Breeding Rice Varieties for Resistance To Stripe Virus Disease

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Rice stripe disease is a virus disease mainly transmitted by *Laodelphax striatellus* FALLÉN. In Japan it was known from old times but not regarded as important. In recent years, with the advancement of cultural period of rice plants for a phase of safety high-productive cultivation and the spread of the directly sown culture for a method of labor-saving cultivation, the damage of rice crop by stripe disease has been on the rapid increase and forming quickly the countermeasure has been requested.

The usual countermeasures are the control of insects vector with insecticides and the avoidance of infestation of insects by the adjustment of cultural period. However, the latter is hard to say positive and the spreading of chemicals is also difficult to prove to be effective in the control of infection because if the vector could be temporarily exterminated by the spreading, a fresh force of insects will come flying immediately after. Consequently the development of resistant varieties was considered as the most effective countermeasure. And among the varieties cultivated actually in Japan no variety resistant to this disease has been found yet.

Since 1962 we have studied aiming at the breeding of resistant varieties for practical use, in close cooperation of the laboratories of plant pathology and breeding. At first, utilizing seedlings a simplified method for testing resistance of varieties was developed.

By this method the mode of inheritance of resistance was elucidated. An effective breeding plan was formed on the basis of these findings and in a short time it was achieved to improve Japanese paddy rice varieties which possessed the superior resistance of Japanese upland rice and varieties of indica type. The results were already reported in detail[11] and the following presents the outline of the report.

Establishing the seedling test method

In testing the resistance of variety to stripe disease, as a method of examining the natural infection in paddy field has several difficulties, the following simplified effective method for seedling test was developed[1),2),11)].

1) Some 30 sprouting seeds of variety tested were sown in a 9-cm Petri dish.

2) Three days after, 150-300 viruliferous nymphs were inoculated into the dish which was covered with a cylindrical glass and was put into the growth cabinet maintained at about 27°C.

3) Two days after, the nymphs were removed and the seedlings were transplanted to a nursery box, which was placed in the green-house or in open-air.

4) 20-25 days after, appearance of the disease was examined and a disease rating index was calculated on the basis of the symptom type and the number of diseased seedlings, and then the index was compared to that of the check variety to determine the degree of resistance (Fig. 1 and 2).

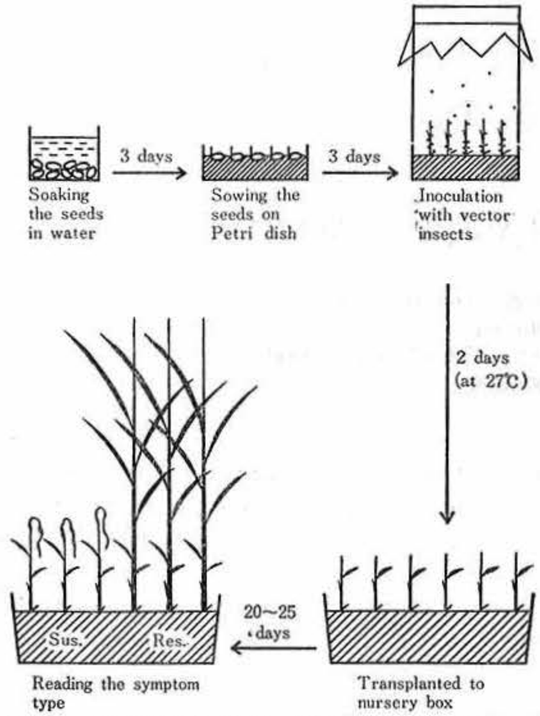

Fig. 1. Procedure of the "seedling test method" for varietal resistance to stripe disease.

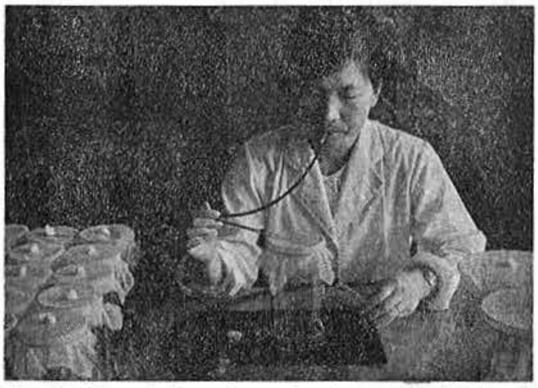

Fig. 2. Inoculation by releasing vector insects into glass cylinder fixed with Petri dish.

This seedling test method can be conducted in laboratories and greenhouses in a short period of time (about a month per test) and at any time in the year. The results of the tests showed a highly accordance with the degree of the disease in paddy field, excepting a few cases.[5] For example, in a case where a trial calculation was carried out from a number of varieties, a positive correlation coefficient ($r=0.882^{**}$) was indicated.

To perform this test method without difficulty, it is necessary to maintain ceaselessly a number of viruliferous nymphs for inoculation. At first, the field collecting of *L. striatellus* was conducted and viruliferous insects were selected and maintained in beakers or rearing cages supplying rice seedlings (Fig. 3). Seven or eight cages were usually grouped in a set and in each cage 2,000 - 5,000 young nymphs, or 500 - 1,500 mature nymphs or adults were maintained with renewals of feed once or twice a week.

Fig. 3. Change of feeding materials in rearing cage using vacuum cleaner.

One cage in a set was used as the exclusive one for oviposition where adult insects were ceaselessly maintained to oviposit with renewals of feed twice a week. And the oviposited rice seedlings were successively transferred to the other cages where the eggs hatch.

For inoculation 2nd or 3rd instar nymphs are most suitable, and after emergence they can be used two or three days after the first renewal of feed. Stripe disease virus is of transoverial passage and, as the generation of viruliferous insects alternates, the proportion of them among the population lowers.

Usually the next generation of viruliferous insects population was observed to contain about 10 per cent virus-free insects[4] so viruliferous insects were reselected from the population every four or five generations for conserving their virulence sufficient for inoculation.

In the seedling tests, the early symptom of stripe disease appeared on the tested seedlings about a week after inoculation, but the final reading of the disease was conducted 20-25 days after transplanting to the nursery box. The symptom types were classified into six grades (Fig. 4). The symptom types A, B and Bt were seen mainly in the susceptible varieties; Cr, C and D, in the resistant varieties.

It was supposed that in the alphabetical order, the reactions of seedlings vary from susceptible to resistant. The degrees of viral proliferation in rice plants decreased actually in this order, too[5].

The disease rating index was calculated from the following formula:

$$\text{Disease Rating Index} = \frac{100A+80B+60Bt+40Cr+20C+5D}{\text{Number of seedlings examined}}$$

(A-D stand for the numbers of seedlings of respective symptom type.)

The gradation of resistance among the tested varieties was determined by the ratio of the disease rating indices for every test (percentage of the index of a tested variety to that of the susceptible check variety). Namely, the ratios 0-29 indicate varieties resistant (R); 30-59, moderately resistant (M); varieties of more than 60, susceptible (S). Rarity of varieties of M grade was seen from the results of the seedling tests.

Screening the sources of resistance gene

To improve resistant varieties, it is necessary to search for the resistance gene or genes sources possible to use as the cross parents. Japanese paddy rice, Japanese upland rice and foreign varieties (428 varieties in all) were

Fig. 4. Symptom types in the "seedling test method" (Black area or dots show discolored parts)

tested for their resistance by the seedling test method[3),5),11)]. Japanese paddy rice varieties, Ponlai varieties and foreign varieties of japonica type were all susceptible; Japanese upland rice varieties and foreign varieties of indica type were mostly resistant, and foreign varieties of intermediate type varied from resistant to susceptible (Fig. 5).

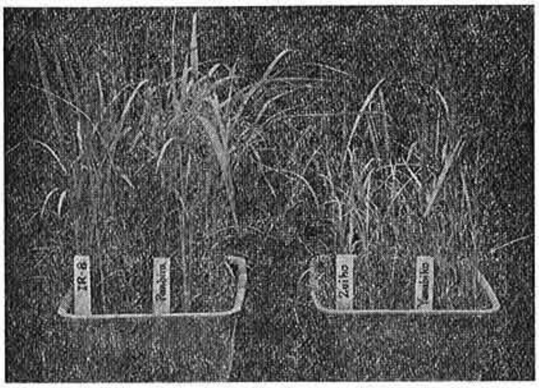

Fig. 5. Inoculated plants 3 weeks after transplanting: (Left—2 varieties are resistant, right—2 are susceptible)

Because no variety possible to use as the resistant parent was found among the existing Japanese paddy rice varieties, Japanese upland rice or foreign varieties of indica type were used as the sources of resistance both of them in remote relation with Japanese paddy rice.

Mode of inheritance of resistance

In the varietal improvement program wherein such remotely related varieties are used, adequate information of mode of inheritance of resistance gene or genes are substantially useful. Therefore, gene analysis of resistance was conducted on four resistant Japanese upland rice varieties and six resistant foreign varieties[6),9),10),11)].

From the segregation ratios of resistance in F_1, Bs, Br, F_2, and F_3 between resistant Japanese upland rice and susceptible Japanese paddy rice, it was considered that resistance of Japanese upland rice is controlled by two pairs of complementary dominant genes St_1 and St_2[9)].

This was proved by the appearance of resistant individual in F_1s derived from cross between the susceptible F_4 lines. As the result of examining relation between resistance and other traits of Japanese upland rice, St_1 gene was seen to link with the waxy endosperm wx and the photosensitive gene Se and to belong to 'wx' linkage group (Group I).

It was indicated that, same as Japanese upland rice, Zenith of six foreign varieties tested controlled its resistance by two pairs of complementary dominant genes, while other foreign varieties of indica type and Indonesian Bulu type controlled their resistance by a pair of incomplete dominant genes (rather close to recessive)[10)].

The pair of genes is influenced in its action by cytoplasm, and there was a genetic relation between seed dormancy and grain breadth. Since the resistance gene of varieties of indica type has a multiple allelomorph relation with St_2 gene of Japanese upland rice, it was named St_2^i. St_2^i gene was seen to show resistance independently, while the poorly active gene St_2 showed resistance only when it coexited with the enhancer St_1, and St_2^i also displayed complete dominance in the case of coexisting with St_1.

From the analysis by the reciprocal translocation method it was assumed that St_1 gene having chromosome 6 belonged to 'wx' linkage group (Group I); St_2 and St_2^i genes having chromosome 12, to 'I-Bf' linkage group (Group V).

Breeding the resistant varieties

Some varieties resistant to stripe disease had been bred up using Japanese upland rice and foreign varieties as the souces of resistance gene or genes. In the case of using Japanese upland rice as the resistant parent, the poly-cross method of back-cross type was applied. Upland rice Kanto 72, derived from the cross between Upland rice Norin 24 and Paddy rice Norin 29, was selected as the parent. Koshihikari was mated with the parent, and Kusabue with F_1 of them. From F_1s of the triple-cross the resistant individuals were selected by the seedling test method, and the poly-cross between resistant triple-

F_1s and Chusei-Shinsenbon, Kibiyoshi, etc. were conducted.

After that, repeating the seedling tests and the field selections on general traits, early fixation of F_1, F_3 and F_5 generations was tried by generation-shortening culture in greenhouses during the winter seasons. Four lines of Chugoku 40 (early), 41 (middle), 42 (late) and 49 (very early) were developed which possess resistance and are equal to the check variety in the yielding ability.

In introducing resistance of varieties of indica type, selections were carried out from 570 lines which, with the object of incorporating the resistance to blast disease of indica type, had been developed by back-cross using foreign varieties as a donor. Of these materials one line, derived from 5 back-crossings between Modan of Pakistani origin and Norin 8, showed resistance to stripe disease and was named St. No. 1. Chugoku 31 was selected from the derived lines of St. No. 1[7].

Since the variety was insufficient for practical use, Sachikaze, Chusei-Shinsenbon, etc. were mated with Chugoku 31 as the parent, and the emergence of F_1, F_2, F_3 and F_4 was conducted in a year by the generation-shortening culture through the bulk-method. After the emergence of F_5, resistance tests by the seedling test method and selections on general traits were carried out, and Chugoku 46 and 51 were developed for practical use.

The resistant varieties improved till now are known to indicate superior resistance in the severely diseased regions, and the development of these resistant varieties are expected to make possible a hopeful prospect to the control of rice stripe disease.

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