New Sources of Resistance in *Vicia faba* to Chocolate Spot Caused by *Botrytis fabae*

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ABSTRACT

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A two-cycle screening technique was used to evaluate 253 faba bean germ plasm accessions for resistance to chocolate spot. In the first cycle in 1985, faba bean accessions were exposed to a mixture of 20 isolates of *Botrytis fabae* collected from a wide range of naturally infected leaves of local susceptible cultivars grown in major faba bean production regions in Syria. Some of the resistant accessions detected in 1985 developed a few coalesced sporulating lesions. To subject resistant materials to a rigorous evaluation, 20 isolates of *B. fabae* obtained from such lesions were used in a second screening cycle in 1986 to inoculate progenies of the resistant accessions detected the previous season. Of the 253 accessions, 14 new, potentially useful sources of resistance to a wide range of isolates of the pathogen were identified.

Faba bean (*Vicia faba* L.) is grown worldwide on approximately 3.6 million ha, with a total production of about 4 million tons annually (5). The great majority of people in the low and middle income classes in Asia, Latin America, and the Mediterranean and Nile Valley regions depend on faba bean as a major source of proteinaceous food (5). In other parts of the world, however, faba bean is used mainly as an animal feed. Although the crop is attacked by a wide range of pathogens, it is widely accepted that chocolate spot, caused by *Botrytis fabae* Sard., is one of the most destructive diseases, sometimes causing complete crop failure (6,8,11,13,17). Unfortunately, attempts in the past to identify useful sources of resistance to chocolate spot (2,4,16) resulted in the detection of only a few genes that were not effective enough to develop acceptable disease-resistant cultivars (4). This is probably due to the systems of variations (3,9,12) by which species of *Botrytis* are generally able to overcome host defense mechanisms (10).

Although a few useful sources of resistance to the four common races of *B. fabae* in the Middle East have recently been identified at the International Center for Agricultural Research in the Dry Areas (ICARDA) (7), there is no guarantee that new races will not appear that could render the present genes for resistance less effective.

The objective of this work was to identify new sources of resistance to chocolate spot from the faba bean germ plasm collection of ICARDA.

MATERIALS AND METHODS

Isolation and inoculum production. Twenty isolates of *B. fabae* cultivars were obtained from a wide range of naturally infected leaves of local susceptible cultivars grown in major production regions in Syria. The leaves were surface-disinfested with 10% sodium hypochlorite solution for 2 min, plated on faba bean dextrose agar (extracts of 200 g of faba bean seed, 20 g of dextrose, and 18 g of agar), incubated at room temperature (20 ± 2 C) for 5 days, then subcultured until pure colonies of *B. fabae* were obtained. Colonies were exposed to three cycles of 12 hr of darkness/12 hr ultraviolet light to induce sporulation (17), then propagated at room temperature. Equal amounts of whole cultures were placed together in a Waring Blendor, blended for 2 min, and passed through two layers of cheesecloth. Leachates were then diluted with tap water until 600,000 spores per milliliter were obtained.

Field evaluations. Faba bean germ plasm accessions were evaluated for resistance to *B. fabae* in the field in 1985 and 1986 at ICARDA's substation near Latakia, in northern Syria. In October of each year, 20 seeds of each accession were planted under insectproof cages, in single rows 2 m long and 50 cm apart, with the local susceptible cultivar ILB 1814 repeated as a standard susceptible check every four test entries. A new two-cycle screening technique was employed (7). In the first cycle in 1985, 253 accessions were evaluated using a mixture of spores of the 20 isolates of *B. fabae* prepared as described. The inoculum was applied with a high-volume sprayer in the evening (1800–2000 hours) to the foliage of 8-wk-old plants at an average of 23 ml per plant. Inoculated plants were then sprinkled with a fine mist of water three times a day, 2 hr each time, until the susceptible local cultivar developed severe chocolate spot symptoms.

Some of the resistant accessions detected in the first cycle developed a few coalesced, sporulating lesions. These lesions were believed to have been induced by highly virulent forms (7,9,15) that seemed to occur at low frequencies in local populations. To subject resistant material to a more rigorous evaluation, spores of 20 isolates of *B. fabae* obtained from such lesions were mixed in equal proportions and applied in a second screening cycle, in 1986, to progenies of the 53 resistant accessions detected the previous season using the same procedures as in the first cycle. The mixed inocula used in the first and second screening cycles were designated IA and IB, respectively. Disease severity in both cycles was recorded 3 wk after inoculation as follows: 1 = no disease symptoms or very few lesions covering up to 1% of the leaf surface; 3 = a few small, discrete lesions covering 1.1–2% of the leaf surface; 5 = lesions common, some coalesced, covering 2.1–5% of the leaf surface and poor sporulation; 7 = large, coalesced sporulating lesions covering 5.1–10% of the leaf surface, some defoliation, and intermediate sporulation;

Fig. 1. (Left) Faba bean accession ILB 3026 highly resistant to chocolate spot compared with (right) susceptible cultivar ILB 1814 3 wk after inoculation in the field.

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and 9 = extensive lesions on leaves covering more than 10% of the leaf surface, severe defoliation, abundant sporulation, stem girdling, and death of most plants.

Laboratory evaluations. A modification of the detached-leaf technique (7,9) was used in two tests. The first test was conducted to determine reactions of the 53 accessions to the mixed inocula IA and IB. Fully expanded leaflets of a similar age were detached from the fifth node position of healthy plants of the 53 accessions. Similar leaflets were detached from the local susceptible cultivar ILB 1814. These leaflets were laid flat on 2-cm-thick moist sponge lining the bottoms of 90 × 40 × 5 cm galvanized metal pans, then inoculated separately with *B. fabae* inocula IA and IB. An Eppendorf digital pipette (Brinkman Instruments, Westbury, NY) was used to deposit 0.1-ml droplets of a suspension containing 600,000 spores of *B. fabae* per milliliter. One droplet was placed on each half of the upper laminal surface of each leaflet, then the pans were covered immediately and incubated at room temperature (20 ± 2 C). The second laboratory test was conducted to study differences in the frequency distribution of virulence between inocula IA of the first screening cycle and IB of the second. Fully expanded leaflets were detached from the fifth node position of healthy plants of the local susceptible cultivar ILB 1814. These leaflets were inoculated separately with each of the 20 isolates of inocula IA and IB, employing the same methods described for the first laboratory test. These tests were repeated twice, with treatments replicated three times. Disease readings were made 5 days after inoculation in both tests.

RESULTS AND DISCUSSION

Of the 253 accessions tested in the first screening cycle in the field, 53 were rated resistant (Table 1) and 200, susceptible. Of the 53 accessions identified in the first cycle and retested more rigorously in the second cycle, 14 remained resistant (Fig. 1) and 39 were rated susceptible. Chocolate spot scores on plants of the local susceptible cultivar, ILB 1814, ranged between 7 and 9 in both cycles, indicating a uniform disease distribution pattern throughout these tests. These results were consistent with those of the first laboratory test (Table 1). The 53 accessions rated resistant to inoculum IA in the first screening cycle in the field were also rated resistant to inoculum IA in the laboratory. However, the 14 accessions rated resistant to inocula IA and IB in the field were also rated resistant to both inocula in the laboratory. The decrease in the number of resistant accessions in both field and laboratory tests from 53 with inoculum IA to only 14 with inoculum IB was apparently due to differences in the degree of virulence between the two inocula. This was shown in the second laboratory test. Inoculum IB, with greater frequency of highly virulent isolates of *B. fabae* (Fig. 2) and narrower variation for virulence (Table 2), induced a significantly greater level of necrosis on ILB 1814 than did inoculum IA. Therefore, the greater virulence of inoculum IB appeared to have enabled pathogenesis in the 39 accessions that were resistant to inoculum IA. Although inoculum IB was more efficient than inoculum IA, it still did not suppress resistance in any of the remaining 14 accessions that had also been rated resistant to inoculum IA. Therefore, the

Table 1. Reaction of faba bean accessions to chocolate spot in the field and in the detached leaf test in the laboratory

Entry number	Accession[a] (ILB number)	Reaction in field[b] 1985 IA	Reaction in field[b] 1986 IB	Reaction in laboratory[c] 1986 IA	Reaction in laboratory[c] 1986 IB
1	2282	R	R	HR	HR
2	3025	R	R	HR	HR
3	3026	R	R	HR	HR
4	3027	R	R	R	R
5	3028	R	R	R	R
6	3029	R	R	R	R
7	3030	R	R	R	R
8	3031	R	R	R	R
9	3033	R	R	R	R
10	3056	R	R	R	R
11	3079	R	R	R	R
12	3091	R	R	R	R
13	3104	R	R	R	R
14	3105	R	R	R	R
15	2268	R	S	R	S
16	2269	R	S	R	S
17	2302	R	S	R	S
18	2320	R	S	R	S
19	3034	R	S	R	S
20	3035	R	S	R	S
21	3036	R	S	R	S
22	3037	R	S	R	S
23	3038	R	S	R	S
24	3047	R	S	R	S
25	3057	R	S	R	S
26	3058	R	S	R	S
27	3059	R	S	R	S
28	3060	R	S	R	S
29	3063	R	S	R	S
30	3064	R	S	R	S
31	3066	R	S	R	S
32	3068	R	S	R	S
33	3069	R	S	R	S
34	3070	R	S	R	S
35	3071	R	S	R	S
36	3072	R	S	R	S
37	3073	R	S	R	S
38	3074	R	S	R	S
39	3075	R	S	R	S
40	3076	R	S	R	S
41	3077	R	S	R	S
42	3078	R	S	R	S
43	3085	R	S	R	S
44	3087	R	S	R	S
45	3089	R	S	R	S
46	3093	R	S	R	S
47	3094	R	S	R	S
48	3095	R	S	R	S
49	3098	R	S	R	S
50	3101	R	S	R	S
51	3106	R	S	R	HS
52	3107	R	S	R	HS
53	3111	R	S	R	HS
54	1814	S	S	S	HS

[a] Accessions originated from Colombia except the local susceptible cultivar ILB 1814.
[b] Resistance (R) denotes 1, 3, or 5 and susceptibility (S) denotes 7 or 9 on the 1–9 disease rating scale (see text).
[c] HR = highly resistant (1–25% necrosis), R = resistant (26–50% necrosis, very poor sporulation), S = susceptible (51–75% necrosis, intermediate sporulation), and HS = highly susceptible (76–100% necrosis, abundant sporulation).

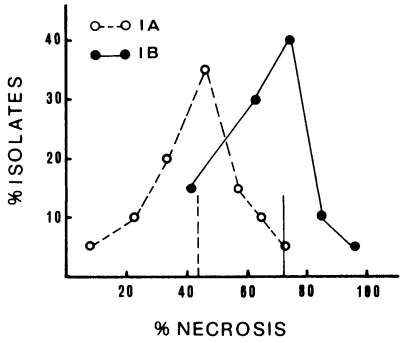

Fig. 2. Frequency distribution of virulence among isolates of *Botrytis fabae* in the mixed inocula IA of the first screening cycle and IB of the second on faba bean line ILB 1814. Means, standard deviations, ranges, and variances for these curves are shown in Table 2.

Table 2. Distribution of virulence among isolates of *Botrytis fabae* of inoculum IA of the first and IB of the second screening cycle on leaves detached from faba bean cultivar ILB 1814 in the laboratory

	Disease severity (% necrosis)[a]			
Inoculum	Mean[b]	SD	Range about mean ($P = 0.01$)	Variance
IA	44.66	16.36	28.30–61.02	267.68
IB	71.16	11.62	59.54–82.78	135.00

[a]0% = No necrosis on leaves, 100% = all of leaf tissue necrotic.
[b]Two means are significantly different at $P = 0.01$ ($t = 10.23$).

two-cycle screening technique separated the 39 accessions with resistance to inoculum IA only from the 14 accessions with resistance to both inocula. The genes carried by accessions in the second group are presumed to confer a broader-based resistance than those carried by accessions in the first group.

These findings are in very close agreement with those reported earlier on the application of the two-cycle screening technique to *B. fabae* (7). The results also agree with those reported by other workers on other host-pathogen systems who indicated that mixtures with a wide range of variation for virulence tend to confound specific with general resistance (14) and that the use of virulent pathogen forms for screening the most resistant individuals helps to detect new sources of horizontal resistance (1).

This study identified new sources of resistance to *B. fabae* that should be useful in stabilizing faba bean production in areas where chocolate spot is serious. These accessions are being grown to produce enough seeds for international testing in different geographic regions around the world and also to provide seeds for breeding programs in the future.

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