

# Inheritance of Resistance to Seed Transmission of Barley Stripe Mosaic Virus in Barley

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## ABSTRACT

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A single recessive gene conditions resistance to seed transmission of barley stripe mosaic virus (BSMV) in barley (*Hordeum vulgare* L.). Among the progenies of crosses of Modjo (resistant) and Vantage (susceptible) barleys infected with the Montana isolate three (MI-3) of BSMV, susceptible  $F_1$  and  $F_2$  seedlings segregated 1 resistant to 3 susceptible plants. Data from  $F_3$  families were used to determine  $F_2$  genotypes, because the low levels of seed transmission were difficult to detect in some of the heterozygous susceptible plants of the  $F_2$  generation. Frequency of seed

transmission by infected homozygous susceptible plants of Vantage (barley) was about 70-80%, indicating that the gene governing susceptibility to seed transmission had a penetrance of 70-80%. Variable expressivity also was associated with this gene since infected seedlings expressed symptoms of variable severity at different growth stages. During this study, the virus was presumed to be genetically stable with respect to seed transmissibility.

Barley stripe mosaic virus (BSMV) caused a serious disease of barley (*Hordeum vulgare* L.) in Montana (5) during the 1950s. Consequently a breeding program was begun in 1956 to develop a cultivar resistant to seed transmission of the virus. Two of the parent barleys selected for the program were cultivars Modjo and Betzes. Genetic analysis of the  $F_1$ ,  $F_2$ , and  $F_3$  progeny from the Modjo  $\times$  Betzes cross suggested that a single allele probably conditioned resistance to seed transmission when a Montana isolate of BSMV was used (Hockett, unpublished).

To clarify further the nature of this resistance to seed transmission of BSMV, we studied its inheritance. Studies on inheritance of resistance to BSMV appear to concern the reaction of barley seedlings to inoculation with the virus (10-12).

## MATERIALS AND METHODS

In April 1974, the resistant barley cultivar Modjo (CI 3212) and the susceptible cultivar Vantage (CI 7324) were planted at Bozeman, MT, in two small plots separated by a 1.5-m alley. One plot consisted of two outside rows of Vantage infected with the MI-3 isolate (4) of BSMV and two inside rows of BSMV-free Modjo. The other plot contained two outside rows of Vantage and two inside rows of Modjo, both BSMV-free. Single seeds from  $S_2$  (self) generation plants of each cultivar were space planted about 7.5 cm apart in these rows. Rows were 3 m long and 0.75 m apart. In the diseased plot when seedlings had three leaves, all plants not manifesting typical symptoms of BSMV were rogued.

Reciprocal crosses of the parents ( $P_0$  generation) Modjo  $\times$  BSMV-free Vantage and Modjo  $\times$  Vantage, infected via seed with BSMV, were made by standard crossing techniques. All crossed and self-pollinated heads of the infected and BSMV-free plants were bagged. In this article seeds that will produce the  $F_1$  generation are referred to as " $F_{(1)}$ " seeds and seeds that will produce the  $P_1$  generation, are referred to as " $P_{(1)}$ " seeds (1).

During the late summer and early fall of 1974,  $F_{(1)}$  seeds from crosses involving diseased plants,  $P_{(1)}$  seeds from infected Vantage, and BSMV-free Vantage and Modjo were planted in the

greenhouse for analysis of seed transmission. The greenhouse temperature was about 22 C and no supplementary light was used. The seedlings were rated for disease symptoms, presence of BSMV antigen as determined by the serodiagnostic technique of Hamilton (7), or both.

In May 1975,  $F_{(1)}$  seeds from the reciprocal crosses of BSMV-free plants (Modjo  $\times$  Vantage, Vantage  $\times$  Modjo), and the three types of  $P_{(0)}$  seeds (Modjo, infected Vantage, healthy Vantage) were sown in the field at Bozeman. Uninfected seeds of the susceptible barley cultivar Black Hullless (CI 666) also were sown and the resulting seedlings served as symptom-positive inoculation controls. Seeds in each of the six treatments were planted in a randomized block design with 10 replicates. Plots consisted of five 3-m long rows, 60 cm apart, with 12 single seeds planted about 25 cm apart in each row. When the seedlings were in the three-to-five-leaf stage, all except infected  $P_0$  Vantage plants were manually inoculated with BSMV by the swab technique. Inoculum consisted of Vantage leaf sap infected with the MI-3 isolate of BSMV. About 2 wk after inoculation, the plants were rated for symptoms, and all symptomless inoculated plants were reinoculated. Readings for symptoms were recorded three times thereafter at about 3-wk intervals. Virus presence in symptomatic plants and absence in symptomless plants were verified by serologic testing of leaves.

During the late summer and autumn of 1975, some  $F_{(2)}$  and  $P_{(1)}$  seeds were sown in the greenhouse or in plastic incubation chambers in the laboratory, and the resulting seedlings were rated for symptoms, tested serologically for virus, or both.

For clarification of some results of the 1975 greenhouse evaluation,  $F_2$  plants were grown in the field in the spring and summer of 1976 so that  $F_3$  families could be evaluated. Fifteen rows were sown with the  $F_{(2)}$  seeds of the Modjo  $\times$  Vantage cross, and six rows were planted with the  $F_{(2)}$  seeds of the reciprocal cross. In addition, four rows each were planted with  $P_{(0)}$  seeds of BSMV-free Modjo and Vantage and infected Vantage. Six rows of BSMV-free Black Hullless were included as controls. All rows were about 3 m long and 30 cm apart. All infected material was spatially isolated from uninfected material. After germination, all  $F_2$  and  $P_0$  seedlings were rated for symptoms via seed transmission and infected plants were marked with a flag. Later, when plants had three to six leaves, all  $F_2$  and Black Hullless control plants were blast-inoculated with the MI-3 isolate of BSMV. The inoculum was

TABLE 1. Seed transmission of barley stripe mosaic virus in generations of barley cultivars and crosses tested in the field and greenhouse

Cultivar or cross	Generation <sup>a</sup>	Test <sup>b</sup>	Total number of seedlings observed			Seed transmission (%)		
			1974	1975	1976	1974	1975	1976
Modjo	P <sub>0</sub>	F	48	0	107	0	...	0
Vantage	P <sub>0</sub>	F	35	0	97	0	...	0
Vantage-i <sup>c</sup>	P <sub>0</sub>	F	26	0	94	73.0	...	77.7
Modjo	P <sub>1</sub>	G	48	156	0	0	0	...
Vantage	P <sub>1</sub>	G	45	119	0	0	0	...
Vantage-i	P <sub>1</sub>	G	47	101	0	80.9	66.3	...
Modjo × Vantage-i	F <sub>1</sub>	G	134	0	0	39.6	...	...
Vantage-i × Modjo	F <sub>1</sub>	G	237	0	0	64.6	...	...
Modjo × Vantage	F <sub>2</sub> <sup>d</sup>	G	0	113	0	...	29.2	...
Vantage × Modjo	F <sub>2</sub> <sup>d</sup>	G	0	158	0	...	25.3	...
Modjo × Vantage	F <sub>2</sub> <sup>e</sup>	F	0	0	1,217	...	...	35.7
Vantage × Modjo	F <sub>2</sub> <sup>e</sup>	F	0	0	402	...	...	32.8

<sup>a</sup>P<sub>0</sub> = parental generation; P<sub>1</sub> = first generation produced from seeds of self-pollinated parental plants; F<sub>1</sub> = first generation produced from seeds of cross-pollinated parental plants; F<sub>2</sub> = second generation produced from seed of cross-pollinated parental plants.

<sup>b</sup>F = field test, G = greenhouse test.

<sup>c</sup>Vantage-i = Vantage barley infected with the MI-3 isolate of barley stripe mosaic virus via seed transmission.

<sup>d</sup>Seedlings from F<sub>2</sub> seeds from representative F<sub>1</sub> plants that had been mechanically inoculated with the MI-3 isolate of barley stripe mosaic virus.

<sup>e</sup>Seedlings from remaining F<sub>2</sub> seeds from many F<sub>1</sub> plants that had been mechanically inoculated with the MI-3 isolate of barley stripe mosaic virus.

TABLE 2. Observed phenotypic vs. expected genotypic ratios for seed transmission of barley stripe mosaic virus in the F<sub>2</sub> generation of reciprocal crosses between barley cultivars Modjo and Vantage

Cross	Expected ratio <sup>a</sup>	Observed ratio (number of plants)		X <sup>2</sup>	P for fit
		No seed transmission	Seed transmission		
Modjo × Vantage	1:3	307	910	0.033	0.80-0.90
Vantage × Modjo	1:3	115	287	2.789	0.05-0.10

<sup>a</sup>No seed transmission/seed transmission.

prepared from infected Vantage by triturating 1 g of leaf tissue in 10 ml of distilled water with a Blendor, straining the triturate through four layers of cheesecloth, and adding 600-mesh Carborundum to give a final concentration in the leaf extract of 2% (w/v). Air pressure for blast inoculation was about 6.5 kg/cm<sup>2</sup> (80 lb/in.<sup>2</sup>), and the gun nozzle was held about 15-30 cm from the seedlings. The blast gun and portable air compressor used were similar to those described by Gardner et al (6). Symptoms were evaluated several times after inoculation; the final evaluation was made just before the ripening stage of plant development. All plants with symptoms due to mechanical inoculation and all asymptomatic plants were marked so that they could be easily distinguished from one another and from plants that had been infected via seed transmission. After the plants were mature, F<sub>3</sub> seeds were harvested from individual plants of known classification and planted in flats in the greenhouse. Resulting F<sub>3</sub> seedlings were rated for symptoms. Some symptomatic and asymptomatic F<sub>3</sub> seedlings were tested serologically to verify determinations based on symptoms.

## RESULTS AND DISCUSSION

Data on seed transmission in generations of barley cultivars and crosses are presented in Table 1. Resistance to seed transmission of BSMV is a recessive trait because each parental plant in the reciprocal crosses involving infected Vantage produced susceptible, infected F<sub>1</sub> seedlings. Seed transmission expressed by the F<sub>1</sub> generation from the infected Vantage × Modjo cross (64.6%) was higher than that from the reciprocal cross (39.6%). This difference was expected because seed transmission of BSMV is usually more frequent through infected ovules than through

infected pollen (8).

Although the frequency of seed transmission in the P<sub>0</sub> and P<sub>1</sub> generations of the homozygous susceptible infected Vantage plants remained relatively high (66.3-80.9%), it never reached 100%. Therefore, the gene governing susceptibility to seed transmission had a penetrance of about 70-80% (2). Furthermore, variable expressivity or phenotypic variability (2), was possibly associated with the seed transmission character. Some seedlings expressed moderate to severe symptoms of infection at emergence, and others showed no symptoms until they had several leaves.

Variable penetrance and expressivity reflect the many factors known to affect seed transmission (3). Some of these are: (i) host genotype; (ii) virus genotype and virus behavior in the host with respect to multiplication, spread, and transmission by pollen, ovules, and embryos; and (iii) environment, particularly temperature.

As a result of incomplete penetrance and variable expressivity, the F<sub>2</sub> plants not infected via seed transmission were difficult to classify as resistant or susceptible. The difficulty was due in part to the limited size of most samples of seeds and in part to evidence of low levels (1-5%) of seed transmission by heterozygous susceptible plants. Therefore, data from F<sub>3</sub> families were used to determine F<sub>2</sub> genotypes (Table 2). Goodness-of-fit to a 1:3 ratio was satisfactory for both crosses.

The relationship between the genetics of resistance to seed transmission of BSMV and the inheritance of barley stripe mosaic reaction (10-12) in barley is unknown. Conceivably, the factor responsible for resistance to seed transmission could also be involved in the resistant reaction to the virus after seedlings are mechanically inoculated with certain viral strains.

Presumably, during this study, the MI-3 isolate of BSMV was genetically stable with respect to seed transmissibility. However, the mutability of this trait has not yet been determined (9).

The resistant gene contributed by Modjo barley probably conditions only for resistance to the seed transmission of MI-3 and other genetically similar isolates of BSMV. This speculation is consistent with the findings of Timian and Sisler (11) regarding resistance expressed as the BSMV reaction in Modjo. They reported that Modjo had different levels of resistance to three apparently unrelated isolates of BSMV inoculated singly. After one inoculation, only 7% of the plants were infected by the California "E" isolate of the virus. In contrast, 70% of the plants were infected by the other two isolates.

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