Variability in Response of Selected Traditional and IRRI Rices to Tungro

Cesar G. Demayo[1] and Isaias T. Domingo[2]

Abstract

Six traditional and six IRRI rices with known genetic background for resistance to the green leafhopper Nephotettix virescens *were planted in five locations in the Philippines for four seasons. Ten-day seedlings were planted at 25 x 25 cm spacing in 2 rows 5 m long alternated with two rows of susceptible TN1 replicated four times every season. At 30 and 60 days after transplanting, tungro-infected hills were counted. The data for both traditional and cultivated rices were subjected to ANOVA. Results showed variability in response of the rices to tungro from locality to locality and from season to season. There was a negative correlation between the number of GLH collected and the frequency of infection on different rices. Population genetic studies on selected GLH populations revealed genetic differences even on populations which are geographically close. These results indicate that variation in response of the rices to tungro was due to differences in virulence of the vectors.*

Key Words: Tubgro, *Nephotettix virescens*, resistance

Introduction

In the Philippines, tungro transmitted by the green leafhopper *Nephotettix virescens* Distant destroyed 70,000 hectares of rice in 1971 and 40,000 hectares in 1972. In recent years, the widespread cultivation and continuous cropping of early maturing, high-yielding varieties and intensive spraying of insecticides intended to increase production have contributed considerably to the increased problem of *Nephotettix virescens* and rice tungro virus (RTV) disease (Ruangsook, 1986). Modern rice cultivars which exhibited tolerance to tungro infection in farmer's fields were observed to be resistant only to the vector and not to RTV. As the vector can adapt to resistant cultivars, tungro infection continues to afflict the rice crop. Breeding rice resistant to insect pests and pathogens usually requires a longer period of time. It therefore concerns specialists in plant breeding, plant pathology, entomology, crop science, and other disciplines when shortly after a variety is released in a commercial level, it succumbs to the herbivore's or pathogen's rapid evolution to breach the variety's resistance mechanism(s). The discovery of these adapted genotypes was believed to occur only if in one or more resistance evaluation tests, the population of *N. virescens* used had the presence of these genotypes. We therefore conducted an investigation to find out whether the existing traditional and IRRI cultivated rices which contain resistance genes against the

This study was funded by a financial grant provided by the International Center of Insect Pysiology & Ecology (ICIPE) and the International Rice Research Institute (IRRI)

[1]Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City

[2] Entomology & Plant Pathology Division, The International Rice Research Institute (IRRI), College, Laguna

green leafhopper will be infected by RTV due to the capabilities of specific genotypes of the vector which may be present in a localized population. We also wanted to assess whether the response of the traditional and IRRI rices to tungro varies in frequency for every locality and whether this is maintained from season to season. As to finding out whether the populations of the vectors were genetically differentiated or not, and whether gene flow will affect the population structure, population genetics of selected populations were conducted. An implication of these studies to the future of resistance in rice was discussed.

Materials and Methods

Experimental Area. Localities of diverse background were selected for this study. Farmers fields in selected locations in Bicol, Cotabato, Palawan, Negros Occidental, and Isabela were selected due to the magnitude of their distance. Bicol and Isabela were at least 800 kilometers away from each other in the Luzon area, Cotabato, Negros Occidental, and Palawan were islands geographically isolated by large distances and seas.

Rices. We evaluated selected cultivars with known genetic background for resistance to green leafhoppers (GLH). For the test on traditional rices, Pankhari 203 (Glh1), Palasithari 601 (Glh2), ARC11545 (unknown), Utri Merah (unknown), Utri Rajapan (unknown), and Ptb8 (glh4) were used. For the IRRI rices IR64, IR66, IR68, IR70, IR72, IR74 (all resistant rices with no specific resistance genes) were tested. TN1 (no resistance gene) was used as susceptible check variety for both tests.

Experimental Design. Field plots were prepared ten (10) days before transplanting of test materials grown in protected seedbeds. Ten-day old seedlings were planted at 25 x 25 cm spacing in two rows 5-m long alternated with two rows of susceptible TN1 replicated four times for three to five seasons (1987 wet, 1988 dry, 1988 wet, 1989 and 1990 wet) depending on the locations. Three seasons were conducted on Bicol, four each for Bacolod, Palawan, Isabela, and five for Cotabato. At 30 days after transplanting, insects were collected and counted, and tungro infection was surveyed. At 60 days, tungro infected hills were counted. Analysis of variance (ANOVA) was conducted on arcsin transformed data.

Population Genetic Studies. The rate of adaptation of the vectors to resistance is dependent on the population structure of the pest. In this study, population structure refers to the extent and range of population subdivision and/or intermixing. For example, large movements of susceptible individuals into treated regions of resistant individuals will influence the rate of development of resistance. Even the most limited gene flow may spread genes conferring resistance from one population to another. If movement among populations is small, then populations are likely to differ in many traits that directly determine pest severity - such as the ability to survive and reproduce on resistant rices and subsequently spreads the RTV it carries. To have an idea of gene flow and the genetic structure and differentiation of populations of the green leafhopper, analysis of gene products (polymorphic enzyme data) as revealed by electrophoresis on a horizontal gel was done. In practice, insects were first directly collected from the field, allowed to feed on caged TN1 rice plants and brought to the laboratory. The insects were then removed from the plant and frozen at -70°C prior to electrophoresis. Microliter amount of crude tissue extracts of squashed whole insects were adsorbed on whatman filter paper strip #3 and inserted directly into the gel slot. The gel was then subjected to direct electric current for four hours at 4°C 40mA/gel slab. After electrophoresis, the gels were sliced, and stained according to the procedure of Shaw

and Prassad (1970).

The genetic interpretation of the data made use of direct scoring of genotypes from the colored spots in the gel. Allele frequencies were obtained from this data. Measures of genetic identity and distance for all pairwise comparisons of populations were tested using BIOSYS-1 (Swofford and Selander, 1981), a computer analysis of genetic variability in populations. Heterogeneity of gene frequencies among populations was tested using the methods of Workman and Niswander (1970). Fst measures of genetic differentiation (Nei, 1973) can range between 0 (no genetic differentiation) and 1 (complete differentiation) (Crow, 1986). In this study, it represents the standardized variance in allele frequencies among local populations (Weir, 1990).

An estimate of gene flow was obtained using the following equation: $Fst = 1/(4Nm + 1)$ where N is the effective size of each population and m is the proportion migrating (Wright, 1951). Thus, a measure of gene flow Fst, or the number of migrants entering and leaving each population each generation (Slatkin, 1985, 1987). The equation assumes an "island model" in which every local population is equally likely to exchange migrants with any other populations.

Results

Means, standard errors of tungro response of the different rices in different seasons from different localities are presented in Tables 1 and 2. Analysis of variance

Table 1. Tungro response of selected IRRI rice types in different localities.

LOCALITY			RICE TYPES						
	SEASON	YEAR	IR64	IR66	IR60	IR68	IR72	IR74	TN1
Bicol	Wet	1987	85.75	66.75	0	0	0	0	99.50
	Dry	1988	88.75	18.25	2.75	3.75	5.25	3.25	94.50
	Wet	1988	92.75	40/50	55.25	0	0	0	96.25
Isabela	Wet	1987	89.25	67.5	0.75	0	1.0	2.75	99.5
	Dry	1988	98	93.25	10.25	9.0	2.0	2.5	94.5
	Wet	1988	100	100	93.5	70.25	75.0	9.5	96.25
	Dry	1989	96.25	90.25	7.0	0	0	0	100
Palawan	Wet	1987	91.75	62.5	28.0	2.25	1.75	0	100
	Dry	1988	100	98.25	6.5	4.0	9.0	0	98.75
	Wet	1988	100	93.25	79.0	12.75	27.0	13.5	100
	Dry	1989	100	2.75	3.5	2.75	0	0	100
Negros	Wet	1987	100	70.0	2.0	13.0	1.0	4.25	100
	Dry	1988	100	89.0	11.0	9.75	5.25	4.75	100
	Wet	1988	100	98.25	100	3.75	0	0	100
	Dry	1989	97.5	87.0	87.0	3.25	0	0	97.5
Cotabato	Wet	1987	100	100	23.0	32.25	5.0	14.0	100
	Dry	1988	100	100	9.0	8.0	77.0	5.75	100
	Wet	1988	100	81.5	2.5	2.0	0	4.5	100
	Dry	1989	100	100	100	87.0	67.0	7.75	100
	Wet	1990	100	100	100	100	94.75	79.75	100

revealed significant differences in tungro response by the different rices for every population and for different seasons (Table 3). It was shown from the analysis that the

Table 2. Tungro response of selected traditional rice types in different localities.

LOCALITY	TRADITIONAL RICE TYPES								
	SEASON	YEAR	Pankhari 203	Palasithari 601	ARRC 11554	Utri Merah	Utri Rajapan	Ptb8	TN1
Bicol	Wet	1987	0	0	0.75	0	3.25	0	99.50
	Dry	1988	0	0.	0	0	0.75	0	94.50
	Wet	1988	0	0	0	0	0	0	96.25
Isabela	Wet	1987	0	0	0	0	0	4.0	99.5
	Dry	1988	0	15.25	0	0	0	7.0	94.5
	Wet	1988	7.25	0	0	0	0	4.0	96.25
	Dry	1989	0	0	0	0	9.5	0	100
Palawan	Wet	1987	0	0	3.0	0	0	0	100
	Dry	1988	0	0	1.25	0	0	25.75	98.75
	Wet	1988	1.25	3.25	0	0	0	80.75	100
	Dry	1989	0	0	0	0	0	0	100
Negros	Wet	1987	4.0	9.25	1.0	3.0	21.75	11.0	100
	Dry	1988	2.0	1.5	0	11.75	0.75	0	100
	Wet	1988	0	0	0	0	0	0	100
	Dry	1989	1.5	0	0	0	2.75	7.75	97.5
Cotabato	Wet	1987	2.0	8.25	2.0	6.0	12.25	71.0	100
	Dry	1988	0	0	0.75	0.75	0.75	4.25	100
	Wet	1988	5.25	0	1.25	2.0	1.25	1.25	100
	Dry	1989	2.0	0	0	0	3.25	86.5	100
	Wet	1990	4.0	0	0	0	5.25	89.75	100

differential responses of the different rices whether traditional or cultivated, were major factors for the differences observed between locations. The unstable response of some of

Table 3. Analysis of variance of tungro response by selected rice types in different locations.

SOURCE	DF	TYPE III SS	MEAN SQUARE	F-VALUE	Pr>F
		Traditional	RiceTypes		
Season (S)	3	0.424	0.14	7.17	0.0001
Locality (L)	4	1.372	0.34	17.4	0.0001
Rice Type ® (RT)	6	134.729	22.45	1139.15	0.0001
L by S	11	2.307	0.21	10.64	0.0001
RTby S	18	0.813	0.05	2.29	0.002
RTby L	24	3.04	0.13	6.52	0.0001
		IRRI RiceTypes			
Season (S)	3	2.985	1.00	21.82	0.0001
Locality (L)	4	7.143	1.79	39.17	0.0001
RiceType (RT)	6	160.311	26.72	585.97	0.0001
L by S	11	14.546	1.32	29.0	0.0001
RTby S	18	7.576	0.42	9.23	0.0001
RTby L	24	5.258	0.21	4.80	0.0001
				0.0001	

the rices in different locations for every season has been the primary factor why there were differences in observed tungro response for every season of tungro response evaluation. The number of *N. virescens* collected in different varieties was observed to be not directly correlated with the extent of tungro response by the plants. Within locations, a detailed analysis of the pattern of tungro infection for every season of tests is presented.

Bicol. In Bicol, a comparison of response of rice types for three seasons has shown differential reactions between the IRRI rices. Rice type by season interaction was highly significant (P>0.001) indicating that differential host response was a major factor for differences in tungro response each of the seasons. A closer look at the data shows that IR64 was consistently susceptible while IR70, IR72, and IR74 were highly resistant for all three seasons. However, IR66 showed susceptibility in 1987 wet season but not in 1988 where it was resistant in the dry season and moderately resistant in the wet season. IR68 was consistently resistant for two seasons but became moderately susceptible in the wet season of 1988. A closer look at the data (Table 1), reveals that a more diverse population of the vector is present in 1988 dry season than in 1987 wet and 1988 wet seasons. Some infections were observed in all of the IRRI rices planted during these seasons. However, in terms of frequency, the number is relatively low since very few infections were observed on the rices.

For the test on traditional rices, the significant rice type effects (P>0.001) were attributed primarily to the susceptibility of the TN1 and some degree of resistance reactions of

Utri Rajapan (Table 2). However, using the standard resistance evaluation, all the traditional rices were observed to be highly resistant in Bicol. Population number of *N. virescens* is not directly correlated with resistance. Population number was high in 1987 wet season but not in the succeeding seasons although the degree of response to tungro was relatively the same. The population of *N. virescens* is not diverse due to low infection of two rices in 1987 and complete noninfection of all the rices in succeding seasons.

Isabela. For the IRRI rices in Isabela, IR64 was consistently showing high susceptibility while IR74 was highly resistant. All other IR rices were observed to be highly resistant in one season but not in others indicating the instability of their resistance factors (Table 1). IR66 was highly resistant in 1989 dry season but was observed to be susceptible in the previous seasons. IR 68, IR70, and IR72 became highly susceptible in 1988 wet season although these rices were highly resistant in three of the four seasons. The population in this location may have diverse genotypes of the vector as shown by differences in response of the resistant rices to tungro. Furthermore, the increase of the frequency of infection in some of the rices in the suceeding seasons indicates that the genotypes that are capable of transmitting the virus in this locality had been maintained.

For the traditional varieties, rice type effects were also significant (P>0.001) although the variability in response was attributed mainly to the susceptibility of the control check of TN1 and the unstable response of Ptb8 (26%) in 1988 dry season and 81% in 1988 wet season. Variation in response of other traditional rice types was not very high and was even absent in Utri Rajapan and Utri Merah in all seasons (Table 2).

Palawan. In this locality, differential response of the IRRI rices was significant (P>0.001). The rice type by season interaction was significant (P>0.001) indicating that variation in host response was an important factor for differences in seasonal response. IR70, IR72, and IR74 showed minor tungro infection in all seasons. Other IR varieties were showing inconsistent response (Table 1). Looking at patterns of infection between hosts and the number of insects collected, no direct correlation between population number and infection rate could be made indicating variability exists within the vector population in their capacity to infect the resistant rices.

In the case of the traditional varieties, a highly significant rice type by season effect was observed (P>0.001) which again indicates that the differential response of the different rices were major reasons for the differences of the pattern of infection observed every season. The variation observed indicates that there exist in the population some genotypes which can feed on the resistant rices and which differ in frequency (Table 2).

Negros Occidental. Significant rice type effects (P>0.001) were observed in all seasons where the test was conducted in this locality. Rice type by locality effects were also significant (P>0.001) which indicates that the host was an important factor for the differences in response of the rices every season. IR64 and IR66 were consistently observed to be susceptible while IR70, IR72, and IR74 were consistently observed to be resistant. IR68 was observed to be resistant in the first two seasons but not in the latter seasons (Table 1). It is interesting to note here that the frequency of infection has a pattern starting from low frequency (2%) to very high frequency (87-100%). There was still no direct relationship between population number and degree of infection. It could be interpreted here that specific genotypes of the vectors which can infect this rice have increased in frequency.

For the traditional varieties, there were differential reactions observed between rice types in some of the seasons and these differences were significant (P>0.001). However, the

extent of response of the rice types was relatively low (<25%) indicating that the rice types were consistently showing high degree of resistance (Table 2). When compared with other locations, the responses of these rices to tungro were consistently high. This location may have a more diverse genotypes of the vector capable of infecting all the traditional rices in certain degrees.

Cotabato. Rice types effects (P>0.001) and seasonal differences (P>0.001) were very pronounced in terms of response to tungro of the IRRI rices in this locality (Table 1). IR64 and IR66 were consistently observed to be highly susceptible to tungro in all seasons. All other IRRI rices were resistant for the first three seasons and showed susceptibility in the fourth season and completely became highly susceptible in the last season of testing. The pattern of infection is not directly correlated with population number. The increasing frequency of tungro infection in selected rices indicates that the geotypes that can infect these rices have increased in frequency.

In terms of the test conducted on the traditional rice types, rice type effects were significant (P>0.001) and were an important factor for the differences observed between seasons as indicated by a strong host x population effects (P>0.001). A closer look at the data (Table 2) indicates that the large variation was contributed by the unstable response of Ptb 8.

Of the five seasons that the different traditional rice types were tested, three seasons (1988 dry, 1989 dry and wet seasons) showed that all six traditional rice types were resistant. Again, the results have indicated no direct relationship between degree of infection and population number. As earlier argued, differential reactions of the rice to tungro in varying frequencies could be due to diversity in vector genotypes capable of transmitting tungro viruses through theit capabiltities of feeding on these resistant rices.

Genetic differentiation among populations. Results of allozyme variation among populations are presented in Table 4. Average heterozygosity within populations was

Table 4. Genetic variability and differentiation at four polymorphic gene loci in all populations of *N. virescens.*

GENE LOCUS	MEAN HETEROZYGOSITY	HETEROGENEITY	Fst
Alkaline phosphatase (Alkp)	0.0319+0.0102	18.09, P>0.001	0.0093
Esterase (Est)	0.1720+0.0542	49.784, P>0.001	0.139
Isocitric dehydrogenase (Idh)	0.0539+0.027	173.47, P>0.001	0.078
Malic dehydrogenase (Mdh)	0.0953+0.026	102.82, P>0.001	0.069
Mean	0.0953	196.28,P>0.001	0.074

0.0953 and ranged from 0.0235 to 0.2720. Genetic differentiation, measured by Fst, was 0.0737, indicating some degree of population subdivision (Table 4). Using the equation for estimating gene flow, Nm was estimated at 8.7134. This estimate of gene flow is appropriate for populations near genetic equlibrium. This value suggests that on the average, about 8.7 individuals are moving in and out of each population each generation.

Among populations, significant differences in gene frequencies were detected among

the populations as indicated by significant heterogeneity of gene frequencies among populations observed in each of the polymorphic loci (Table 4). These differences indicate that gene flow is insufficient to maintain strict genetic similarity among these populations even if they were geographically close. The geographic structure of the populations of *N. virescens* investigated is genetically distinct populations with some gene flow among them.

Discussion

This study show that there exists variation in response of resistant traditional and IRRI culltivated rices to tungro from location to location and the frequency differs from season to season. The major effect of time is dependent on the variation in response from each locality as shown by a very significant population x season interaction. Within populations, a strong host x population effect indicates that the differences in response from each locality are dependent on the differential reactions of the different rices. The variation in response of the resisatnt rices was believed to be caused by the existence from each of the localities, genotypes of the insects which could cause the transmission of the tungro virus by being able to feed on the resistant rices. The frequency of the virulent genotypes of *N. virescens* in natural populations varies as shown by differences in the percentage of tungro infection exhibited by each of the resistant rices. The discovery of *N. virescens* which have adapted to rices with specific genes for resistance (Rapusas and Heinrichs, 1983, 1985; Taulu et al, 1987), the variability in virulence of diferent microgeographic and macrogeographic populations of the pest (Heinrichs et al, 1986), the genetic differences of adapted genotypes (Demayo, 1990) and the existence of genetic differentiation even between geographically close populations (this study) further strengthen the idea that the nature of the vector has a very big role in tungro transmission.

However, one should consider whether the existence of genetic flexibility in the pest populations to overcome the resistance factors present in the plant are geographically restricted, thus the chances of finding them is small. If widely and evenly dispersed geographically but made up of only a small fraction of any single population, it would most likely remain undetectable as shown in this study on the traditional rices.

Scientists involved in breeding for resistance should consider that the discovery of these genotypes even in small frequencies would soon be noticeable few years after commercial use of the rice variety and when natural selection would have favored an increase of the frequency of these adapted genotypes as shown in the case of IR66, IR68, and IR70 varieties.

References Cited

Crow, J. 1986. Basic concept in population, quantitative, and evolutionary genetics. W.H. Freeman, New York.

Heinrichs, E., H. Rapusas, G. B. Aquino, and F. Palis. 1986. Integration of host plant resistance and insecticides in the control of *Nephotettix virescens* Distant, a vector of rice tungro virus. J. Econ. Entomol. 79: 437-443.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. PNAS. 70:3321-3323.

Rapusas , H. and E. Heinrichs. 1983. Plant age and levels of resistance to green leafhopper, *Nephotettix virescens* Distant, and tungro virus in rice varieties. Crop Prot. 1:91-98.

Ruangsook, B. 1986. Genetic analysis of resistance to green leafhopper, *Nephotettix virescens* Distant in some selected rice varieties. Ph. D. thesis. University of the Philippines at Los Banos, College, Laguna. 77p.

Shaw, C. And R. Prassad. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. Biochem. Genet. 4:297 - 320.

Slatkin, M. 1985. Gene flow in natural populations. Ann. Rev. Ecol. Syst. 16:393-430.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236: 787-792.

Swofford, D. and R. Selander. 1981. Biosys 1.7. Department of Genetics and Development, Univ. Illinois. Urbana-Champaign.

Taulu, L., S. Sosromarsono, I. Oka, and E. Guhardja. 1987. Adaptation to green leafhopper, *Nephottetix virescens* to several varieties of rice. Pages 58-65 In Proceedings, Workshop on tungro virus, 24-27 September 1986, South Sulawesi, Indonesia.

Weir, B. S. 1990. Intraspecific differentiation. In D.M. Hillis and C. Moritz. Molecular Systematics, Sinauer, Sunderland, Mass.

Workman, P. and J. Niswander. 1970. Population studies on Southwestern Indian Tribes II. Local genetic differentiation in the Papago. Am. J. Hum. Genet. 22:24-49.

Wright, S. 1951. The genetical structure of the populations. Genetics. 16:97-159.