Resistance in Faba Bean (*Vicia faba*) to Bean Yellow Mosaic Virus

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ABSTRACT

Gadh, I. P. S., and Bernier, C. C. 1984. Resistance in faba bean (*Vicia faba*) to bean yellow mosaic virus. Plant Disease 68: 109-111.

Sixty-eight open-pollinated faba bean selections resistant to bean yellow mosaic virus (BYMV) in preliminary field experiments were evaluated further in the field from 1978 through 1981. The selections were inoculated with the "mosaic" strain of BYMV mechanically and by exposure to viruliferous pea aphids (*Acyrthosiphon pisum*) in separate experiments. Most selections were heterogeneous for disease reaction. Repeated testing and reselection increased the number of symptomless plants in the progeny of accessions 2N138, 2N23, 2N295, and 2N85. The number of resistant (symptomless and mild mosaic) plants also increased in the progeny of accessions 2N101, 2N65, and 2N425. One hundred twenty-five selections from the 1978 field trials were evaluated further and self-pollinated in the greenhouse in 1978 and 1979; inbred lines from accessions 2N138, 2N295, 2N23, 2N65, 2N76, and 2N2 were symptomless. All plants of the inbred line from accession 2N138 remained symptomless in aphid-inoculated field trials. Five additional lines had 70–90% symptomless plants. When superior selections and inbred lines were inoculated with the "necrotic" strain of BYMV, only accession 2N138 was immune under field and greenhouse conditions. In addition, many selections and inbred lines were heterogeneous with a few plants resistant to both virus strains.

Bean yellow mosaic virus (BYMV) is a common and destructive pathogen of faba bean (broad bean) (*Vicia faba* L.) in many areas of the world (5,9). The disease has been observed frequently in the province of Manitoba since faba bean was introduced in 1970 (4). Yield losses as high as 96.3% have occurred as a result of severe early infections in faba bean (7). In Manitoba, transmission of BYMV in seed has not been demonstrated and the virus is principally transmitted from perennial forage legumes (clovers and vetches) to faba beans by pea aphids (*Acyrthosiphon pisum* Harris) (7).

Resistance, when available, is the most efficient means of control. However, earlier attempts by Nitzany and Cohen (11), Kaiser et al (10), and Fiedorow (6) to find resistance to BYMV in faba bean were not successful. Resistance to BYMV has been found and used successfully in pea (3,8,16,17,19), in bean (1,2,14), in cowpea (15), and in soybean (13).

In view of the desirability of developing faba bean cultivars resistant to BYMV, an evaluation of the faba bean germ plasm collection at the University of Manitoba was made in the field from 1975 through 1977. About 360 accessions from different regions of the world were exposed in the field to natural infestations of BYMV-viruliferous pea aphids (C. C. Bernier, *unpublished*). Although none of the accessions appeared uniformly resistant to the virus, 68 accessions had several plants free of symptoms or with mild mosaic only. Faba bean is partially cross-pollinated and cultivars and lines are restricted populations of several genotypes (12,18). Because incidence of BYMV throughout the experimental plots was high and spread was excellent among susceptible cultivars spaced throughout the trial in each year, variability in disease reactions within accessions were probably due largely to genetic resistance. However, some of the variability may also have been caused by preferential feeding by aphids, uneven dispersal of aphids throughout the plots, or time of infection.

In this study, accessions previously identified as having some resistant plants (symptomless or mild mosaic) were subjected to more rigorous evaluation and selection under conditions of mechanical and aphid inoculation in the field followed by further testing and selfing of progeny in the greenhouse. Progeny from superior selections from the greenhouse were subsequently evaluated in the field to determine if greenhouse results correlated with the field results.

Contribution 640, University of Manitoba, Winnipeg, Canada.

Accepted for publication 7 August 1983.

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MATERIALS AND METHODS

Field evaluation. During 1978, 68 accessions and selections resistant in preliminary experiments at Winnipeg were evaluated in separate trials in which they were mechanically inoculated and exposed to viruliferous pea aphids. Because of limited seed quantities, single-row nonreplicated plots were used except for 20 accessions, which were replicated three times in a randomized complete block design in the mechanical inoculation test. Fifty-two resistant single-plant and bulk selections were made and the progeny evaluated again in 1979, when they were mechanically inoculated only. Twenty-two selections with sufficient seed were replicated twice. Selected progeny from the 1979 field trials were retested in 1980 and 1981 along with some of the superior inbred lines developed in the greenhouse during 1978 and 1979. Planting in all years was done by the end of May or early June at the University of Manitoba. Plots were located away from areas where natural infection was known to occur and surrounded by plots of cereal or rapeseed. Fifteen to 20 seeds per selection were planted in single-row plots 1.5 m long and 60 cm apart. In tests where aphids were used to inoculate the plants, two rows of the virus-susceptible cultivar Diana were planted at the ends of each plot to act as virus-spreader rows.

Greenhouse evaluation. Progeny from 125 selections made from the 1978 field trials for symptomless plants or plants showing only mild symptoms were tested and reselected at 27 C in 1978 and 1979. Five to 10 scarified seeds per selection were planted in clay pots 12 cm in diameter using a 2:1:1 (v/v) mixture of soil, sand, and peat moss. Fertilizer and insecticides were applied as necessary to ensure good growth and seed yield and freedom from aphids and mites. Selected plants were moved to controlled-environment rooms with a diurnal temperature of 21–15 C and a 16-hr photoperiod and allowed to self-pollinate.

Virus strains, inoculum preparation, and method of inoculation. Mild strain "M" and a severe strain "S" of BYMV used by Frowd and Bernier (7) (hereafter referred to as "mosaic" and "necrotic" strains, respectively) were used in testing field and greenhouse materials. Symptoms caused by the mosaic strain ranged from veinclearing, leaf mottling, mosaic, and bronzing to severe stunting, stem necrosis, and premature pod splitting in more susceptible plants. The necrotic strain caused severe mosaic, numerous minute dull brown necrotic spots in young leaves leading to tip or vascular necrosis, and rapid death of young plants. The mosaic strain is more common in the province of Manitoba and was used in all tests, whereas the necrotic strain was used to evaluate only the superior selections

tested in the 1979 greenhouse and 1980 field trials.

For inoculum preparation, young BYMV-infected leaves of greenhouse-grown plants of the cultivar Diana were harvested 6–8 and 10–14 days after inoculation with necrotic or mosaic strain, respectively. Leaves were ground in 0.1 M phosphate buffer pH 7.4 (1:10, w/v) and the sap was filtered through two to four layers of cheesecloth and mixed with Carborundum (600-mesh) at 2% (w/v).

In the 1978 field trials, accessions were mechanically inoculated by rubbing freshly prepared inoculum on four young leaves of 1-mo-old test plants with a cotton swab or pipe cleaner. Leaves were washed with water immediately after inoculation. In 1978 and 1981, selections were exposed to BYMV-infective aphids by releasing 10 to 12 pea aphids (*A. pisum*) on each plant of the cultivar Diana in the spreader rows. Viruliferous aphids were reared on caged BYMV-infected plants of the susceptible cultivar Diana in the greenhouse. Aphids were released two or three times to ensure rapid buildup of large aphid populations on the spreader rows.

In 1979 and 1980, faba bean selections were mechanically inoculated in both field and greenhouse tests with an air-brush at a pressure of 276 kPa (40 psi). In all greenhouse experiments, plants were mechanically inoculated at the four- to six-leaf stage and kept at 27 C until symptoms appeared.

Disease assessment. Rating scales from 0 to 4 were developed to assess individual inoculated plants in the field and greenhouse separately for symptoms of mosaic, necrosis, and stunting, where 0 = no symptoms and 4 = severe symptoms. Evaluation of plants for resistance was made on the basis of host reactions to all symptom types. Plants with a 0 rating in all three symptom types were termed highly resistant (HR) and plants with a maximum rating of 1 in any of the three types were termed moderately resistant (MR). Plants with a rating of 0 or 1 were selected for further studies. Plants with a rating of 2 or more in any one type were considered susceptible and progeny were not advanced. In all tests, selected symptomless plants were assayed for the presence of virus by inoculating susceptible Diana plants in the greenhouse at 27 C with sap from test plants extracted in 0.1 M phosphate buffer, pH 7.4 (1:10, w/v). Host reactions were termed immune if virus was not recovered.

RESULTS

In general, selections used as susceptible controls developed severe symptoms of the disease in all tests in all years. Accessions, including those selected previously for resistance, expressed variable symptoms.

In the 1978 field tests, most selections and accessions were heterogeneous for disease reactions. Symptomless plants were observed in very few selections, but plants with mild mosaic symptoms were observed in many selections. The number of symptomless plants was greater in accessions previously selected as symptomless (2N425-3, 2N101-3, 2N4-1, and 2N44-1) than in those not previously selected (2N425-1, 2N101-1, and 2N101-2) or those selected on the basis of mild symptoms (2N425-2, 2N101-4, 2N4-2, and 2N44-2). BYMV was not detected in symptomless plants of eight of the 27 accessions assayed. Thus, plants immune to BYMV were identified in selections 2N43-2, 2N138-1, 2N85-1, 2N1-2, 2N65-1, 2N23-3, 2N101-3, and 2N425-1. Most of the accessions reacted similarly in the mechanically inoculated and aphid-inoculated trials. However, progeny from a few accessions, such as 2N138-1, 2N40-3, 2N5-1, 2N101-3, 2N63-1, and 2N76-2, showed a higher percentage of resistant plants in the aphid test than in the mechanically inoculated test, whereas accessions such as 2N23-1, 2N40-2, 2N85-2, 2N4-1, 2N44-1, and 2N78-1 had more resistant plants in the mechanically inoculated test than in the aphid test. To avoid selecting plants that might have escaped infection in the aphid-inoculated tests, most of the 150 single or bulk plant resistant selections (0–1 rating) were made in the mechanical inoculation test.

Considerable variation existed again in the 1979 field tests among and within accessions for disease reactions although many selections had more symptomless plants than in the previous year. Thus, selections 2N43-2, 2N85-1, 2N101-1, 2N295-2, and 2N138-1, which had less than 6.9% symptomless plants in 1978, had 20, 35.7, 44.7, 36.4, and 77.8% symptomless plants, respectively, in 1979.

Progeny from 25 superior selections were retested in 1980 and progeny of 10 of these retested in 1981 by aphid inoculation. To illustrate the efficiency of selection, data for 10 accessions that were reselected in each of the 4 yr of field testing (1978 through 1981) are summarized in Table 1. Only the HR or MR plants of each accession (converted to the percentage values) are presented. Progress in terms of obtaining uniformly symptomless selections was more apparent in selections from 2N23, 2N85, 2N138, and 2N295, although selections from 2N101, 2N65, and 2N425 also had considerably more resistant (HR and MR) plants in 1980 and 1981 than in the previous 2 yr. The selection from 2N43 had fewer resistant plants in 1981 than in the previous 2 yr. In reviewing the performance of accession 2N43, we found that this accession had been more sensitive to infection by aphids than to mechanical inoculation in 1978. This may explain the sharp decline in number of resistant plants in this accession in 1981, when accessions were exposed to viruliferous aphids, than when mechanically inoculated.

By 1981, all plants in the progeny of the field selection from accession 2N138 were symptomless (Table 1). In addition, selections from 2N23, 2N101, and 2N295 had more than 70% symptomless plants in 1981 compared with fewer than 10% in 1978. Most of the selections, however, were still heterogeneous after 4 yr of selection. Differential response of accessions to cross-pollination and aphid infestation might account for the differences in obtaining uniformly resistant selections. Most of the symptom-

Table 1. Effectiveness of selection for resistance to the mosaic strain of bean yellow mosaic virus in the progeny of 10 faba bean accessions evaluated in the field from 1978 through 1981

Accession	Country of origin	Disease reaction[a]	Percent plants resistant			
			1978[b]	1979[c]	1980	1981
2N23	France	HR	6.7	2.5	66.7	73.3
		MR	26.7	37.5	33.3	20.0
2N43	Afghanistan	HR	4.9	20.0	55.6	0.0
		MR	7.3	52.0	27.8	36.4
2N85	Germany	HR	10.8	35.7	57.1	61.5
		MR	32.4	28.6	28.6	23.1
2N101	Czechoslovakia	HR	2.6	33.3	85.7	76.9
		MR	20.5	16.7	14.3	15.4
2N138	Czechoslovakia	HR	6.9	77.7	94.1	100.0
		MR	6.9	5.6	5.9	0.0
2N65	USSR	HR	6.7	37.9	30.4	35.3
		MR	20.0	24.2	52.2	47.1
2N295	USSR	HR	0.0	34.6	25.0	85.7
		MR	57.2	42.3	68.8	14.3
2N425	Egypt	HR	0.0	16.7	16.7	61.5
		MR	17.7	33.3	33.3	30.8
2N53	Syria	HR	0.0	4.0	0.0	5.9
		MR	0.0	0.0	0.0	5.9
Diana	Germany	HR	0.0	14.3	0.0	10.0
		MR	0.0	21.4	0.0	10.0

[a] HR = Highly resistant with a maximum of 0 rating and MR = moderately resistant with a maximum of 1 rating in any of the symptom types. Disease reaction scale 0–4: 0 = no symptoms, 4 = severe symptoms.
[b] Based on three replicates.
[c] Based on two replicates.

less plants observed in 1979 through 1981 were found to have latent infections to BYMV when assayed on susceptible Diana plants. However, symptomless selections from 2N138, 2N4, 2N23, and 2N425 were virus-free and considered immune.

Progeny of 125 single and bulk plant selections from the field test in 1978 were tested in the greenhouse and 55 superior, self-pollinated selections were retested in 1979. One inbred line from each of accessions 2N138, 2N295, and 2N23 remained symptomless in both years, 35 days after inoculation with the mosaic strain. Progeny from these three accessions also had the highest percentage of symptomless plants in the field program (Table 1). In 1979, inbred lines from three additional accessions, 2N65 from the USSR and 2N76 and 2N2 from the United States, were also symptomless. Five inbred lines had all plants moderately resistant and 12 inbred lines were heterogeneous for disease reactions. Most of the HR inbred lines and field selections were of European origin (Table 1).

In 1981, 36 inbred lines were tested in the field to the mosaic strain by aphid inoculation. All plants of the inbred line from accession 2N138 remained symptomless. Five additional inbred lines had 70–90% symptomless plants. The other lines were still heterogeneous after two cycles of self-pollination. The two susceptible lines in the test had fewer than 10% symptomless plants.

Eighteen superior selections from the field testing program and 22 S_2 inbred lines from the greenhouse program were evaluated against the necrotic strain in the field and greenhouse, respectively. Progeny from the open-pollinated selection 2N138-1-1-1 and the S_2 inbred line 2N138-1GH-3-5 were immune to BYMV in the field and greenhouse, respectively, whereas the progeny from another open-pollinated selection, 2N138-1-1-2, had only mild symptoms in the field. Many of the lines were again heterogeneous with only a few resistant plants. Some selections from accessions 2N23, 2N295, 2N65, and 2N85 with resistance to the mosaic strain were found to be highly susceptible to the necrotic strain in the field and greenhouse tests.

DISCUSSION

The procedure for testing and selecting single plants for resistance (symptomless or mild mosaic) to BYMV was effective in the field and more so in the greenhouse, where plants were self-pollinated. Selection for genotypes that developed severe mosaic and stunting without stem or vascular necrosis in response to BYMV infection was also effective. Selection efficiency was high in accessions 2N23, 2N85, 2N101, 2N138, and 2N295 and resulted in greater uniformity and an increasing number of symptomless plants over 4 yr of testing (Table 1). In the other accessions, the number of symptomless plants increased only slightly or not at all although the number of resistant (symptomless and mild mosaic) plants increased with each succeeding year. Open pollination or genetic linkage might explain the lack of progress in these accessions. With the exception of selections from 2N138, the selections were still heterogeneous at the end of 4 yr. In comparison, 2 yr of greenhouse selection resulted in 11 lines that were uniformly resistant to the mosaic strain. These inbred lines remained resistant when tested in the field by aphid inoculation.

Although the emphasis was to identify lines resistant to the mosaic strain, the selected genotypes were also inoculated with the less common but more severe necrotic strain of BYMV. Only the selections from accession 2N138 were found to be uniformly resistant to this strain. Several additional selections were heterogeneous mixtures with a few resistant plants, which could be useful for further selection. Because one selection was resistant to both strains of BYMV, whereas other selections were resistant to the mosaic strain and not to the necrotic strain, resistance to each strain is probably controlled by a different factor(s).

It will be of interest to test the BYMV-resistant faba bean selections in other countries where BYMV occurs to observe whether the resistance will hold.

ACKNOWLEDGMENTS

The first author was supported by a Government of India scholarship. We wish to thank Richard Smith and Lorraine Harrison for technical assistance.

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