BREEDING METHODS AND PROCEDURES EMPLOYED AT IRRI FOR DEVELOPING RICE GERM PLASM WITH MULTIPLE RESISTANCE TO DISEASES AND INSECTS

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Rice is the host of over 60 diseases and more than 100 insect pests. Some of these are of major international importance. During the last ten years major changes have occurred in the varietal composition and cultural practices for rice. High yielding varieties are now planted in approximately 50% of the 130 million hectares occupied by rice all over the world. These varieties are characterized by early maturity, photoperiod insensitivity, short stature, high tillering, and dark green erect leaves. A relatively small number of improved varieties have literally replaced hundreds of traditional cultivars, thereby reducing the genetic variability of the crop.

In the wake of the introduction of varieties with improved plant type, farmers have started using improved cultural practices such as the application of more fertilizers and the establishment of higher plant populations per unit area. Development of irrigation facilities and availability of early maturing, photoperiod-insensitive varieties have enabled the farmers in tropical Asia to grow successive crops of rice throughout the year covering large areas.

Reduced genetic variability, improved cultural practices, and continuous cropping with rice, intended for increased rice production, have increased the genetic vulnerability of the crop. Within the last few years serious outbreaks of diseases and insect pests have affected rice in several countries. Very little research has been done on the chemical control of rice diseases in the tropics. Chemical control of high insect populations for prolonged periods in tropical climates where insect generations overlap throughout the year is very expensive. Social and economic conditions in the tropics present other obstacles to the chemical control of rice diseases and insects. The use of host resistance to control diseases and insects is the most logical approach to overcome these production constraints. Therefore, major emphasis has been placed at IRRI on developing germ plasm with multiple resistance to major diseases and insects. Varieties and breeding lines with resistance to as many as five diseases and five insect species have been developed. This paper describes the breeding methods and procedures employed in developing disease and insect resistant rice.

Major diseases and insects of rice

In most of the rice growing areas, more than one disease or insect cause serious yield losses. In Latin America, for example, blast, hoja blanca, and *Sogatodes oryzicola* are the factors limiting rice production. In Africa, blast and stemborers take serious toll of rice yields. In Asia, where 92% of the rice is produced and consumed, more than a dozen diseases and insects cause losses of epidemic proportions. In tropical Asia, disease and insect problems are most serious because of year round favorable climate and long history of rice cultivation conducive to the development of many diseases and pest organisms. One year there may be an epidemic of bacterial blight, the next year green leafhopper and tungro may cause serious damage and the following year an outbreak of brown planthopper and grassy stunt may be observed. Therefore, to minimize the yield losses from diseases and insects, varieties with multiple resistance to most of the major diseases and insects are required. Five diseases (blast,

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sheath blight, bacterial blight, tungro, and grassy stunt) and five insects (brown planthopper, green leafhopper, white backed planthopper, stemborer, and gall midge) are of common occurrence in most of the countries of tropical and subtropical Asia. Our efforts are concentrated on developing germ plasm with multiple resistance to these major diseases and insects.

Sources of resistance

Before a breeding program for resistance can be launched, sources of resistance to the various diseases and insects are identified. Our scientists have screened world's germ plasm collection of rice for resistance to the major diseases and insects and have identified sources of resistance to most of them. High levels of resistance to blast (Ou et al., 1975), bacterial blight (Ou et al., 1971), tungro (Ling, 1969), brown planthopper (Pathak, 1972), green leafhopper (Cheng and Pathak, 1972), white backed planthopper (IRRI, 1977), have been found amongst the cultivated germ plasm. Resistance to grassy stunt was not found amongst the cultivated varieties. However, one accession of *Oryza nivara* was found to be highly resistant (Ling et al., 1970). High levels of resistance to stemborer and sheath blight have not been found. Varieties with moderate level of resistance to stemborer have been identified (Pathak et al., 1971).

Improving the sources of resistance

The donor parents have poor plant type, typical of tall traditional varieties of the tropics. As a first step the sources of resistance were transferred to improved plant type background characterized by IR8. This conversion was carried out by crossing the donor parents with an improved plant type parent. Between 1965 and 1969 TN1, IR8, IR24, and IR262-43-8 were used extensively as improved plant type parents. Several dwarf lines with resistance to disease or insect in question and having good grains were selected from each cross. By 1970, improved plant type lines with resistance to individual diseases and insects became available.

Developing germ plasm with multiple resistance

The crossing program was expanded in 1970 and crosses were made amongst these

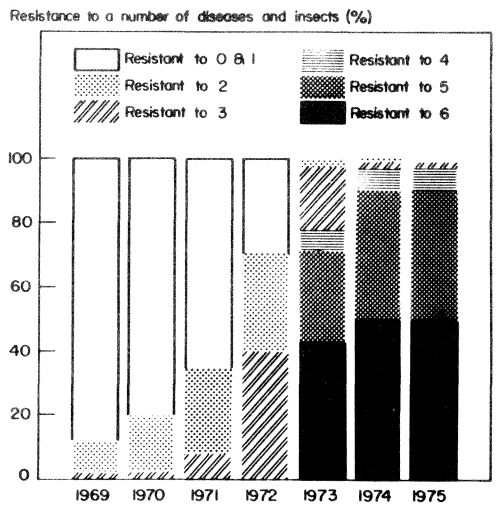

Fig. 1. Changes in proportion of entries in IRRI's annual replicated yield trials with multiple resistance to important diseases and insects in the Philippines (blast, bacterial blight, tungro, grassy stunt, brown planthopper, and green leafhopper). Each year's trial consisted of at least 185 entries.

breeding lines with resistance to different diseases and insects. By the end of 1973, breeding lines with high yield potential and resistant or moderately resistant to as many as five diseases and four insect species became available. The progress made in developing germ plasm with multiple resistance is shown in Figure 1. As shown in the figure, 87% of the entries in the replicated yield trials conducted during the 1969 wet season were either susceptible to all of the six diseases and insects (blast, bacterial blight, tungro, grassy stunt, brown planthopper, and green leafhopper) or resistant to only one of them. Only 2% of the entries were resistant to three diseases and insects. The proportion of entries with multiple resistance gradually increased and in the replicated trials conducted in 1975, 90% of the entries were either resistant to five diseases and insects or to all six of them.

These breeding lines with multiple resistance have high yield potential, excellent grain quality, and early to medium growth duration. Many of these lines were evaluated in replicated yield trials at several locations in the Philippines and in several other countries. On the basis of their superior performance and multiple resistance six were named varieties (IR26 in 1973; IR28, IR29 and IR30 in 1974; and IR32 and IR34 in 1975). In 1975, IRRI changed its policy on naming the varieties. No varieties are now named by IRRI. However, we continue to supply the germ plasm with multiple resistance to the scientists working in the national programs. This germ plasm is either utilized in the hybridization programs or is directly used for commercial production. Thus, the Government of the Philippines released four of the multiple resistant IRRI lines as varieties under the names of IR36, IR38, IR40, and IR42. The disease and insect ratings of IRRI named varieties and the IRRI lines named by the Philippine Government are shown in Table 1. The progressive increase in the level of resistance of varieties from IR8 to IR42 is evident.

Table 1. Disease and insect resistance reactions of varieties named by IRRI and those named by the Philippine Government.

	Disease and Insect Reactions*							
	Blast	Bacterial Blight	Grassy Stunt	Tungro	Green Leaf-hopper	Brown Plant-hopper	Stem-borer	Gall Midge
IR5	MR	S	S	S	R	S	MS	S
IR8	S	S	S	S	R	S	S	S
IR20	MR	R	S	MR	R	S	MR	S
IR22	S	R	S	S	S	S	S	S
IR24	S	S	S	S	R	S	S	S
IR26	MR	R	MS	MR	R	R	MR	S
IR28	R	R	R	R	R	R	MR	S
IR29	R	R	R	R	R	R	MR	S
IR30	MS	R	R	MR	R	R	MR	S
IR32	MR	R	R	MR	R	R	MR	R
IR34	R	R	R	R	R	R	MR	S
IR36**	R	R	R	R	R	R	MR	R
IR38**	R	R	R	R	R	R	MR	R
IR40**	R	R	R	R	R	R	MR	R
IR42**	R	R	R	R	R	R	MR	R

* S- Susceptible; MS - Moderately susceptible; MR - Moderately resistant; R - Resistant. Reactions based on tests conducted in the Philippines for all diseases and insects except gall midge. Screening for gall midge was done in India.

** - Named by the Philippine Government.

Breeding methods employed

We have almost exclusively employed the pedigree method of breeding for developing the multiple resistant germ plasm. With this method the selection can be based on comprehensive records of the disease and insect reactions of each line and, in the case of F_4 and later generation lines, on the reaction of ancestral lines as well. The bulk method is not used as it does not permit the concurrent screening for a number of diseases and insects. The backcross method has not been employed extensively because of a lack of suitable recurrent parents. A few backcrosses were made in the crosses with *O. nivara* for grassy stunt resistance; IR8 and IR24 were used as recurrent parents. After three or four backcrosses, we obtained grassy stunt resistant breeding lines similar to the recurrent parents, but they lacked resistance to other important diseases and insects such as tungro, bacterial blight, and brown planthopper. These breeding lines were again used in hybridization programs as sources of grassy stunt resistance. However, numerous suitable recurrent parents with multiple resistance, including the named varieties such as IR28, IR32, IR34, IR36, IR38, IR40, and IR42 are now available and are being used as recurrent parents in a backcrossing program to transfer resistance to white backed planthopper from N22 and Dharial.

We are also using backcross method to develop isogenic lines with different genes for resistance to brown planthopper.

Pedigree method of breeding is eminently suited to disease and insect resistance programs if the resistance is governed by major genes. In rice, resistance to blast (Kiyosawa, 1974), bacterial blight (Khush, 1977), tungro (Shastry et al., 1972), grassy stunt (Khush and Ling, 1974), green leafhopper (Athwal et al., 1971; Siwi and Khush, 1977), brown planthopper (Athwal et al., 1971; Lakshminarayana and Khush, 1977), and gall midge (Satyanarayanaiah and Reddi 1972) is under major gene control. Therefore, it was possible to combine genes for resistance to six or seven major diseases and insects together in such a short period.

For traits governed by polygenes, the pedigree method of breeding is not so suitable. Resistance to stemborers and sheath blight appears to be under polygenic control. For these two traits we are using a diallele selective mating system proposed by Jensen (1970). This method involves: (1) crossing a number of moderately resistant parents in all possible combinations; (2) intercrossing the F_1 populations so obtained in all the possible combinations; (3) screening the double-cross F_1 progeny for resistance; and (4) intercrossing the selected plants found to have better resistance than either of the parents. The crossing, screening, selection and recrossing will be continued until minor genes from different sources are accumulated and the intensity of the trait is built up.

We are also exploring the feasibility of employing the rapid generation advance method for improving the traits governed by polygenic variation. Early generation populations from multiple crosses involving three or four parents with minor gene resistance are crossed and propagated in bulk using the rapid generation advance method. With this method it is possible to grow 3 - 4 generations in a year. No selection is practiced during this period. At F_5 or F_6 the bulk population is exposed to the disease or insect pressure and individuals with better level of resistance are identified and grown in progeny rows for further evaluation.

Breeding procedures

As a result of ten years of experience on breeding for disease and insect resistance we have developed a set of procedures for handling the donor parents, making the crosses, growing and screening of segregating populations and concurrent evaluation of the materials for agronomic traits and grain quality. These procedures are briefly reviewed here.

Donor parents. Each season we plant the donor parents into a hybridization block. The hybridization block consists of 200 - 250 entries and includes newly identified unimproved donor parents as well as breeding lines with resistance to specific diseases and insects. The

entries of the hybridization block are planted five times during each season at bi-weekly intervals. This schedule insures the availability of donor materials for crossing work at different times during the crossing season.

Hybridization. A large number of crosses are made each season. In producing the single cross F_1 hybrids, each donor parent or breeding line is crossed with a number of other breeding lines. Thus, a set of single cross F_1 progenies is available for making the double or top-crosses in the next season. All the F_1's involving the same donor parent or breeding line are grown together in the F_1 nursery. The best ones are used for making the top-crosses or double crosses. The top-cross parent is selected to complement the deficiency of the single cross F_1 hybrid. Thus, if one parent of the single cross F_1 is resistant to blast and bacterial blight and the other is resistant to tungro, the top-cross parent should have resistance to brown planthopper and green leafhopper. It is desirable to use the top-cross parents which are homozygous for resistance. All the F_1 plants then inherit the trait. It is also desirable to use improved plant type breeding line or variety as top-cross parent. If the unimproved tall donor is used as a top-cross parent all the F_1 progenies of the top-cross are tall and only ¼ of the F_2 progeny would be of short stature.

In making a top-cross or double cross a fairly large number of F_1 seeds (300 - 400) are obtained. This allows us to sample gametic variability of the single cross F_1 hybrids. The screening starts from the F_1 generation. Let us consider a double cross between four parents of which A is resistant to bacterial blight, B is resistant to grassy stunt, C is resistant to brown planthopper, and D is resistant to green leafhopper. All these traits are under the control of dominant genes that segregate independently of each other. About 400 seeds from the double cross A/B/ / C/D are obtained. The seeds are germinated and inoculated with grassy stunt virus in the greenhouse. Approximately 50% of the seedlings are susceptible and are eliminated. The remaining 200 are transplanted in the field and inoculated with bacterial blight at the age of about 60 days. About 50% of these which are susceptible are destroyed. The remaining 100 plants are harvested separately. Two small seed samples are taken from each and are progeny-tested for resistance to brown planthopper and green leafhopper. Those carrying the brown planthopper resistance gene (50%) and the ones carrying the green leafhopper resistance gene (50%) are identified. The F_2 populations are grown only from those carrying both genes (25 to 30 plants). Thus, by judicious, and timely screening, the original F_1 sample of 400 is reduced to 25 - 30 plants. All the F_2 populations grown from these plants segregate for the four resistance genes.

F_2 populations. Most of the F_2 populations are grown without insecticide protection and are thus exposed to the onslaught of leafhoppers and plant hoppers. Generally, enough inoculum of the virus diseases is present on the IRRI farm and we get high disease incidence in the F_2 populations. It is not uncommon to see all the F_2 plants of certain cross combinations infected with tungro virus. These populations are discarded., Most of the F_2 populations are artificially inoculated with bacterial blight in the field and susceptible plants are destroyed.

F_2 populations from the individual F_1 plants of top-crosses or double crosses are grown separately and selections are made only from agronomically suitable populations. We grow about 2000 to 5000 F_2 plants of each cross combination and 100 to 500 plants are selected from promising combinations for growing in the pedigree nurseries.

Pedigree nurseries. We generally obtain 25 - 30 gram seeds of each plant selection from the F_2. This sample is divided into 5 - 6 sets of 5 gm each. One set is used for planting the pedigree nursery We grow one single 5 meter long row consisting of 25 plants, for each plant selection. The remaining seed sets are used for testing for resistance to blast, grassy stunt, green leafhopper, brown planthopper, grain quality, and sometimes for other traits such as drought tolerance or

for tolerance to some injurious soil conditions. The pedigree rows are inoculated with bacterial blight in the field. Most of the pedigree nursery is planted without insecticide protection to encourage the build up of green leafhopper population. To insure the tungro infection we grow tungro susceptible variety all around the borders of the pedigree nursery. Diseased plants from the previous nursery are maintained and occasionally transplanted around the border of the nursery to provide the tungro inoculum. Thus, we are able to screen for tungro and bacterial blight in the field. Data on blast are obtained from blast nursery screening, and screening for resistance to grassy stunt, green leafhopper and brown planthopper is carried out in the greenhouse. The data from various screening trials are recorded in the pedigree nursery book before the selections are made in the field. The main selection criteria, besides the agronomic characteristics (maturity and yield potential) and grain quality are the resistance ratings for various diseases and insects.

Three plants are individually harvested from the promising rows. Seeds from the remaining plants of each selected row are bulk harvested. Total seed harvest of each selected plant is again divided into 5 - 6 sets of 5 gm each. One set is used for replanting in the pedigree nursery and other sets are used for disease and insect screening. The three plant selections from each common source are planted together for comparison. Data from the ancestral row of previous generation are transferred to the new book where the new data are recorded for the reaction of each row. Thus, in F_4 and later generations data from two seasons are available for each pedigree row to facilitate the selection work. These procedures are repeated for several generations till the breeding lines become uniform in maturity, height and other traits.

We grow about 60,000 pedigree rows each year. All of them are screened for resistance to major diseases and insects.

Yield trials. Selected F_5, F_6 or later generation rows are evaluated for yield in replicated trials. Each season we test for yield about 400 entries. These entries are also screened for resistance to the same major diseases and insects. In addition, all the entries in the yield trials are inoculated with sheath blight in the field and evaluated for stemborer resistance in the screenhouse.

Promising materials with high yield, good grain quality, and having multiple resistance to diseases and insects are entered in the international nurseries for dissemination of the seed to other programs. The bulk seed of the early generation lines is also supplied to the scientists in the national programs.

Summary

In the rice improvement work at IRRI major efforts are devoted to developing germ plasm with multiple resistance to major diseases and insects. Screening techniques have been developed, sources of resistance have been identified, genetics of resistance has been investigated, genes for resistance have been transferred to improved plant type background and improved germ plasm with resistance to as many as five major diseases and four insect species has been developed. The breeding methods and procedures employed in our program are discussed.

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Discussion

S. Okabe, Japan: It took a long time for the IRRI breeders to establish high-yielding varieties which have multiple resistance genes. It is highly possible that these varieties might become susceptible due to changes in disease races or insect biotypes. In order to promptly meet the requirement of new varieties with other type of resistance, do you utilize any special breeding procedure at IRRI?

Answer: Yes, we are now emphasizing the development of varieties and improved germplasm with horizontal resistance. This is particularly the case with resistance to brown planthopper. We are utilizing a process of recurrent selection for accumulating polygenes for resistance from different parents into improved varieties.

H. Fujimaki, Japan:

1. How are you going to cope with the occurrence of new biotypes in various diseases and insect pests?

2. Do you believe in the existence of stable horizontal resistance to a specific disease or insect?

Answer:

1. We are incorporating diverse genes for resistance to each disease and insect in our breeding materials. If materials carrying a specific gene for resistance become susceptible due to the development of races or biotypes, we will utilize other genes which are effective against these.

2. Yes, I do believe in the existence of horizontal resistance. Resistance governed by

polygenes is stable and effective against all the races or biotypes of diseases and insects. Its level may not be high, however it is moderate.

J. T. Rao, India: How do the multi-resistant varieties behave with reference to quality, particularly the minor points of quality?

Answer: All of our multiple resistant varieties have excellent grain quality. We have not encountered any difficulty in combining genes for disease and insect resistance with serious quality characteristics.

K. Kawano, Colombia: It seems that there is an amazingly narrow genetic variability in the materials developed at IRRI during the 1960's. What would have happened if IRRI had tried to incorporate much greater genetic diversity since the beginning?

Answer: It is difficult to answer this question. The composition or the parentage of crosses largely depends upon the breeding objectives and the germ plasm availability. In the early years IRRI did not have as wide germplasm resources as in the later years. Moreover, the objective in the earlier years was the improvement of plant type and yield potential. However the objectives were greatly modified to include grain quality, disease and insect resistance and tolerance to environmental stresses. Therefore, a large number of varieties were used in crosses as donors for various traits.