 × *O. nivara*	54	68	61	61	1.607	30–20
IR24 × *O. nivara*	169	187	178	178	0.910	40–30
IR773 × *O. nivara*	494	515	504.5	504.5	0.437	60–80
			BC_3F_1			
IR8 × *O. nivara*	70	72	71	71	0.028	90–80
IR20 × *O. nivara*	36	37	36.5	36.5	0.014	95–90
IR24 × *O. nivara*	64	69	66.5	66.5	0.188	70–60
IR773 × *O. nivara*	49	44	46.5	46.5	0.269	70–60

nivara (16 seedlings) were not infected. In contrast, the F_1 progenies of IR22 × *O. nivara* (49 seedlings), IR24 × *O. nivara* (70 seedlings), and IR773 × *O. nivara* (93 seedlings) had 6.1, 5.7, and 5.4% infection, respectively. The F_1 progenies of all crosses exhibited a level of resistance comparable to the resistance of the resistant parent *O. nivara*, which had an average of about 6% infected seedlings (6).

Segregation ratios for resistance in the F_2 populations of all six crosses agreed with the expected monohybrid ratio of three resistant plants to one susceptible (Table 1). The resistance of *O. nivara* to grassy stunt virus appeared to be governed by a single dominant gene. These results confirm findings in previous studies (3,5,7).

Progenies of all the backcrosses (BC_1, BC_2, and BC_3) segregated into a ratio of one resistant to one susceptible plant, which also indicated monogenic resistance Table 2).

The monogenic nature of inheritance of resistance to this virus has important practical implications for rice breeding. Breeders can select from the progenies of crosses between *O. nivara* and high-yielding varieties and obtain progenies with the gene for resistance as early as the F_2 generation. F_3 plants from resistant F_2 plants should be inoculated with the grassy stunt virus by the mass screening method using *N. lugens* to determine lines with homozygous resistance to the grassy stunt virus. Plants with desirable agronomic traits selected from the homozygous resistant plants can be used as parents in crosses with susceptible high-yielding varieties, instead of with the agronomically undesirable *O. nivara*, in breeding for resistance to the grassy stunt virus.

Results from the backcrosses showed that resistance could be quickly introduced into varieties that were agronomically desirable but susceptible to the grassy stunt virus. From the second and third backcross populations, many resistant individuals possessing desirable characteristics similar to those of the recurrent susceptible parents were selected. This success indicated that genes for grassy stunt virus resistance could be combined with genes controlling desirable agronomic characters. Backcross lines IR1737 ($IR24^4$ × *O. nivara*) and IR1917 ($IR20^4$ × *O. nivara*) were used in synthesizing IR28, IR29, IR30, IR32, IR36, IR38, IR40, and other new, major varieties developed at the International Rice Research Institute.

Screening of varieties for resistance to grassy stunt virus should be done continually to identify varieties with resistance genes different from the genes of *O. nivara*. These resistant varieties could be used in the hybridization program in case the grassy stunt virus mutates. Another alternative is to cross varieties with 20–30% resistance and to test their hybrids against the grassy stunt virus to determine whether the progenies will have increased levels of resistance.

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