Induced Mutation for Resistance to Barley Yellow Mosaic Virus

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Yellow mosaic of barley is a disease caused by a soil-borne virus, barley yellow mosaic virus (BYMV).[2] It was recorded for the first time in Okayama Prefecture, western part of Japan, in 1940, but was not important in those days.[5] The disease damage became serious since around 1950 and the infected acreage gradually increased in many affected prefectures. The distribution of the disease now covers all over Japan except Hokkaido. European breeders informed us that since the first detection of the disease in the Federal Republic of Germany in 1978 the disease has been spreading more and more widely, prevailing now in Germany, England, France and Belgium.

The high losses in yield endanger the cultivation of two-rowed and six-rowed barley in the virus-infested areas. So far, no effective and economic control of the disease by chemical agents is available, nor is it to be expected in the near future. Growing resistant cultivars is the only means of avoiding the yield losses due to the virus infection. A large variation in resistance to the disease among barley cultivars was recognized. Mokusekko 3 was found to show high resistance at all locations and years tested and since then it has often been used as a parent in crossings for developing a resistant cultivar. However, it originated from China and has a genetic background so different from those of main cultivars in Japan. Genetic resources available for breeding of resistant cultivars are still rather limited, particularly for two-rowed barley. Thus, it is an urgent task to increase the spectrum of resistant varieties.

Induced mutations provide a useful tool for enlarging such spectrum. The authors found a mutant highly resistant against BYMV among a collection of early-maturing mutants induced by different physical and chemical mutagens. Here, the derivation and inheritance of the mutation are briefly reported.

Derivation and characteristics of the mutant

Using a winter habit six-rowed variety "Chikurin Ibaraki 1" of barley (*Hordeum vulgare* L.) as a material, 61 early mutants were obtained from a total of about 120,000 plants of M_2 or M_3 population by the treatment of seeds or plants with gamma-rays, thermal neutrons, or chemical mutagens.[9] At the end of October 1978, twenty-four seeds for each of the mutants and the original variety were sown in a field heavily infected with BYMV. In early spring of the next year the plants of the original variety showed discolorations or yellowing of leaves, a symptom known to be caused by BYMV. Although the infected plants recovered leaf greenness a little with the rise of temperature in April, they still showed isolated streak symptoms and much reduced growth. Some of the most infected plants were lethal. All mutants except one named Ea52 were found to be similarly susceptible to the original variety. Plants of

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Plate 1. A mutant Ea52 which is highly resistant to BYMV (at the center)

Ea52 showed a normal appearance and no inhibition of growth, proving to be highly resistant to the virus (Plate 1).

Ea52 was screened as an early mutant from M_3 after irradiation of a plant at a vegetative stage with 250 R of gamma-rays in the gamma-field. It has a heading time about 5 days earlier than the original variety in field. Its earliness was revealed to be due to the fact that its critical daylength below which differentiation of spike primordia is completely suppressed was shortened by mutation. No other characters than earliness and the resistance to the disease are recognized to be altered by mutation in comparison with the original variety.

Inheritance of the resistance

To investigate the inheritance of the resistance of Ea52 to BYMV, Ea52 was crossed as male with an early mutant Ea17, which was obtained from the same original variety by treating the seeds with a chemical mutagen ethyleneimine. Ea17 has a similar degree of earliness due to a reduction in the critical daylength by mutation. When grown in a heavily infested field, Ea17 was found to be susceptible to the virus at a degree similar to the original variety. Mosaic appearance of yellowing of leaves in early spring was marked for the mutant. Culm length at maturity and the number of spikes per plant were decreased to about one half and one third respectively, of the values of Ea52 (Table 1), although the two mutants showed no difference in these characters in a normal field.

Table 1. Average culm length at heading time and number of spikes of Ea 52, Ea 17 and the original variety when grown in a field heavily infested by BYMV

	Culm Length (cm)	No. spikes per plant
Mutant Ea 52	65.47±3.38	12.82±1.71
Mutant Ea 17	31.47±2.31	4.95±0.82
Original variety, Chikurin Ibaraki 1	30.95±2.12	3.50±0.58

The seeds of Ea17, Ea52 and F_2 from a cross between them were sown in an infested field at the end of October. All plants of

Table 2. Segregation of resistant and susceptible plants in F_2 from a cross between a susceptible early mutant Ea 17 and the resistant mutant Ea 52

		Resistant	Susceptible	Total
F_2*	1	16	43	59
	2	13	41	54
	3	15	42	57
	Total	44	126	170
Mutant Ea 52		20	2	22
Mutant Ea 17		0	22	22
Original variety, Chikurin Ibaraki 1		0	22	22

Susceptibility was determined by the symptoms of BYMV, i.e. leaf yellowing and stunting.

* Three F_2s each derived from one F_1 plant were tested.

Heterogeneity d.f.=2, $\chi^2=0.14$, $p>0.90$

Segregation (1 : 3) d.f.=1, $\chi^2=0.16$, $p>0.60$

Ea17 and the original variety were found to be highly susceptible to the disease as judged by yellowing of leaves (Table 2), while all but two plants of Ea52 resistant and normal in appearance. Segregation of resistant and susceptible plants was recognized in the F_2. The ratio of resistant to susceptible plants was 44 : 126, which showed a good fit to 1 : 3 expected from the single gene inheritance. No intermediate types were observed. Resistance gene of Ea52 was completely recessive to the original allele.

Segregation in F_2 of the degree of damage caused by the disease was also recognized in plant weight after harvesting (Fig. 1). Distribution was bimodal. Nine F_2 plants were lethal due to the disease. The ratio of resistant to susceptible plants was 46 : 115, again in good agreement with the 1 : 3 ratio. Except for a few plants, resistance as judged by plant weight showed a coincidence with the resistance observed in the symptoms.

To see whether or not the resistance to the virus is associated with the earliness of Ea52 in inheritance, heading time of the resistant F_2 plants was compared with that of the plants of Ea52 (Fig. 2). As the plants of Ea17 and the susceptible F_2 group showed more or less a delay in heading date due to the disease, with extreme ones failing to head

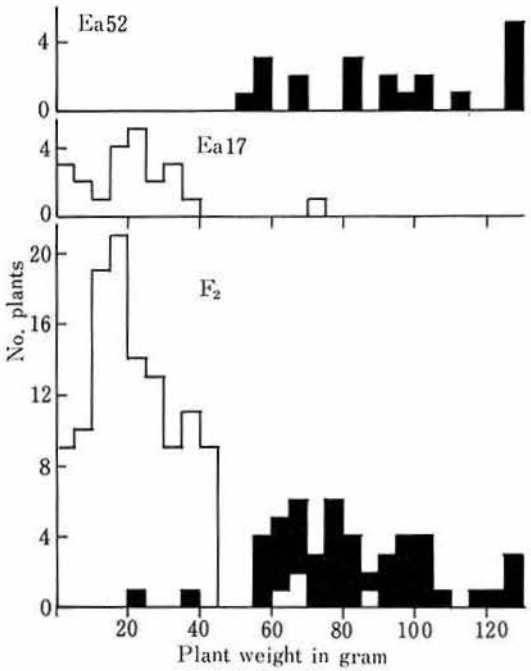

Fig. 1. Distribution of plant weight for F_2 from a cross Ea 17 (susceptible) × Ea 52 (resistant)

Open and solid colums show the plants with and without external symptoms, i.e. leaf yellowing and stunting, respectively.

or lethal, the data for these plants were excluded. The original variety showed heading date from 20th to 26th April, with 23rd on the average, when grown in a normal field. If Ea52 and Ea17 have a single mutated gene for earliness in common, then no segregation of heading time in F_2 is expected. If the earliness of Ea52 is pleiotropy of resistance to the disease, all of the resistant F_2 plants are expected to be early even when the earliness genes of Ea52 and Ea17 are not allelic to each other. Frequency distribution of heading date of the resistant F_2 plants in Fig. 2 shows that plants earlier than the earliest plant of Ea52 were segregated and that there were observed many plants with heading time not differing from that of the original variety. It is concluded from the results that Ea52 and Ea17 have different genes for earliness and that the resistance to the virus carried by Ea52 is not due to pleiotropic effect of

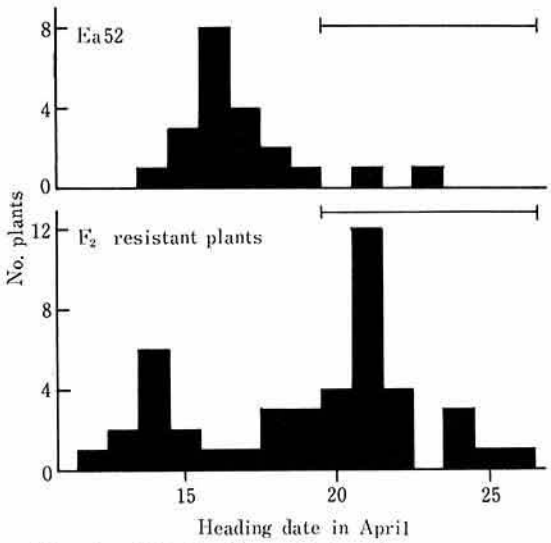

Fig. 2. Distribution of heading time on plant basis of Ea 52 and the resistant plants of the F_2 from a cross Ea 17 × Ea 52

Data of the susceptible parent Ea 17 and the susceptible F_2 plants were omitted since the plants showed more or less delay in heading due to the disease damage. The bars show the range of heading time for the original variety.

the earliness gene of the mutant.

Existence of the virus in leaf cells of plants of Ea52 and the original variety was surveyed by direct negative method under electron microscope. The plants of the original variety were proved to have particles of BYMV and Soil-Borne Wheat Mosaic Virus (SBWMV) as well while apparently healthy plants of Ea52 were free from both viruses. Stunted plants which appeared among the plants of Ea52 with a relatively low frequency were found to have SBWMV, but no BYMV.

Discussion

Since the success in the induction of mutations for resistance to powdery mildew (*Erysiphe graminis* f. sp. *hordei.*) by Freisleben and Lein[1] many mutations for disease resistance have been obtained in different crop plants. Almost all of them, however, are relating to diseases caused by fungus or bacterium. Mutations for resistance to virus disease was seldom reported and believed to be very difficult to be screened. As far as the authors are aware, resistance to Y-virus in tobacco[3] and resistance to bean golden mosaic virus in *Phaseolus vulgaris* L.[8] are all such cases so far obtained by mutation. The mutation for resistance to BYMV reported here is an additional important case and the first as mutagen-induced resistance to a soil-borne disease.

Screening of barley varieties for the resistance to BYMV has been carried out since 1960's.[4,5,6] Takahashi et al. found that Mokusekko 3 and Mihorihadaka 3 have resistance to the virus.[7] They further revealed that the resistance of Mokusekko 3 is controlled by a partial dominant gene Y_{m1} located on chromosome 4 and that the resistance of Mihorihadaka 3 is due to another partial dominant gene Y_{m2} on chromosome 1. In contrast to these genes the mutated gene for resistance in Ea52 is completely recessive to the original allele when the mutant is crossed as male to a susceptible strain. Crossing of the mutant with Mokusekko 3 and Mihorihadaka 3 in recent experiments revealed that the mutated gene is not allelic to Y_{m1} or Y_{m2}, either.[10] The mutant will be useful as another genetic source for the resistance to the virus in further barley breeding plans.

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References

1) Freisleben, R. & Lein, A.: Über die Auffindung einer mehltauresisten Mutante nach Röntgen-bestrahlung einer anfällingen reinen Linie von Sommergerste. *Naturwissenschaften*, 30, 608 (1942).
2) Inouye, T. & Saito, Y.: Barley yellow mosaic virus. Description of plant viruses, No. 143. Commonwealth Mycological Institute, Kew, England (1975).
3) Koelle, G.: Genetische Analyse einer Y-

Virus-(Rippenbräune) resisten Mutante der Tabaksorte Virgin A. *Züchter*, 31, 71–72 (1961).
4) Saito, Y. & Okamoto, H.: Studies on the soil-borne virus diseases of wheat and barley. V. Variation in varietal resistance of wheat and barley. *Bull. Nat. Inst. Agr. Sci.*, Series C, 17, 75–102 (1964) [In Japanese with English summary].
5) Takahashi, R. et al.: Studies on resistance to yellow mosaic disease in barley. I. A screening test for resistance of two-rowed and six-rowed barleys. *Nogaku-Kenkyu*, 51, 135–152 (1966) [In Japanese].
6) Takahashi, R. et al.: Studies on resistance to yellow mosaic disease in barley. II. Comparison of varietal reactions to the viruses of different sources and relation between disease symptoms and damage. *Nogaku Kenkyu*, 52, 65–78 (1968) [In Japanese].
7) Takahashi, R. et al.: Studies on resistance to yellow mosaic disease in barley. III. Inheritance and linkage studies of the resistance. *Nogaku-Kenkyu*, 53, 153–165 (1970) [In Japanese].
8) Tulmann Neto, A. et al.: Attempts to induce mutants resistant or tolerant to golden mosaic virus in dry beans (*Phaseolus vulgaris*). *In* Induced Mutations against Plant Diseases, IAEA, Vienna, 281–290 (1977).
9) Ukai, Y. & Yamashita, A.: Early mutants induced by radiations and chemicals in barley. I. Frequencies of mutations and brief description of the selected mutants. *Jpn. J. Breed.*, 29, 255–267 (1979) [In Japanese with English summary].
10) Ukai, Y. (Unpublished).

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