

Induction of Mutation for Powdery Mildew Resistance in Two-Rowed Barley

By ISAO YAMAGUCHI* and ATSUSHI YAMASHITA **

Institute of Radiation Breeding, National Institute of Agrobiological Resources
(Ohmiya, Naka, Ibaraki, 319-22 Japan)

Many attempts to induce mutations for disease resistance have been continued from the first success of an induction of powdery mildew resistance in barley,³⁾ and resulted in the release of varieties resistant to fungus, bacterial and virus diseases.

However, as the frequency of mutations for disease resistance is extremely low, it is hoped to find out highly efficient mutagenic treatments in induction of resistant mutations, and also establish an efficient system to screen a large number of materials.

The present authors have established an efficient system to isolate mildew resistant mutants from a large number of two-rowed barley seedlings, and compared the mutagenic efficiency of γ -rays and ethylene imine (EI) in inducing resistant mutants to powdery mildew.¹²⁾

Method of screening a large number of seedlings for powdery mildew resistance

The pathogen of barley powdery mildew (*Erysiphe graminis* DC. f. sp. *hordei* Marchal) is an obligate parasite: its hypha cannot be cultured on artificial media, and its spores cannot be stored. The optimum temperature for the seedling growth and disease infection

is 15–20°C. Presence of water droplets on leaf surface at the time of inoculation lowers the level of infection. The special screening system and the nursery beds were designed to satisfy these necessary conditions.

M₁ spikes are sown in rows on seedling beds. At the time when the first leaf of M₂ plants expands about 10 days after sowing, mildew spores produced on diseased plants which have been prepared in advance, are sprayed onto the test plants for the inoculation. The disease symptom appears 7–8 days after the inoculation and a large number of spores are produced after 10 days. At this time, the screening for resistance is made. The spores remaining on the diseased plants can be used to inoculate the second set of test plants sown 10 days after the sowing of the first one, without preparing an additional inoculum source.

The powdery mildew pathogen used for the screening was the race IX,⁶⁾ given by Prof. U. Hiura, the Institute of Agricultural and Biological Science, Okayama University. The degree of infection was rated into 5 classes from the type 0 (highly resistant) to the type 4 (susceptible) according to Mains and Dietz.⁷⁾ Response of the original varieties to the race IX was type 4 in all cases.

Induction of disease resistance mutation

Screening tests for the disease resistance was carried out for 5 years. Original variety was Azuma Golden in the experiment from 1973 to 1975, and in the experiment from 1976 to 1977, Azuma Golden, Fuji Nijou, and

Present address:

* Okinawa Branch, Tropical Agriculture Research Center
(Ishigaki, Okinawa, 907-01 Japan)

** Department of Molecular Biology, National Institute of Agrobiological Resources
(Yatabe, Ibaraki, 305 Japan)

Table 1. Total number of M₂ plants tested for mildew resistance

Varieties	Mutagens	Number of M ₂ plants tested					Total number of M ₂ plants M ₁ spike progenies	
		1973	1974	1975	1976	1977		
Azuma Golden	γ-rays	73,739	139,953 ^{a)}	156,832	15,010	21,285	406,869	44,318
	EI		305,461	186,146 ^{b)}	43,547	38,345	573,499	42,281
Fuji Nijou	γ-rays				26,870	48,853	75,723	6,680
	EI				23,440 ^{c)}	27,424 ^{d)}	50,865	6,215
Nitta Nijou No. 1	γ-rays				39,685	27,608	67,293	8,430
	EI				40,521 ^{e)}	29,647 ^{f)}	70,168	8,615
Total	γ-rays	73,739	139,953	156,832	81,565	97,746	549,885	59,428
	EI		305,461	186,146	107,508	95,416	694,532	57,111

a) M₃ of chronically irradiated materials, b) ML-3A, c) ML-4F, d) ML-9F and ML-13F, e) ML-7N, f) ML-10N and ML-12N.

Table 2. List of resistant mutants for powdery mildew

Mutant lines	Year	Original varieties	Mutagenic treatment	Segregation in M ₁ spike progenies		Reaction type to race IX	Remarks
				S	R		
ML-3A	1975	Azuma Golden	EI 0.06%	—	1	0/(4)	<i>ml-o</i> gene
ML-4F	1976	Fuji Nijou	EI 0.06%	4	1	0/(4)	<i>ml-o</i> gene
ML-7N	1976	Nitta Nijou No. 1	EI 0.06%	4	1	1-2	necrosis
ML-9F	1977	Fuji Nijou	EI 0.04%	15	1	0/(4)	<i>ml-o</i> gene
ML-10N	1977	Nitta Nijou No. 1	EI 0.04%	10	3	1-2	
ML-12N	1977	Nitta Nijou No. 1	EI 0.04%	13	4	0	chlorosis
ML-13F	1977	Fuji Nijou	EI 0.06%	9	2	0/(4)	<i>ml-o</i> gene

Nitta Nijou No. 1 were used.

Dose of γ-rays was from 10 to 30 kR, and concentration of EI was from 0.02 to 0.06% (for 2-hr treatments). Both mutagens were applied to seeds at 2-3 different levels varying slightly by years, except that the γ-ray treatment in 1974 was chronic irradiation on growing plants.

In the isolated field M₁ plants were densely grown to prevent outcross and to assure the independent mutational event in each M₁ plant. The total number of M₂ plants subjected to the mutant screening was 980,000 for Azuma Golden in 5 years. 130,000 for Fuji Nijou and 140,000 for Nitta Nijou No. 1 both in 2 years, totaling 1,240,000 plants (Table 1). Out of them, 1 plant of 1 line, 7 plants of 3 lines and 8 plants of 3 lines were found to be resistant mutants in Azuma

Golden, Fuji Nijou, and Nitta Nijou No. 1, respectively, giving a total of 16 plants from 7 mutant lines (Table 2). Mutation rate was 6.0×10^{-5} , and 1.3×10^{-3} based on the number of M₁ spike progeny and M₂ plant, respectively. These values are of a similar level to those reported^{2,4,5)} for powdery mildew resistance mutation.

All the resistant mutants were obtained by EI treatments. This result may simply be interpreted as indicating that the mutagenic effect of EI was generally higher than that of γ-rays, and consequently the resistance mutation occurred only by the EI treatment. However, it is noteworthy that 3 out of 7 resistant mutants were found in the few mutagenic treatments which gave chlorophyll mutation rate lower than 6% and were comparable to those for gamma-rays.

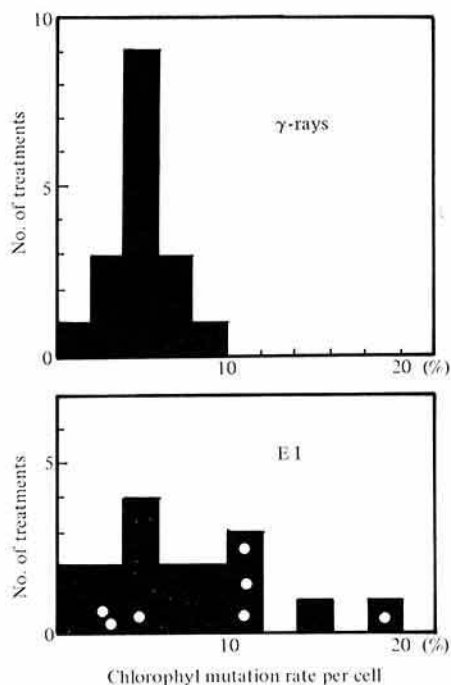


Fig. 1. Histogram of treatments of average chlorophyll mutation rate per cell. Each open circle in the figures stands for the resistant mutants induced.

Frequency distribution of all the treatments of EI and γ -rays against the chlorophyll mutation rates is shown in Fig. 1, in which the occurrence of resistance mutation is indicated by white circles. There is a treatment showing as high a chlorophyll mutation rate as 18% in the EI group, whereas the rate was lower than 10% in all the γ -ray treatments. In the EI group, 3 lines out of 7 lines of resistant mutants were obtained within a range of chlorophyll mutation rate similar to that of γ -ray, i.e., lower than 10%. This fact shows that EI is more effective than γ -ray in inducing resistance mutation. This result gives an additional case to several reports^{9,10,13} dealing with comparison of different mutagens for economic characters on the basis of relative rate against chlorophyll mutation rate.

Frequency of resistant mutation per M_2 plants in EI treatment was 0.17×10^{-5} for Azuma Golden, 13.8×10^{-5} for Fuji Nijou,

and 11.4×10^{-5} for Nitta Nijou No. 1. Azuma Golden gave as low a rate as 1/70–1/80 that of the other two varieties. Not only the mutation rate, but also the genes of mutants differ with different varieties, as shown later. In inducing disease-resistance mutation, therefore, careful attention must be paid in selecting original varieties for mutation work.

The M_1 spike progeny method, adopted in the present screening for mildew resistance is theoretically criticized to give a lower probability in obtaining mutants than the one spike-one grain method. However, the deleterious mutation which is simultaneously occurred in the genome of a resistant mutant can not be separated from the resistant mutant when the latter method is applied.

Out of 7 resistant mutants 3 mutants were associated with male sterility, albina and yellow stripe, but resistant mutant without these deleterious characters could be isolated in progenies which were raised from the normal heterozygous individuals in M_1 spike progeny.

There were 4 progenies from each of which only one resistant mutant was identified. In the screening for the resistance, it is easy to detect the resistance mutation when more than two mutant plants were segregated, and there is a high possibility of overlooking resistance mutation when only one resistant plant was segregated.

In addition, the M_1 spike progeny method requires far less labor for collecting and sowing the screening materials than the one-spike-one grain method. From these results, the former seems to be more efficient than the latter as a screening method of resistance mutation for powdery mildew in two-rowed barley.

Characteristics of resistant mutant lines

1) *ML-3A*

This line was obtained from Azuma Golden (Plate 1). It shows the immuno-type resistance, without showing necrosis or hypha under ordinary inoculation conditions, but it sometimes develops one to several large

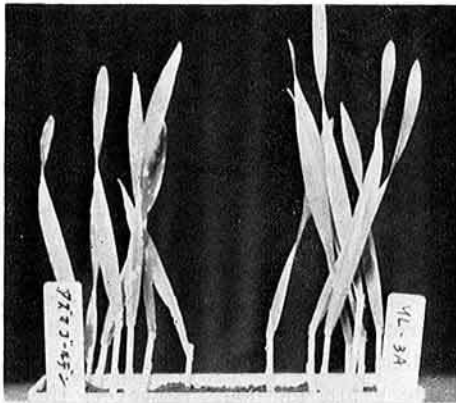


Plate 1. Reaction of a mutant line to powdery mildew
 Left: original variety Azuma Golden.
 Right: resistant mutant line ML-3A.

colonies, when an extremely large amount of spores are inoculated. This reaction is the same as the type 0/(4), defined by Nover.⁸⁾ It was proved that the resistance of this line was caused by a single recessive gene, *ml-o*, by crossing to a tester line MC20.

2) *ML-4F*, *ML-9F*, and *ML-13F*

They were obtained from Fuji Nijou. Their reaction to race IX was similar to that of ML-3A except that the number of colonies developed by a heavy inoculation was slightly abundant in ML-9F. Resistant gene of these three lines are allelic to *ml-o*.

The mutation of *ml-o* gene has also been reported elsewhere,^{2,3,5,11)} and it is known that all of mutants are resistant to all the races collected upto date in the world. Actually ML-3A was also resistant not only to race IX used for the screening, but also to race XIV, which has accumulated all virulent genes existing in Japan, except race IX, by repeated crossings undertaken in Prof. Hiura's laboratory. Induction and utilization of such kind of resistance mutation may be useful in overcoming the problem of resistance breakdown due to appearance of new races.

All the lines with *ml-o* gene develop small and yellow-brown flecks on upper leaves just

before heading. Their development was more severe under higher temperature, and the number and size of flecks in the field condition differed each other among the mutant lines.

3) *ML-7N*, *ML-10N*, and *ML-12N*

They were obtained from Nitta Nijou No. 1. Although hypha is not recognized, ML-7N develops large necrosis on leaf surface, whether inoculated or not, and its growth is very poor. ML-10N showed a few hypha, no colony formation, small necrotic spots, and grade of resistance: type 1-2. ML-12N is highly resistant (type 0), without showing hypha, but it develops a large number of small chlorosis, whether it is inoculated or not. The resistance of these three mutant lines depends on single recessive genes differing from each other. They are non-allelic to the *ml-o* gene.

The four lines with mutated *ml-o* gene are being used as a new gene source in the breeding of resistant varieties against powdery mildew disease in barley breeding stations. Development of promising resistant varieties is expected.

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(Received for publication, April 4, 1983)