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# VARIATION IN VARIETAL REACTION TO RICE TUNGRO DISEASE: POSSIBLE CAUSES

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# VARIATION IN VARIETAL REACTION TO RICE TUNGRO DISEASE: POSSIBLE CAUSES[1/]

ABSTRACT

Variation in varietal reaction to rice tungro disease is encountered among locations and over time, particularly in field tests. The variation may be due to *mechanical* errors wherein a variety shows a reaction in the field but is noted differently in a notebook or publication, or to different reactions of rice varieties in the field. The former could be caused by negligence in handling test materials, misidentification of the disease, difference in level of ability to score the reaction, and insufficient replicates; the latter, by existence of different biotypes of tungro vectors or tungro strains among locations or tests, and variation in tungro disease pressure to the variety in the tests. Except for variation caused by biotypes and strains which is under investigation by a Rice Tungro Virus Collaborative Project, the possible causes are discussed with suggestions on minimizing or reducing the errors.

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# VARIATION IN VARIETAL REACTION TO RICE TUNGRO DISEASE: POSSIBLE CAUSES

When rice tungro disease occurs in the field, or after artificial inoculation, rice varieties vary in percentage of infected plants which in turn vary in disease severity in terms of growth retardation or yield reduction (Ling 1969). Because tungro is a systemic infection, the percentage of infected plants reflects the degree of resistance of a variety. The disease severity reveals only the tolerance of a variety to the disease after infection. It can be used only for supplementary rating of the resistance. To rate the disease resistance of a rice variety to tungro, a scale is essential. The international scale of the Standard Evaluation System (SES) for tungro resistance in the field measures such resistance on the basis of percentage of infected plants (Table 1, IRRI 1979). The system is arbitrary because the number of groups and the percentage of infected plants for each scale can be varied according to investigators' desire (Ling 1972).

Table 1. International scale of the Standard Evaluation System for resistance of rice varieties to tungro disease in the field (IRRI 1979).

| Group | Scale | Description |
|---|---|---|
| Resistant | 0 | No infection |
| | 1 | <1% of plants infected |
| | 2 | 1-5% of plants infected |
| | 3 | 6-10% of plants infected |
| Intermediate | 4 | 11-20% of plants infected |
| | 5 | 21-30% of plants infected |
| | 6 | 31-40% of plants infected |
| Susceptible | 7 | 41-60% of plants infected |
| | 8 | 61-80% of plants infected |
| | 9 | 81-100% of plants infected |

Variation in varietal reaction to rice tungro disease has been encountered from place to place and from time to time, particularly in field tests. For example, in 1975 eight varieties, including TN1, which is generally considered highly susceptible, showed all three groups of reaction -- resistant, intermediate, and susceptible -- in different locations and at different times (Table 2).

To investigate the variation, a Rice Tungro Virus (RTV) Collaborative Project was initiated in 1978. Its objective is to identify causes of the variation in varietal reaction with emphasis on experiments to investigate variation caused either by

Table 2. Field reaction of IR varieties to rice tungro disease in various locations and at various times, 1975.

| | Location and time | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | | | | C | | | D | | E | | | F | | | | G | H | |
| Variety | 1 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 4 | 1 | 1 | 2 | 3 |
| IR20 | 2 | - | 3 | 8 | 8 | - | 6 | 6 | - | 9 | - | 3 | 3 | - | 8 | 6 | 9 | 5 | - | 5 | 6 |
| IR28 | 2 | S | 3 | 6 | 6 | S | 9 | 9 | R | 9 | R | 3 | 0 | R | 2 | 3 | - | 1 | S | 6 | 6 |
| IR29 | 2 | R | 3 | 6 | 6 | S | 9 | 9 | R | 9 | R | 3 | 0 | R | 4 | 3 | - | 1 | S | 3 | 4 |
| IR30 | 2 | S | 3 | 6 | 6 | R | 6 | 5 | R | 9 | S | 5 | 7 | R | 5 | 4 | - | 4 | S | 5 | 5 |
| IR32 | 1 | - | 0 | 6 | 6 | - | 9 | 9 | - | 5 | - | 5 | 5 | - | 5 | 5 | 8 | 2 | - | 6 | 6 |
| IR34 | 0 | - | 7 | 9 | 9 | - | 9 | 8 | - | 9 | S | 4 | 5 | R | 3 | 2 | - | 1 | S | 1 | 2 |
| IR36 | 1 | - | 3 | 5 | 5 | - | 9 | 7 | - | 4 | - | 4 | 0 | - | 3 | 2 | 5 | 2 | - | 3 | 4 |
| TN1 | 3 | - | 3 | 9 | 9 | - | 9 | 9 | - | 9 | - | 9 | 9 | - | 9 | 9 | 9 | 9 | - | 6 | 7 |

biotypes of RTV vectors or RTV strains. The project now has seven collaborators in five countries.

Variation in varietal reaction can be caused by factors other than the biotypes of the vectors and strains of the virus. It can be due to *mechanical* error wherein a variety's reaction is noted differently in a notebook or in publication, or to different reactions of rice varieties in the field. The former could be caused by negligence in handling test materials, misidentification of the disease, scoring ability of the enumerator, and insufficient replicates; the latter, by difference in biotypes of tungro vectors and tungro strains, and variation in tungro pressure. This paper points out factors, other than biotypes and strains, that could cause the variation.

## NEGLIGENCE IN HANDLING TEST MATERIALS

Negligence in handling test materials, such as impurity of rice seeds and mislabeling of rice varieties, could cause variation in varietal reaction to tungro disease. The reason is that nonidentical materials may not have identical reaction to the disease in the field. Sufficient care in handling should minimize such variation.

## MISIDENTIFICATION OF THE DISEASE

Misidentification of the tungro disease could also cause the variation. Tungro symptoms consist of

stunting of the plant; mottling, yellowing, and slight twisting of the leaves; and delay in flowering. The common diagnostic symptoms are stunting and yellowing. Stunting varies by degree according to rice variety, plant age at the time of infection, and nutrient conditions. Yellowing also varies by degree according to rice variety, light intensity, and nutrient conditions. The yellowing may sometimes be temporarily masked. Stunting and yellowing, on the other hand, are common symptoms of rice plants brought about by chemical injury, direct insect damage, and nutrient disorders (e.g., sulfur deficiency). Unless the symptoms are clearly diagnostic, additional experiments are needed to confirm tungro identification.

1. Positive transmission results with virus-free vectors provide the most solid evidence for identification, particularly the demonstration of transitory virus-vector interaction (Ling and Tiongco 1977).

2. Persistency of tungro symptoms can be demonstrated after the diseased plants are transplanted in soil with all nutrients and kept under different environmental conditions, or after ratooning. Although persistency of symptoms is not unique for tungro, it illustrates the systemic infection of the disease.

3. Accumulation of starch in leaf blades can be determined by iodine tests, particularly after the removal of chlorophyll by boiling alcohol. Tungro-infected plants often have starch accumulation in the leaf blades (IRRI 1967).

4. Examination under an electron microscope of dip preparations or ultrathin sections of diseased materials for virus particles is the most positive way of identifying tungro.

Recently, Japanese scientists (Hibino et al 1978, Saito 1977a, 1977b) reported two kinds of virus particles associated with tungro disease. The particles were isometric (30 to 33 nm in diameter) and bacilliform (35 x 150-350 nm) with rounded ends. When negatively stained with phosphotungstic acid, the bacilliform particles were mostly 31 x 100-200 nm (Saito, unpublished data). The symptoms of the disease were severe on rice plants infected with both kinds of particles, and moderate on plants infected with only the bacilliform particles. Only slight stunting occurred on plants infected with only the isometric particles. The isometric particles were serologically related to rice waika virus, which occurs in Japan. Consequently, existence of both kinds of particles in the diseased materials would be the criterion for the identification of tungro disease.

Proper identification of the disease is essential in reducing variation in varietal reaction to the tungro disease.

SCORING ABILITY

The ability of enumerators to score varietal reaction to tungro disease could also cause the variation. A variety's reaction in the field may be scored differently by the same individual or group of enumerators and noted differently in the notebook or in publication. An enumerator can use the international scale of the SES (Table 1) to score the reaction of a number of entries of test materials. If he takes the reading of the same entries twice before any change of reaction occurs in the field, his scoring ability can be determined by comparing the two scores of the same entries. When the entries are grouped according to the differences in score between two readings, the frequency of the entries in each group reveals his scoring ability.

The scoring ability of four enumerators tested in 1976 (Table 3) revealed that the scoring ability varied among individuals, and no enumerator scored identically in 2 readings for each of the 561 varieties. The greatest variation occurred when the difference in scores between 2 readings reached 5, indicating that the variety was resistant in 1 reading and susceptible in the other. The actual varietal reaction was the same in the field. If the difference within one (±1) in score of any entry between 2 readings was considered acceptable, the average scoring ability of the 4 enumerators was only 85% reliable. Hence, the other 15% of the readings were not acceptable because the difference in score between 2 readings was greater than one (±1).

Table 3. Frequency (%) of 561 rice varieties grouped by difference in score between 2 readings of their reaction to tungro disease. Readings were made by four enumerators in 1976.

| | Frequency (%) by difference in score between two readings in the range of | | | | | |
|---|---|---|---|---|---|---|
| Enumerator | 0 | ±1 | ±2 | ±3 | ±4 | ±5 |
| A | 42.8 | 75.8 | 93.1 | 98.8 | 99.7 | 100 |
| B | 46.4 | 83.1 | 97.2 | 100 | | |
| C | 48.1 | 86.3 | 100 | | | |
| D | 59.4 | 96.3 | 99.7 | 100 | | |
| Av | 49.2 | 85.4 | 97.5 | 99.7 | 99.9 | 100 |

A varietal reaction may be recorded differently by various enumerators. For instance, 6 enumerators recorded readings of reaction to tungro disease for 28 rice varieties at the Central Rice Research Institute, India, at the same hour in 1978 (IRRI 1978). Except for 1 variety, which each of the 6 enumerators scored 3, varieties received different scores from the enumerators. The maximum difference in score for a variety among the readings from 6 enumerators ranged from 1 to 5 (Table 4). The worst case was the appearance of 6 missing scores for 5 varieties by 3 enumerators. If the same

Table 4. Difference in scores of each entry of varietal reaction to tungro disease given by six enumerators (IRRI 1978).

| Maximum difference in scores | Range of scores of an entry[a] | Entries No. | Entries % |
|---|---|---|---|
| 0 | 3-3 | 1 | 4 |
| 1 | 3-4, 4-5 | 2 | 7 |
| 2 | 2-4, 3-5, 4-6, 5-7, 6-8, 7-9 | 12 | 43 |
| 3 | 3-6, 4-7, 6-9 | 4 | 14 |
| 4 | 3-7, 4-8, 5-9 | 3 | 10 |
| 5 | 3-8 | 1 | 4 |
| Unknown | ?-5, ?-8, ?-9 | 5 | 18 |
| Total | | 28 | 100 |

[a]Scores given by six enumerators; ? = missing score.

standard of acceptance was applied, only 54% of the readings had a difference within 2 (±1) in score of an entry among the 6 enumerators.

The above two examples illustrate that the variation in varietal reaction was not due to variety's reaction *per se* in the field but to the scoring ability of an individual or a group of enumerators.

To reduce such variation, it is essential to prepare a *clip note* before taking the reading of varietal reaction in the field. Because it is sometimes difficult to immediately convert the number of diseased plants to percentage without calculation, the first step is to count the number of rice hills of an entry in the field and then calculate the number of diseased plants for each scale. The *clip note* for a 168-hill entry is in Table 5.

Table 5. *Clip note* for converting number of diseased hills of an entry with a total of 168, 158, 148, 138, and 128 hills to the international scale of the Standard Evaluation System for tungro resistance.

| Scale | Diseased hills (no.) of an entry out of a total of 168 | 158 | 148 | 138 | 128 |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 1 | 1 | 1 | 1 |
| 2 | 2-9 | 2-8 | 2-8 | 2-7 | 2-7 |
| 3 | 10-17 | 9-16 | 9-15 | 8-14 | 8-13 |
| 4 | 18-34 | 17-32 | 16-30 | 15-28 | 14-26 |
| 5 | 35-51 | 33-48 | 31-45 | 29-42 | 27-39 |
| 6 | 52-68 | 49-63 | 46-59 | 43-55 | 40-51 |
| 7 | 69-101 | 64-95 | 60-89 | 56-83 | 52-77 |
| 8 | 102-135 | 96-127 | 90-119 | 84-111 | 78-103 |
| 9 | 136-168 | 128-158 | 120-148 | 112-138 | 104-128 |

Because missing hills sometimes occur in the field, the *clip note* includes 158, 148, 138, and 128 hills per entry or 10, 20, 30, and 40 missing hills per entry.

The enumerator counts the number of tungro-infected hills of an entry and checks the clip note to obtain a proper score of the international scale for the entry. For example, if there are 158 hills (or 10 missing hills) and 50 diseased hills, in the *clip note*, the scale for the entry is 6.

In cases where the diseased hills of an entry are too many, it saves time to count the healthy ones and subtract them from the total number of hills to get the number of diseased hills.

Recording the reading by another person in the field would increase the efficiency of taking the data but it often creates errors. For instance, when the enumerator reads 3 for an entry, the recorder may write another number for the entry in the notebook or enter 3 in another entry. If it is feasible, it would be better to tape record the readings and transfer the data to the notebook later in the office. This method has been found to have less errors.

The scoring ability of an enumerator can be improved by practice. Statistical analysis show that the *errors* or unreliable scoring ability, expressed by variance or coefficient of variance, decreases with increasing experience of the enumerator in terms of number of entries scored previously (Table 6).

Table 6. Comparison of *errors* or unreliable scoring ability of four enumerators with their experience in terms of number of entries scored previously.

| Enumerator | Experience | Mean score | Error Variance | Error cv(%) |
|---|---|---|---|---|
| A | 0 | 5.3 | 0.8846 | 17.6 |
| B | 1,000 | 5.0 | 0.5903 | 15.3 |
| C | 2,000 | 4.6 | 0.4654 | 14.7 |
| D | 40,000 | 5.8 | 0.2678 | 8.8 |

## INSUFFICIENT REPLICATES

In sufficient replicates, which refer to number of test plants per entry, number of replicates in a test, and number of repeated tests, are a possible cause of variation in varietal reaction to rice tungro disease.

Because the SES for varietal resistance to tungro disease is based on percentage of infected plants (Table 1), the percentage figures are limited when

the number of test hills is too small. For example, if there is only one test hill, the percentage of infected plants can only be 0 or 100. If there are only 10 test hills, aside from no infection, the minimum percentage of infected plants is 10, which is scale 3 in the SES (Table 1). In other words, there is no way for 10 test hills to show a reaction of scale 1 or 2, which respectively indicates less than 1% or 1 to 5% infected hills. Consequently, it is essential to have more than 100 hills in a single test for an entry to show a <1% infection.

Although the reason remains obscure for the variation in reaction of a variety to tungro disease in replicates or repeated tests, no variety shows a constant reaction to the tungro disease either in the greenhouse or in the field. When Pankhari 203 and TN1 were tested for their resistance to tungro disease in the greenhouse by the mass screening method, the infection ranged from 0 to 20.7% and from 76.9 to 100%, respectively (Fig. 1, Ling 1968). In the field, IR5, IR8, IR26, IR28, IR34, and IR1561-228-3 individually showed a range of reaction to tungro disease in 381 to 814 replicates and repeated tests (Fig. 2, IRRI 1977). Each variety had its own frequency distribution by its reaction to tungro disease. However, average percentage of infection of Pankhari 203 was much lower than that of TN1 in the greenhouse whereas the average reaction of IR34 and IR28 were much lower than that of the other varieties in the tests.

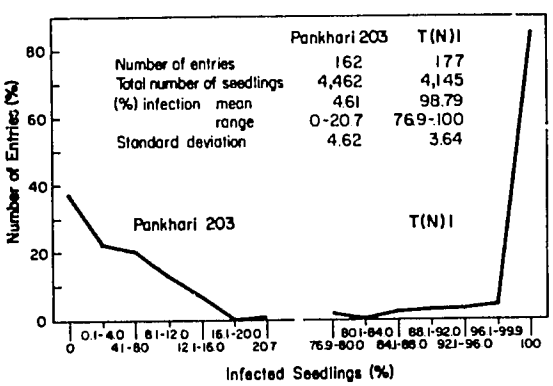


Fig. 1. Frequency distribution of replicates of Pankhari 203 and TN1 by the percentage of tungro-infected seedlings tested by the mass screening method (Ling 1968).

In screening for varietal resistance to a disease, emphasis is given to the resistant variety. Hence, once a variety showed a susceptible reaction in one test, the variety is categorized in the susceptible group and it is generally not tested again. On the other hand, if a variety showed a resistant reaction in one test, the variety is often subjected to more tests before it is categorized in the resistant group.

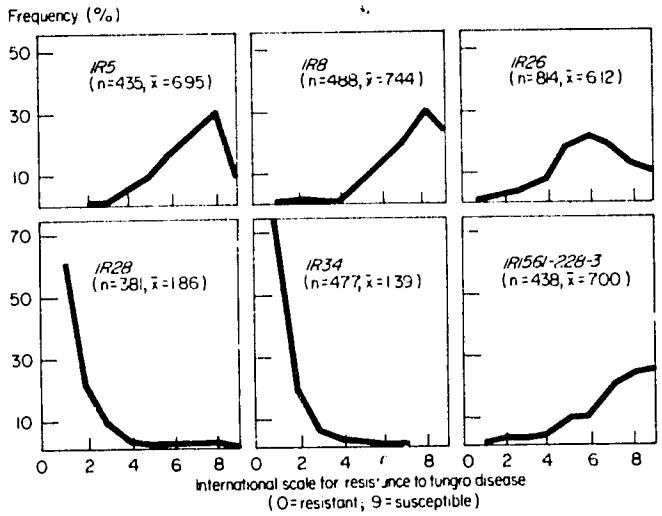


Fig. 2. Frequency distribution of replicates of rice varieties by their field reactions to tungro disease (IRRI 1977).

In testing varietal resistance to a disease, particularly for comparison, statistical procedure dictates replications as a minimum requirement for a valid experimental design. Without replication, there is no way to measure the experimental error. Consequently, increasing the number of test plants per entry, number of replicates in a test, and number of repeated tests increases accuracy in varietal reaction to the tungro disease. An average of the reactions of a variety would be more reliable than its reaction in a single test.

## TUNGRO PRESSURE

Another possible cause of variation in varietal reaction to rice tungro disease is nonidentical pressure on the crop by the pathogen. Tungro pressure varies among test fields, from place to place in a field, and from time to time.

A rice plant has to be inoculated with tungro to become infected. Because tungro virus is transmitted by insect vectors, the amount of tungro inoculation on rice plants in an area is determined by population density and inoculation efficiency of viruliferous insects. The percentage of viruliferous insects is determined by the available quantity, quality, and location of virus source. Although the precise way of measuring the amount of tungro inoculation in the field remains to be investigated, the amount of tungro inoculation on a test variety could be similar to that of other varieties or lines adjacent to the test variety. Therefore, the tungro pressure can be expressed by the average reaction (based on the international scale of the SES, Table 1) of other rice varieties or lines that are

grown adjacent to the test variety or line (IRRI 1977, Ling et al 1977).

When variation of tungro pressure occurs in a field, replicates of a variety may show different reactions in different spots in the field. For example, the reactions to tungro disease of four rice varieties varied among their replicates in a field in Lanrang, Indonesia (Table 7, IRRI 1978). The variation was not due to scoring ability of individual enumerators because the scores given were the consensus of a group of six enumerators.

Table 7. Reactions to tungro disease of replicates of four rice varieties at the Central Research Institute for Agriculture, Lanrang, Indonesia, determined by a group of six enumerators (IRRI 1978).

| Variety | Replicates (no.) | Score | |
|---|---|---|---|
| | | Range | Av |
| IR26 | 8 | 4-7 | 5.8 |
| IR34 | 8 | 2-4 | 3.1 |
| IR2863-38-1-2 | 6 | 3-8 | 4.3 |
| TN1 | 7 | 8-9 | 8.9 |

Theoretically, when there is no tungro pressure in a field, tungro infection of any rice variety cannot occur in the field. When the tungro pressure reaches a maximum level, a test variety should have received the maximum tungro inoculation to show its highest susceptible reaction. Consequently, the reaction of a variety to tungro disease, in terms of either the international scale or percentage of infected hills, increases as the tungro pressure increases (Fig. 3, IRRI 1977). In general, the relation between reaction (Y) of a variety and tungro pressure (x) can be expressed by $Y = a + bx$. However, the $a$ and $b$ values may not be identical among rice varieties. Therefore, the reactions of two rice varieties may be different under various tungro pressures. For instance, if variety A has small $a$ value but greater $b$ value than variety B, variety A should be more resistant to the tungro disease than variety B under a low tungro pressure. On the contrary, variety A becomes more susceptible than variety B under a high tungro pressure. Nevertheless, a variety having small $a$ and $b$ values

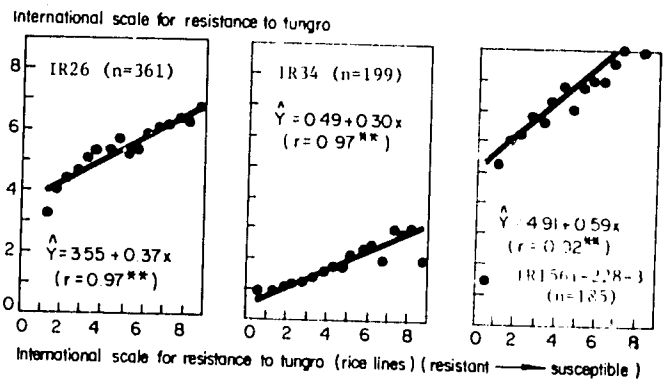


Fig. 3. Field reactions to tungro disease of three rice varieties at different tungro pressures as expressed by the average reactions of other adjacent rice varieties and lines (IRRI 1977).

would be more desirable as far as resistance to tungro disease is concerned.

It is difficult to minimize variation in varietal reaction due to tungro pressure. However, more replicates and repeated tests should provide a greater chance for a test variety to encounter all levels of tungro pressure.

## CONCLUSION

There is no simple solution for eliminating variation in varietal reaction to rice tungro disease. The variation may be due to *mechanical* errors or due to actual difference in the variety's reaction in the field. The former is controllable and efforts can be made to minimize or to reduce the error. The latter is uncontrollable. Repeated tests would provide a greater chance for a test variety to be exposed to various tungro pressures in the field. The average of the tests would be a more reliable indication of the variety's reaction. The other two possible causes -- variation due to RTV vectors and RTV strains -- are being investigated by the RTV Collaborative Project. However, if the biotypes and strains were different among locations, a variety that is constantly resistant in a location or region would still be useful in reducing tungro disease incidence in the location or region through its resistance.

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