

# Variation in Embryo Infection and Seed Transmission of Barley Stripe Mosaic Virus Within and Between Two Cultivars of Barley

T. W. Carroll and S. R. Chapman

Department of Botany and Microbiology and Department of Plant and Soil Science, respectively, Montana State University, Bozeman 59715.

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## ABSTRACT

Atlas (C.I. 4118) and Hypana (C.I. 11772) were inoculated with type strain of barley stripe mosaic virus at the three-leaf stage in field studies. At jointing, plants not showing symptoms were rogued. At maturity, 10 random infected plants of each variety were harvested individually, and a composite sample of 100 random heads of each variety was taken.

Embryo infection of seed of the individual plants, based on a maximum sample of 50 seeds, and of the composite sample were determined serologically. In the greenhouse, seed transmission was assayed in seedling progeny of the field-grown plants and com-

posite sample as the ratio of seedlings expressing visible symptoms of barley stripe mosaic to total seedlings emerged.

Hypana had a lower level of embryo infection and seed transmission than Atlas. Using heterogeneity  $X^2$ , with the level of incidence in the composite samples as the expected value, it was verified that there is significantly greater plant to plant variation in Atlas than in Hypana. The same pattern of variation was detected for both embryo infection and seed transmission. *Phytopathology* 60:1079-1081.

Many factors influence seed transmission of barley stripe mosaic virus (BSMV) in barley. Among these, environmental conditions such as air temp were found to be important by Singh et al. (10). In greenhouse tests with tolerant barley varieties, more seed transmission occurred at 20 and 24 C than at 16 C. Certain viral strains transmit to a greater extent in a given barley variety than do others (9, 11). Furthermore, the barley host has been shown to have an influence on the passage of the virus from the parent plant to progeny through seed. Infection with BSMV prior to heading results in a higher percentage of seed transmission than does infection with BSMV following heading (3, 4, 5, 7, 10). Moreover, marked differences in seed transmission of BSMV have been detected in different cultivars or varieties of barley. The amounts of transmission reported vary from 0 to 75% (3, 4, 5, 7, 9, 10, 11). It is presumed that variation in transmission is due in part to genetic differences between varieties. The purpose of this investigation was to determine if genetic variation resulting in differences in embryo infection and seed transmission occurs not only between varieties but also within varieties.

**MATERIALS AND METHODS.**—Determinations were made by comparing patterns of embryo infection and seed transmission for the type strain of BSMV by using progeny (seed) from several individual field grown plants of each of two barley varieties. Embryo infection was studied along with seed transmission because: (i) Inouye (8) showed that seed transmission of BSMV is caused primarily by embryo infection; and (ii) Hamilton (6) obtained excellent correlation between infection detected by serological seed testing and by visual inspection of seedlings from seed of the same lot. In our studies we used both serological seed testing (6) and the seedling test of Afanasiev (1). Preliminary results have been published (2).

Atlas barley (C.I. 4118) was selected because it is a differential host for two strains of BSMV which are currently being investigated at Bozeman for their seed transmissibility. Hypana (C.I. 11772) was chosen as the second host because it is a newly released variety in Montana and studies on the seed transmission of BSMV in it have not yet been reported. The type strain (A.T.C.C. No. 69) of BSMV was used because of the extensive documentation of the culture.

Field seedings were made by hand to insure accurate spacings of single plants on a 30.5-cm grid. Plants of both varieties in the three-leaf stage were inoculated manually with BSMV at Bozeman, Montana, 30 May 1968. At jointing, plants not showing symptoms were rogued. At maturity, 10 random infected plants of each variety were harvested individually, and a composite sample of 100 random heads of each variety was taken. For 17 of the individual field plants and the 100 random heads, embryo infection based on a sample of 25 or 50 seeds was determined serologically. For Atlas field plants No. 7, 9, and 10, embryo infection was determined serologically for a sample of 18, 13, and 13 seeds, respectively.

For 18 of the individual field plants and the 100 random heads, seed transmission of BSMV was assayed in the spring and summer in greenhouse studies. Twenty-five or 50 seeds/field-grown plant or from the composite sample of 100 heads were planted, and all emerged seedlings scored for symptoms of barley stripe mosaic. For Atlas field plants No. 9 and 10, seed transmission was determined for samples of 12 seedlings each.

For both embryo infection and seed transmission, the seed from individual field-grown plants represents a progeny test of each plant; since the embryo and seedling tests were carried out under constant conditions in the laboratory, variation among seed lots from differ-

TABLE 1. Observed and expected values and approximate chi-square ( $\chi^2$ ) analysis of embryo infection by barley stripe mosaic virus in Atlas and Hypana barley based on progeny tests of infected field-grown plants<sup>a</sup>

Field plant	Atlas				$\chi^2$	Hypana				$\chi^2$
	Infected		Healthy			Infected		Healthy		
	Observed	Expected	Observed	Expected		Observed	Expected	Observed	Expected	
1	0	7.0	50	43.0	8.1	0	3.5	25	21.5	4.1
2	0	7.0	50	43.0	8.1	0	3.5	25	21.5	4.1
3	5	3.5	20	21.5	0.7	0	3.5	25	21.5	4.1
4	4	3.5	21	21.5	0.1	0	3.5	25	21.5	4.1
5	21	7.0	29	43.0	32.5	5	3.5	20	21.5	0.7
6	0	7.0	50	43.0	8.1	2	3.5	23	21.5	0.7
7	3	2.5	15	15.5	0.1	0	3.5	25	21.5	4.1
8	0	7.0	50	43.0	8.1	0	3.5	25	21.5	4.1
9	3	1.8	10	11.2	0.9	0	3.5	25	21.5	4.1
10	7	1.8	6	11.2	17.1	0	3.5	25	21.5	4.1
Total	43	48.1	301	295.9	83.8 <sup>a</sup>	7	35.0	243	215.0	34.2 <sup>b</sup>

<sup>a</sup>  $\chi^2_s = 83.8 = \text{sum of } 10 \chi^2 \text{ values}$ ;  $\chi^2_p = 0.6 = \chi^2 \text{ based on totals}$ ;  $\chi^2_h = 83.8 - 0.6 = 83.2^b$ ;  $\chi^2_s = 34.2 = \text{sum of } 10 \chi^2 \text{ values}$ ;  $\chi^2_p = 26.0 = \chi^2 \text{ based on totals}$ ;  $\chi^2_h = 34.2 - 26.0 = 8.2$ ;  $\chi^2_h = \chi^2_s - \chi^2_p$  measures variation among field-grown plants.

<sup>b</sup> Probability of chance deviation from expectation 1% or less.

ent field grown plants reflects, in part, genetic differences among the individual field grown plants.

RESULTS AND DISCUSSION.—Observed levels of infection (Table 1) indicate that Hypana had a lower level of embryo infection than Atlas. To test uniformity of transmission among plants within each variety an approximate chi-square ( $\chi^2$ ) test was employed. The expected level of transmission for each variety was based on the level of infection detected in embryos from seed from the composite samples of heads from each variety. This expected level (E) was compared with the observed level of embryo infection (O) in seed from individual field-grown plants (progeny of field-grown plants). A  $\chi^2$  value for seed from each field-grown plant was calculated by  $\chi^2 = (O - E)^2/E$  (infected) +  $(0 - E)^2/E$  (noninfected). In addition, data were pooled for all field-grown plants and a pooled  $\chi^2$  ( $\chi^2_p$ ) was calculated. The  $\chi^2$  values calculated for

the data from individual field grown plants were summed to yield a summed  $\chi^2$  ( $\chi^2_s$ ). The deviation  $\chi^2_s - \chi^2_p = \chi^2_h$  (heterogeneity  $\chi^2$ ); this is a measure of the heterogeneity of embryo infection of individual field grown plants. When  $\chi^2_h$  is significant, all plants do not follow the same pattern of embryo infection. This difference is presumably due in part to genetic differences among plants. The validity of the expected level of infection is measured by  $\chi^2_s$ . When  $\chi^2_s$  is significant, the model for expectation does not fit the biological circumstances adequately.

When  $\chi^2_h$  is significant, it is expected that no single model for expected levels of infection would fit the observed data. Heterogeneity  $\chi^2$  ( $\chi^2_h$ ) indicates there is greater plant to plant (genetic) variation within Atlas than within Hypana:  $\chi^2_h = 83.2$  vs.  $\chi^2_h = 8.2$ , respectively. A satisfactory fit to expectation  $\chi^2_s$  was not obtained for either variety, but the fit was much better

TABLE 2. Observed and expected values and approximate chi-square ( $\chi^2$ ) analysis of seed transmission by barley stripe mosaic virus in Atlas and Hypana barley based on progeny tests of infected field-grown plants<sup>a</sup>

Field plant	Atlas				$\chi^2$	Hypana				$\chi^2$
	Infected		Healthy			Infected		Healthy		
	Observed	Expected	Observed	Expected		Observed	Expected	Observed	Expected	
1	0	3.8	44	40.2	4.2	0	2.1	25	22.9	2.3
2	0	3.9	45	41.1	4.3	0	2.0	23	21.0	2.1
3	9	1.8	12	19.2	30.8	0	2.0	24	22.0	2.2
4	7	1.6	12	17.3	19.0	0	2.0	24	22.0	2.2
5	17	4.0	29	42.0	46.3	3	2.1	22	22.9	0.4
6	0	4.0	46	42.0	4.4	0	2.1	25	22.9	2.3
7	5	2.1	19	21.9	4.4	0	2.0	24	22.0	2.2
8	0	4.2	48	43.8	4.6	0	2.0	24	22.0	2.2
9	5	1.0	7	11.0	16.5	0	2.1	25	22.9	2.3
10	5	1.0	7	11.0	16.5	0	1.9	22	20.1	2.0
Total	48	27.4	269	289.5	151.0 <sup>b</sup>	3	20.3	238	220.7	20.2 <sup>c</sup>

<sup>a</sup>  $\chi^2_s = 151.0 = \text{sum of } 10 \chi^2 \text{ values}$ ;  $\chi^2_p = 17.0 = \chi^2 \text{ based on totals}$ ;  $\chi^2_h = 151.0 - 17.0 = 134.0^b$ ;  $\chi^2_s = 20.2 = \text{sum of } 10 \chi^2 \text{ values}$ ;  $\chi^2_p = 16.1 = \chi^2 \text{ based on totals}$ ;  $\chi^2_h = 20.2 - 16.1 = 4.1$ ;  $\chi^2_h = \chi^2_s - \chi^2_p$  measures variation among field-grown plants.

<sup>b</sup> Probability of chance deviation from expectation 1% or less.

<sup>c</sup> Probability of chance deviation from expectation 5% or less.

for Hypana than Atlas:  $\chi^2_s$  for Hypana is less than  $\chi^2_s$  for Atlas: 34.0 vs. 83.8.

Following the same model for deriving expected levels of embryo infection, the same approximate  $\chi^2$  tests were used in the analysis of seedling infection. The level of seedling infection was higher in Atlas than in Hypana (Table 2). As in the embryo study, Atlas showed more plant to plant (genetic) variation than Hypana:  $\chi^2_h = 134.35$  vs.  $\chi^2_h = 6.14$ , respectively. Goodness of fit to expectation,  $\chi^2_s$  was closer for Hypana than Atlas: 20.2 vs. 151.0.

From these data the following conclusions are drawn: (i) Both the embryo test and seedling tests indicate the same pattern of variation within and between varieties; (ii) the difference between Atlas and Hypana with respect to embryo infection and seed transmission is due in part to greater genetic variation in Atlas; and (iii) to avoid genetic heterogeneity of response to the type strain of BSMV, a highly inbred host line must be used. At present, highly inbred strains of several host barley varieties are being developed to eliminate genetic variation within hosts so that patterns of embryo infection and seedling transmission of various strains of BSMV can be studied without the disrupting effects of host genetic variation.

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