10. FIELD RESISTANCE OF THE RICE PLANT TO *PIRICULARIA ORYZAE* AND ITS TESTING METHOD

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Introduction

Resistance of the rice plant to the blast pathogene, *Piricularia oryzae* Cavara, is generally considered to be of two types; one is called "racial resistance" or "true resistance", and the other "field resistance", "partial resistance" or "tolerance". "Racial resistance" is characterized as a hypersensitive reaction of the host to infection, and plants possessing resistance of this kind can be classified as resistant or susceptible according to their reactions to specific races of the pathogene. "Field resistance" has been considered to include all types of resistance other than the hypersensitive type controlled by the major genes, and the plants with "field resistance" only are not immune to any races of the pathogene.

Breeding for blast resistance in Japan has advanced by the following three steps; the first step was by evolving resistant varieties by hybridization of Japanese paddy rice, the second by the use of resistant varieties derived from upland rice and the third by the use of resistant varieties derived form Chinese and indica varieties. Recently, some so-called "highly resistant varieties" with the genes of foreign rice varieties have been developed and recommended to farmers for practical use. Actually, these varieties which had been hitherto thought as highly resistant suffered very severely from blast during their second or third year of recommendation. These phenomena suggested the existence of different pathogenic races of the fungus, and the inversion of varietal resistance was thought to be caused by the rapid propagation of a special race which was virulent to those varieties.

The history of breeding for resistant varieties to patato late blight caused by *Phytophthora infestans* De Bary indicates that "field resistance" played an important part in controlling the disease instead of "racial resistance". Hence the possiblities of utilizing "field resistance" have to be explored in rice breeding for blast resistance.

This paper deals with the methods for assessment of relative field resistance of varieties together with the high level of field resistance shown by St. 1 and Chugoku 31.

Evaluation of Field Resistance under Mudland Conditions

Some varieties and lines given in Table 1 which would have representative characteristics in racial resistance and field resistance were grown in the paddy field, and their level of field resistance were evaluated. Though the occurrence of blast in the testing field was not so severe, the varietal difference among Japanese varieties was indicated clearly as shown in Fig. 1. The outbreak of blast in the varieties derived from Chinese varieties was delayed as compared with that in the Japanese varieties, and was not uniform in the field. Accordingly the varietal difference was not so markedly clear among these varieties as shown in Fig. 2. These phenomena indicate that a comparison of field resistance of the varieties has to be done among the varieties with the same racial resistance.

In this experiment, it appeared that the racial resistance gene *Pi-a* in the Japanese varie-

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Table 1. The varieties and lines used in tests and their genes of racial resistance

Varietal or line	Foreign variety from those resistant genes induced	Resistant type or genes of resistance
St. 1	Modan	Shin 2 type (+)
Ginga	—	
Norin 22	—	
Koshihikari	—	
Norin 8	—	
Moko ine	—	
Homarenishiki	—	Aichi-Asahi type (*Pi-a*)
Fujiminori	—	
Shuho	—	
Norin 17	—	
Aichi Asahi	—	
Norin 18	—	
Jukkoku	—	
Chugoku 31	Modan	Kanso 51 type (*Pi-k*)
Tatsumi Mochi	Reishiko	
Sanin 68	To-to	
Kanto 51	Reishiko	
Mangetsu Mochi	To-to	
Senshuraku	Reishiko	
Kusabue	Reishiko	
Kongo	Hokusei Taimai	(*Pi-a*) (*Pi-k*)
Yukara	Reishiko	

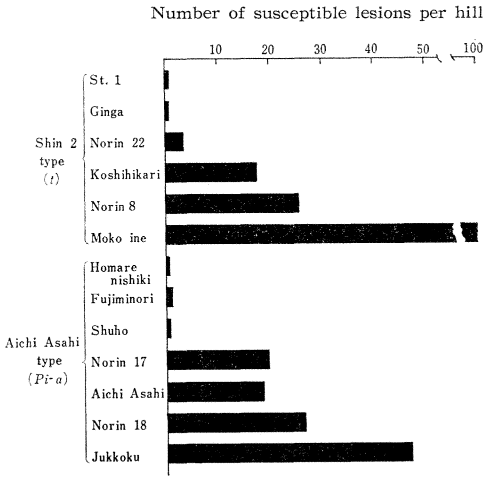

Fig. 1. Field resistance of each variety and line to blast in paddy field under natural condition (1)

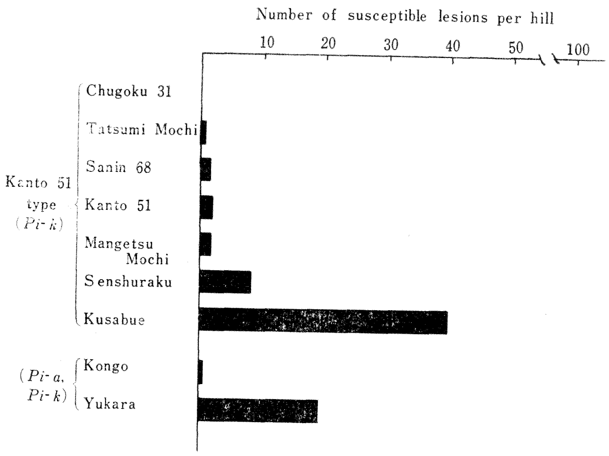

Fig. 2. Field resistance of each variety and line to blast in paddy field under natural conditions (2)

ties, had little influence on the degree of resistance, so Shin 2 type without the racial resistance gene and Aichi-Asahi type with *Pi-a* gene were handled as one group for comparison of field resistance in Japanese varieties.

From Fig. 1 and 2, it appeared that the Japanese varieties, Mokoine, Norin 8, Norin 17, Norin 18 and Jukkoku have a low level of field resistance, especially Mokoine which has the lowest level, and Ginga, Norin 22, Homarenishiki, Fujiminori and Shuho have a fairly high level of field resistance, and St. 1 has the highest level of them all. Among the varieties possessing the racial resistance gene *Pi-k* from Chinese varieties, Kusabue and Yukara have a low level of field resistance, and Senshuraku also possess a comparatively low level, but Kongo and Chugoku 31 have a much higher level of field resistance than the others.

These results from field tests indicated that there was evidently different levels of field resistance among the varieties with the same racial resistance, and that the severe damage of the so-called "highly resistant varieties" might be caused by their lack of field resistance and by the rapid propagation of pathogenic races. St. 1 and Chugoku 31 which were selected by the authors as resistant varieties for stripe disease appeared to possess a high level of field resistance.

Evaluation of Field Resistance under Upland Nursery Conditions

The level of field resistance and its fluctuation in each variety were examined using the varieties and lines shown in Table 1 together with other varieties under upland nursery conditions where blast was epidemic. The tests were repeated six times from June to September in 1966 a National Chugoku Agricultural Experiment Station. Fig. 3 shows a progressive status of disease severity in representative varieties. The level of field resistance was then evaluated by the next procedure.

1. The varieties were grouped by their genotypes of racial resistance. In this experiment the racial resistance gene *Pi-a* appeared to have no effect on the degree of resistance, the varieties without racial resistance gene and those with the gene *Pi-a* were grouped in one, and the varieties with the gene *Pi-k* and those with both the genes *Pi-a* and *Pi-k* were classified into the other group respectively

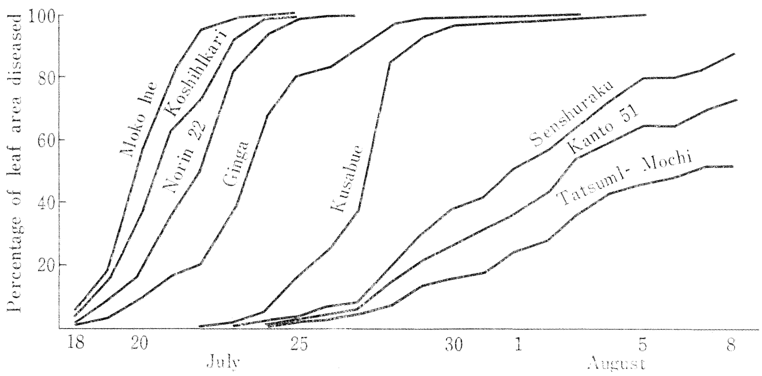

Fig. 3. Change of the percentage of leaf area diseased on seedlings used in the upland nursery bed

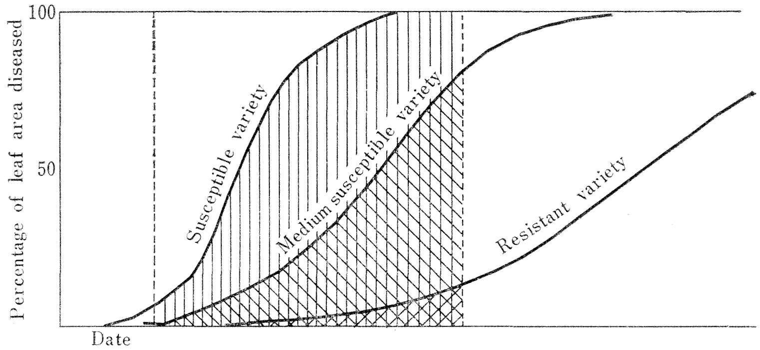

Fig. 4. Daily progress of percentage of leaf lesions diseased on seedlings used in the upland nursery bed (Model figure)

2. Observations were made on the percentages of diseased leaf area in total leaf area during the period from a day when more than half the varieties in each racial resistance group showed more than 5 percent of disease leaf area ratio, to a day when more than half the varieties in each group showed more than 90 percent of diseased leaf area ratio.

3. Daily percentages of diseased leaf area in variety were summed up for getting the approximate quantity of the integral calculus of the curved line shown in Fig. 4. The disease rating index of each variety was calculated in each group by the following formula.

$$\text{Disease rating index} = \frac{\text{Summed up value of given variety}}{\text{Maximum summed up value in the group}} \times 100$$

The average value of disease rating indices of each variety in six tests are given in Fig. 5 and 6. Though the tendency of the varietel difference in the upland nursery bed resembled considerably those in the paddy field, there were some differences in the following points; in the paddy field, the difference of field resistance in the low level varieties was clear, but on the other hand in the high level ones the difference was not so clear; in the upland nursery bed, the low level ones were not clear, but those in the high level, was very clear. This distinction might be due to the difference of severity of disease occurrence. Consequently, there was a little difference between the test in the upland nursery bed and those in the paddy field fundamentally.

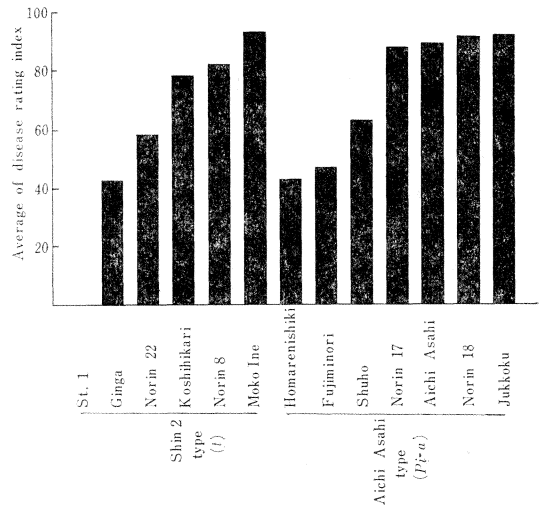

Fig. 5. Field resistance of each variety and line to blast tested in the upland nursery bed (Average value of 6 tests)

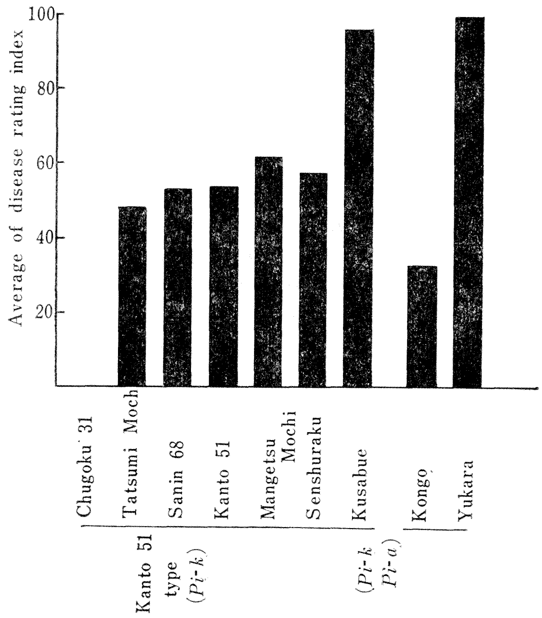

Fig. 6. Field resistance of each variety and line to blast tested in the upland nursery bed (Average value of 6 tests)

In the upland nursery bed, the lines St. 1 and Chugoku 31 showed a few susceptible lesions on their leaves, but the number of lesions was extremely less than those on the other varieties. This indicated that field resistance of both lines was of the highest level which had never been found in Japanese paddy varieties.

Appearance of field resistance in each variety showed some variations in six nursery tests as shown in Fig. 7 for *Pi-a* and without the racial resistance gene group. For determining the condition for observing the clear expression of field resistance in the upland nursery

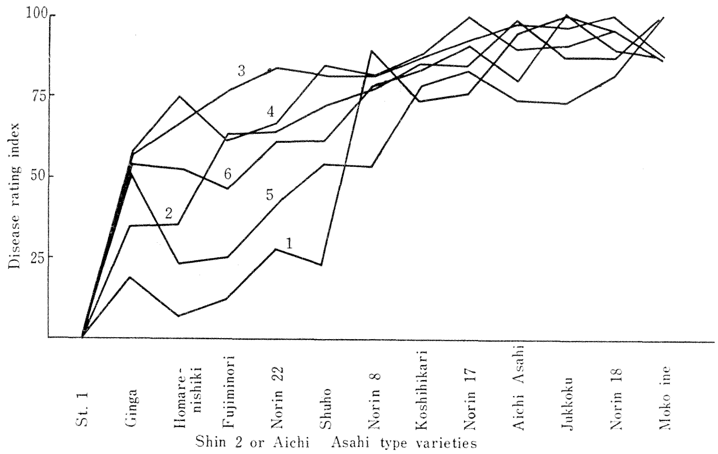

Fig. 7. Variation of disease rating index in each test

bed, the correlation coefficients between the next item A and the other ones were calculated:

(1) The difference between the average value of disease rating indices in the high field resistant varieties (Ginga, Homarenishiki, Fujiminori, Norin 22 and Shuho) and that in the low field resistant ones (Koshihikari, Aichi-Asahi, Norin 18, Jukkoku and Moko-ine).

(2) The number of days from the beginning to the end of the observed poriod.

(3) The number of days from seeding to the beginning of the observed period.

(4) The number of days from seeding to the end of the observed period.

The correlation coefficients were as follows:

(1):(2)=0.15, (1):(3)=0.96 and (1):(4)=0.86

These indicated that the period from seeding to the beginning of disease occurrence was closely related with the appearance of field resistance in consequence of the effect of plant ages probably. Therefore, 20 to 30 days from seeding to the beginning of disease occurrence was desirable to evaluate the field resistance in each variety.

From these data, we can say that it is possible to evaluate the field resistance of given varieties by the upland nursery bed method especially to select high field resistant lines in the rice breeding program, when the pathogenic races of the varieties are prevalently distributed in the testing field, and the level of field resistance in given varieties must be compared with each other in the varieties belonging to the respective racial resistant groups.

Field Resistance Evaluated by the Seedling Inoculation Method

Relative field resistance of rice varieties to blast was evaluated in an upland nursery bed where the pathogenic races of the fungus were prevalent, but this testing method was influenced by weather conditions such as temperature and rain fall, and could not be performed throughout the year. Moreover, the comparison of field resistance among the varieties belonging to different racial resistant group was impossible, and field resistance of the varieties possessing racial resistance to the races of the fungus existent in the testing field could not be evaluated, because these varieties had no lesions.

Therefore, a better method of testing for field resistance was adapted. For this purpose, the seedling inoculation method and the factors affecting the expression of field resistance were investigated.

Seedlings of the varieties and lines which showed different levels of field resistance and also racial resistance were inoculated with the spore suspensipn of standard races; Hoku 373

(N—1), Ken 60—19 (C—1) and Ken 53—33 (T—1). Inoculation test were repeated seven times.

The varieties and lines shown in Fig. 8 had no racial resistance gene to the inoculum used, because the gene *Pi-a* has no effect to N—1, C—1 and T—1 races. Therefore, the average number of susceptible lesions per plant might indicate the level of field resistance in each variety. The varieties shown in Fig. 9 had the gene *Pi-k* or *Pi-a* and *Pi-k*, which showed racial resistance to the race N—1. Therefore, Hoku-373 (N-1) developed no susceptible lesions on the plants belonging to this group. Since the pathogenicity of the races, Ken 60—19 (C-1) and Ken 53—33 (T-1), was not disturbed by the existance of the resistance genes *Pi-a* and *Pi-k*, the data given in Fig. 9 indicates the level of fieldresistance in this group.

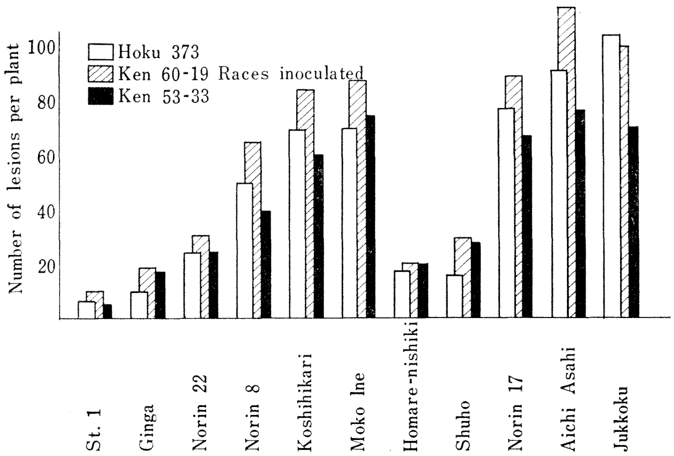

Fig. 8. Test of field resistance by the seedling inoculation method

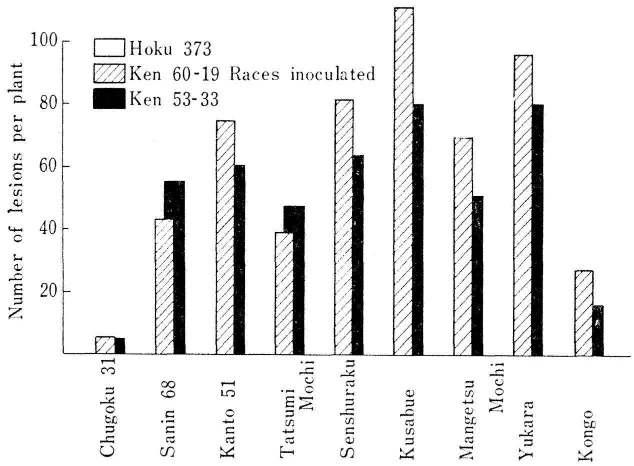

Fig. 9. Test of field resistance by the seedling inoculation method

The order of the level of field resistance in each variety evaluated by the seedling inoculation method coincides considerably well with that of the paddy field and upland nursery ded. Accordingly it seems that field resistance of given varieties could be evaluated by the seedling inoculation method when the pathogenic races are inoculated to given varieties.

The lines st. 1 and Chugoku 31 also showed excellent field resistance in this seedlings inoculation method.

The relation between nitrogen application and the number of susceptible lesions per plant in the varieties with the different levels of field resistance is shown in Fig. 10. The data indicates that high application of nitrogenous fertilizer increases the number of susceptible lesions in the seedlings inoculated, and that the heavy application of ammonium sulphate (10 g per 35×27×12 cm pot) is a desirable condition for deciding the varietal difference of field resistance. Fig. 11 shows the relation ship between the number of susceptible lesions per plant and the leaf-ages of seedlings tested. Field resistance of seedlings appears to become higher, as the leaf-ages of seedlings tested progress. Hence the leaf-age adaptable for seedling inoculation might be the seventh of a given plant.

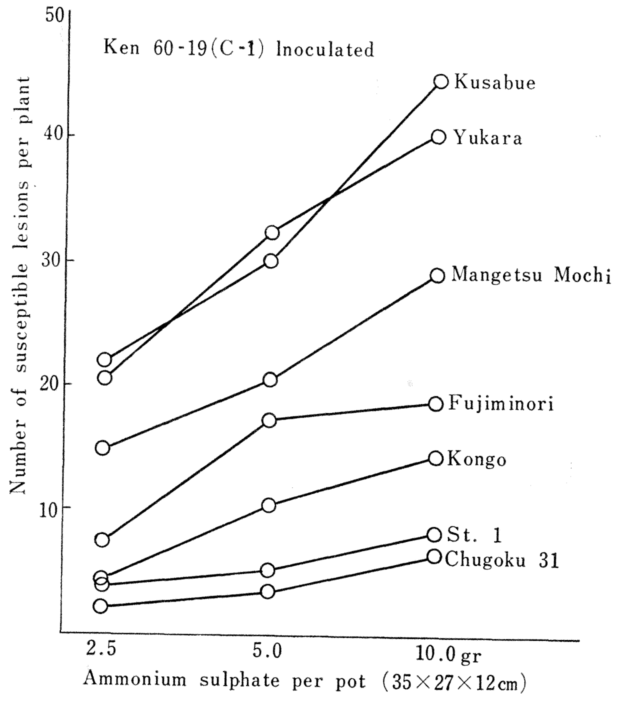

Fig. 10. The relation between field resistance and nitrogenous fertilizer application

The number of susceptible lesions per plant was also influenced by the concentration of inoculum used. When the seedlings were inoculated by too dense or too thin concentration of inoculum, it was difficult to determine the varietal difference of field resistance, because the number of susceptible lesions was either too much or inadequate. The data given in Fig. 12 show that the optimum concentration of inoculum is 100,000 to 250,000 spores per 1 *ml* of water.

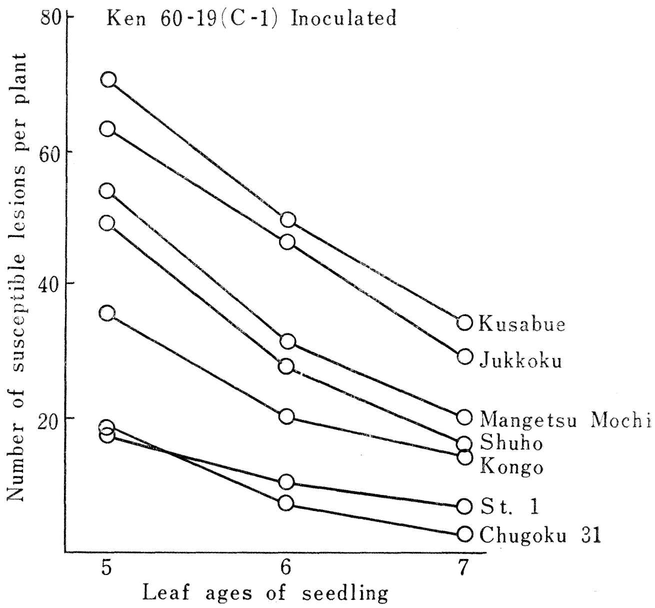

Fig. 11. The relation between field resistance and leaf ages of seedlng

For evaluating the degree of field resistance in given plants, there are different means, i. e., the percentage of diseased leaf area in total leaf area, the number of susceptible, medium and resistant lesions respectively and the total number of lesions. Among them, the number of susceptible lesions is the most convenient and effectual means and can be easily done by the seedling inoculation method.

Judging from these experiments, a seedling inoculation method for evaluating the level of field resistance can be decided for practical use as follows;

(1) Inoculum is selected among the races possessing the pathogenicity to the varieties tested.

(2) Heavy application of nitrogenous fertilizer (10 g per pot).

(3) The seventh leaf-age of a given plant is suitable for inoculation.

(4) Concentration of inoculum is 100,000 to 250,000 spores per 1 *ml* of water.

This seedling inoculation method is characterized by its use all around the year in the greenhouse and by comparison of field resistance among different racial resistant varieties with pathogenic races to entries.

IV. High field resistance in St. 1 and Chugoku 31.

Two lines, St. 1 and Chugoku 31 were derived from the orthodox fifth backcross involving Norin 8 as a recurrent parent and Modan as a donor for the purpose of establishing stripe resistant Japanese paddy rice. Norin 8 is a Japanese paddy variety susceptible to both blast and stripe, while Modan is a typical indica variety of Pakistan origin and characterized by high resistance to blast and stripe. Fortunately, these two lines have a high level of resistance not only to stripe but also to blast as already shown in the above experiments.

Which type of resistance do these lines have, "racial resistance" or "field resistance"? As mentioned already, "racial resistance" is characterized as a hypersensitive reaction of host to infection, and the plants possessing racial resistance show immunity to specific races corresponding to the resistance gene. Table 2 shows seedling reactions of these two lines and some other varieties to each differencial blast fungus. St. 1 has no racial resistance gene similar to its recurrent parent Norin 8, and Chugoku 31 has one racial resistance gene *Pi-k* which shows resistance to N race group but susceptible to C and T race groups.

Table 2. Reaction of some lines and varieties to each differential blast fungus

Variety or line	Inoculum							Genotype presumed
	P-2b	Ken53 -33	Ina-72	Hoku-1	Ken54 -20	Ken54 -04	Ina-168	
St. 1	S	S	S	S	S	M	S	Shin type (+)
Norin 22	S	S	S	S	S	M	S	
Norin 8	S	S	S	S	S	M	S	
Moko Ine	S	S	S	S	S	S	S	
Homarenishiki	S	S	R	S	S	MR-M	R	Aichi Asahi type (*Pi-a*)
Shuho	S	S	R	S	S	MR-M	R	
Aichi Asahi	S	S	R	S	S	S	R	
Norin 18	S	S	R	S	S	M	R	
Chugoku 31	M	S	S	R*h*	R*h*	R*h*	—	Kanto 51 type (*Pi-k*)
Kanto 51	M	S	S	R*h*	R*h*	R*h*	—	
Senshuraku	M	S	S	R*h*	R*h*	R*h*	—	
Kusabue	M	S	S	R*h*	R*h*	R*k*	—	

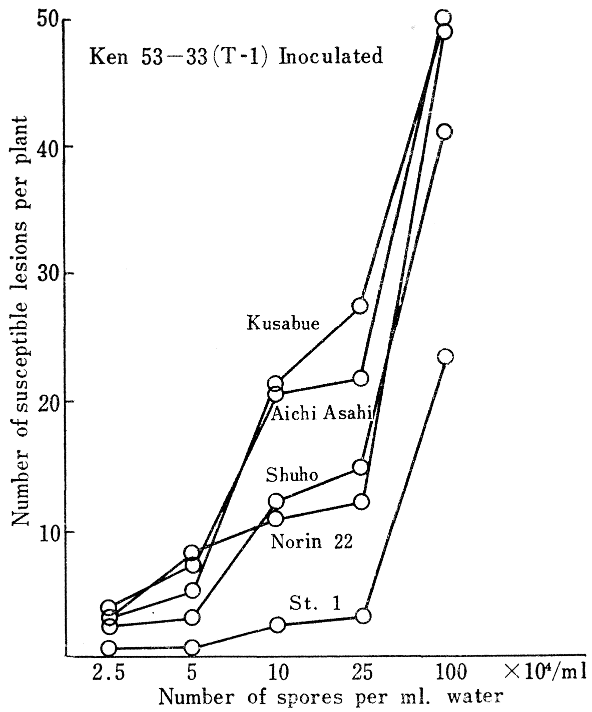

Fig. 12. The relation between number of lesions and concentration of inoculum

In the experiment performed in the paddy field and the upland nursery bed, the pathogenic races to the racial resistance gene *Pi-k* were found, and St. 1 and Chugoku 31 developed a few susceptible lesions. Therefore, the high level of resistance shown by both lines might be caused by the existence of " field resistance ".

Various kinds of isolates of blast fungus collected as an inoculum were examined whether they infected severely to both lines or not. In all the inoculation tests with these isolates, the number of susceptible lesions on both lines was always few compared with those on the other varieties. Then, some fougus strains isolated from the susceptible lesions of St. 1 and Chugoku 31 were inoculated to the respective lines. The number of susceptible lesions was similar to those by their original fungus strains throughout all the tests as shown in Fig. 13 and 14. The data on strains reisolated from St. 1 and Chugoku 31 inoculated with the above isolates showed the same result on the original ones. This was the proof that the level of field resistance showe by both St. 1 and Chugoku 31 was constantly high and stable regardless of the fungus stain.

A knowledge of the mode of inheritance of field resistance shown by St. 1 and Chugoku 31 is required in order to study the feasibility of breeding resistant varieties against blast disease, and it is complelled to use these field resistance for practical breeding programs.

Seedling reaction to the blast fungus in the F_1, F_2 and F_3 generations between two lines and the other low field resistant varieties was determined by the seedling inoculation method and the seedling injection method. Reaction of the F_1 plants was inclined to a high field resistant parent, suggesting the dominance of field resistance. In the F_2 generation, about 3/4 of seedlings tested were classified as highly field resistant. This indicates that high field resistance shown by both St. 1 and Chugoku 31 is controlled by one dominant gene, *Pi-F*. And the data of the F_3 generation supported this proposed genic system.

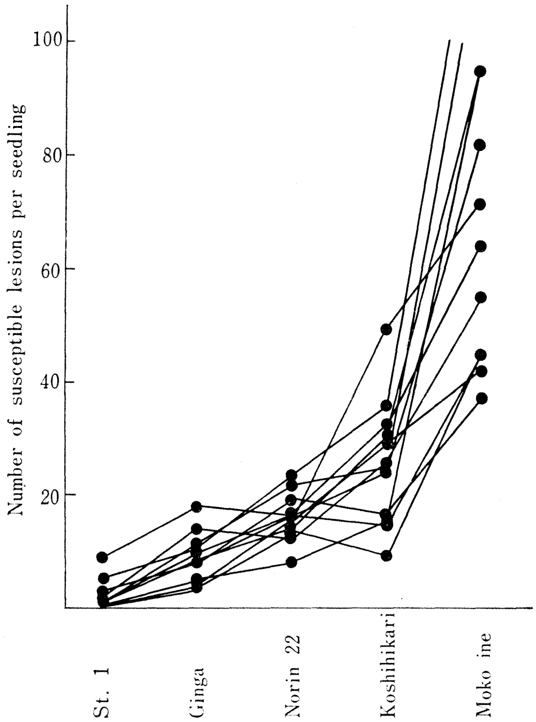

Fig. 13. Results of inoculation with the blast fungi isolated from St. 1

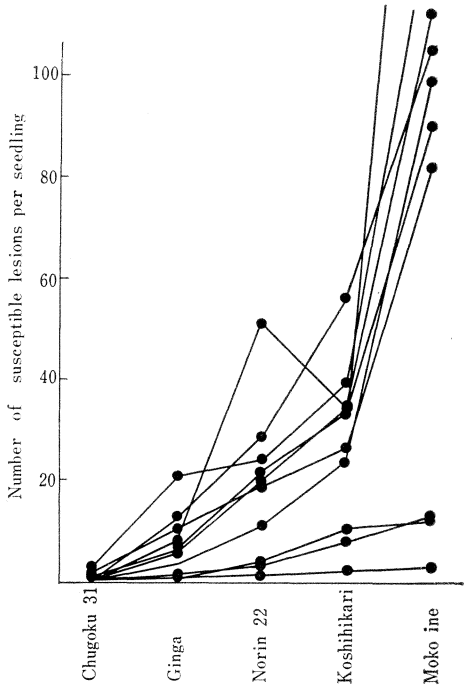

Fig. 14. Results of inoculation with the blast fungi isolated from Chugoku 31

As to the general idea of field resistance to potato late blight, it was known that field resistance was attributed to resistance to penetration, resistance to spread of mycelium in the host, length of the period of incubation and quantity of sporulation. According to this idea, resistance of St. 1 and Chugoku 31 may be considered to be attributed to resistance to penetration, but this assumption in the case of field resistance to blast in rice plant is unsatisfactory to explain the resistance. In future studies, therefore, the knowledge of the mechanism of expression of field resistance should be clarified.

As it has been recognized that the so-called " highly resistant varieties " without field resistance suffered very severely from blast, we think that resistance based solely on racial resistance genes becomes unstable by the existence of pathogenic races, and does not offer much promise of permanent, practical benefit, as field resistance does. Namely, for developing highly stable resistant varieties to blast, it is neccessary to combine the high level of field resistance with some racial resistance in the varietal improvement program.

Discussion

D. V. M. Abeygunawardena, Ceylon: How would you determine field resistance to neck blast? May I also ask whether there is a positive correlation in field resistance to leaf blast and neck blast.

Answer: The varietal difference in resistance to neck blast is usually determined by the percentage of diseased necks in the field accelerated in blast occurrence. Generally speaking, there are high positive correlation between the leaf blast resistance and the neck blast resistance. The situation of racial and field resistance to neck blast is not yet known well, being left as a challenging problem for the future.

S. Yoshimura, Japan: You have mentioned from the experimental result carried out last year, that two varieties St. 1 and Chugoku 31 were field resistant varieties. In this year, I have missed to listen which variety has tested, but one of these varieties was reported to be severely diseased in Fukushima Exp. Sta. If so, the variety St. 1 may be concluded not to be field resistant variety. What do you think about this point?

Answer: We'd like make a re-examination of the fungus strain from Fukushima. If the result is in that case, we'll have to change our mind about the resistance of St. 1. So far as we know, the varietal resistance to blast may be devided into two categories, racial resistance and field resistance. The former depends upon the fungus strains and is determined qualitatively by lesion types, while the latter is independent of fungus strains and determined quantitativety by lesion number or area. If St. 1 is attacked severely by a specific strain of fungus, it may be assumed to have a new type of quantitative resistance varying with the fungus strains.