Evaluation of Fava Bean for Resistance to *Ascochyta fabae* and Development of Host Differentials for Race Identification

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ABSTRACT

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A total of 372 populations of fava bean (*Vicia faba*) were evaluated for resistance to *Ascochyta fabae* under field and growth room conditions. In the field, fava bean populations varied considerably in reaction to two isolates of *A. fabae*. After three cycles of testing and mass selection, the level of heterogeneity was reduced, but no population was homogeneous for resistance. A total of 18 and 11 populations were identified with >80% plants resistant to isolates A and Y^1, respectively, and two populations had >80% plants resistant to both isolates. The level of homogeneity was improved with continuous selfing in the growth room. After four cycles of testing and selfing, seven and eight inbred lines (S_4) were homogeneous for resistance to isolates A and Y^1, respectively, and five were homogeneous to both isolates. Eight inbred lines differentiated 10 isolates of *A. fabae* into seven races.

Ascochyta blight, caused by *Ascochyta fabae* Speg., is a common and occasionally destructive disease of fava beans (*Vicia faba* L.) in the Middle East (11), Europe (4,5), and New Zealand (8). The fungus infects all aboveground plant parts, including the seed (9,12). The main sources of inoculum are infected seed (8,9) and infested stubble (23). Yield losses of 35–40% are common (4,8,9), and losses as high as 90% have been reported (10). In Manitoba, the disease was observed soon after the introduction of fava bean in the 1970s (2,3,14), and all commercial cultivars showed varying degrees of susceptibility (3,15). *A. fabae* did not survive from one season to the next when infected plant debris was plowed down (17) but did survive when such debris was left on the soil surface in the field (5,23). The use of certified seed and crop rotation in Manitoba has been effective in keeping the disease incidence at low levels. In dry areas, where fava beans are often grown in short rotations, these measures are not as effective because the crop debris is usually not degraded between seasons.

Attempts to control Ascochyta blight by treating seed with fungicides have not been completely effective (3,8,10,14,22). There are no completely resistant cultivars, but variations in degree of resistance have been reported on fava beans in naturally infected plants (5) and in artificially inoculated plants (13,15,17). Variability among isolates of *A. fabae* has not been investigated extensively. Single-conidium isolates of *A. fabae* from seed of fava bean cultivars obtained from various regions of the world, then evaluated in the field in western Canada, were classified into 10 groups based on morphological characteristics in culture (15). Differences in virulence among isolates of *A. fabae* from Poland were also recognized (7). Recently, resistance was found among a germ plasm collection of fava bean lines, and eight isolates of *A. fabae* were classified into four races (11).

Fava bean is a partially cross-pollinated crop, and individual cultivars were found to include several genotypes that differ in reaction to any given isolate of *A. fabae* (15) or the rust fungus *Uromyces viciae-fabae* (Pers.) Schroet. (19). Fava bean lines with homogeneous reaction to rust were developed after three cycles of testing and selfing and then were used in genetic analysis (19,21). Selfing, however, may reduce vigor and interfere with selection of agronomic traits (K. Y. Rashid, *unpublished*).

The objectives of this study were to identify fava bean populations with resistance to isolates A and Y^1 of *A. fabae* in the field and to develop inbred lines that can be used as a set of host differentials for race identification. Preliminary results have been reported (20).

MATERIALS AND METHODS

Field evaluation. A total of 300 fava bean open-pollinated and mass-selected populations were tested for resistance to isolate A of *A. fabae* at the University of Manitoba campus farm in 1981. In addition, 72 populations were tested for resistance to isolate Y^1 at a separate location. Each population was tested in a single 2.5-m row (25–30 plants) with rows 30 cm apart. Plants were inoculated 3 wk after emergence by applying a conidial suspension of the appropriate isolate in water (1×10^6 conidia per milliliter) with a pressure sprayer at the rate of 3–4 ml per plant. Inoculation was always done after sunset. Plots were irrigated before inoculation, and plants were enclosed in polyethylene overnight to maintain leaf wetness. Scoring for disease reaction was done 2 wk after inoculation on a scale of infection-type reaction of 0–5 on stems and leaves, where 0 = no infection, 1 = flecking or bronzing, 2 = localized lesions <2 mm in diameter with no pycnidia, 3 = localized lesions 2–3 mm in diameter with pycnidia, 4 = spreading lesions 3–5 mm in diameter with pycnidia, and 5 = coalescing lesions >5 mm in diameter with pycnidia. Only plants with scores of 0–2 were classed as resistant. To improve homogeneity for disease reaction, all susceptible plants were removed.

In 1982, 50 progenies from populations with resistance to isolates A or Y^1 were evaluated further by inoculating plants with the two isolates. Isolate A was applied first, and isolate Y^1 was applied on the new plant growth 8 days later. Plants were scored for reaction to each isolate 2 wk after inoculation. Again, all susceptible plants were removed to further improve homogeneity for resistance.

In 1983, 23 progenies from populations with resistance to isolate A or Y^1 were retested with A and Y^1 in separate trials. In another trial, 34 progenies were inoculated sequentially with Y^1 and A.

Growth room evaluation and development of inbred lines. Progenies from 35 single-plant selections with resistance to isolates A or Y^1 in Manitoba and 42 selections rated as resistant to *A. fabae* at the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria, were evaluated further in the growth room for reactions to isolates A and Y^1. Ten seeds from each selection were planted in 15-cm-diameter clay pots (five seeds per pot) using a 2:1:1 (v/v) mixture of soil, sand, and peat. When

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3 wk old, seedlings were inoculated as described above, placed in a mist chamber for 24 hr (16), then moved into a growth room under high-intensity fluorescent light (16 hr day, 380 $\mu E \cdot m^2 \cdot s^1$) and a night/day temperature of 15/20 C. Infection-type reactions were scored on stems and leaves 2 wk after inoculation. Individual plants with infection type 0, 1, or 2 were selected and selfed for seed increase and further progeny testing. Additional plants with good vigor and seed yield but susceptible to isolate A or Y^1 were also selected and selfed for further testing to other isolates of *A. fabae*. Four cycles of selfing and testing against each isolate were completed in order to obtain homogeneous inbred lines. For 3 yr, all inbred lines were evaluated for reactions under field conditions to 10 isolates of *A. fabae*: A, Y^1, B, C, D, E, X, X^1, Y, and Z (15).

Development of host differentials and race identification. A total of 31 S_4 inbred lines were tested individually for reaction to the 10 isolates under growth room conditions as described above. A representative set of eight inbred lines (S_5) was selected as differentials. Ten seedlings from each inbred line were sequentially inoculated with two isolates at 8-day intervals and scored for reaction to each isolate 2 wk after inoculation. Ten isolates were used on each of the eight inbred lines. This was repeated once, and to ensure that infection caused by the first isolate did not affect host reaction to the other isolate, each combination of line and two isolates was assessed in a third experiment by reversing the order in which the plants were inoculated with the two isolates.

Inoculum preparation. The 10 single-conidium isolates of *A. fabae* used in this study were recovered from seed of fava bean cultivars that had been introduced and evaluated in the field in western Canada (15). Inoculum was prepared and increased by flooding Difco potato-dextrose agar with conidial suspension in sterile water. Excess water was decanted and dishes were incubated at 18 C for 10 days. The dishes were flooded with sterile water, and conidia (pycnidiospores) were gently brushed off the surface of the agar and decanted into a container (15). The conidial concentration was adjusted to 1×10^6 conidia per milliliter.

RESULTS

Field evaluation. All populations expressed considerable variation in reactions to isolates A and Y^1 of *A. fabae*. Populations with >80% plants resistant to either isolate A or Y^1 were observed only after one or more cycles of testing and mass selection (Table 1). Many plants in the different populations were resistant and moderately resistant. Some plants had resistant stems and moderately susceptible leaves. Although three cycles of testing and mass selection further reduced the level of heterogeneity, no population was homogeneous for resistance (Table 1). By the third year of the study, 18 and 13 populations, respectively, showed >80% and 60–80% plants resistant (stems and leaves) to isolate A, and 11 and 13 populations, respectively, showed >80% and 60–80% plants resistant (stems and leaves) to isolate Y^1. Two and 10 populations, respectively, showed >80% and 60–80% plants resistant to both isolates.

Growth room evaluation and development of inbred lines. All S_1 and S_2 progenies from 77 single plant selections were heterogeneous in their reactions to either isolate A or Y^1, but by the fourth cycle of testing and selfing, a greater proportion of the progenies tested was homogeneous. Seven S_4 inbred lines (14013-2, 14427-2, 14588-1, 14989-2, 2-1, 5-5, and 7-5) were homogeneous for resistance to isolate A, eight lines (14399-1, 14475-1, 14986-4, 14998-1, 15041-2, 15067-1, 1-21, and 3-14) were homogeneous for resistance to isolate Y^1, and five lines (14434-2, 15025-2, 15035-1, BPL2485-3, and 9-1) were homogeneous for resistance to both isolates.

Additional S_4 inbred lines were identified with resistance to one of the 10 isolates of *A. fabae*: 28 lines to A, 19 lines to B, 16 lines to C, 7 lines to D, 7 lines to E, 18 lines to X, 13 lines to X^1, 18 lines to Y, 42 lines to Y^1, and 15 lines to Z. Furthermore, three lines (15025-2, BPL2485, and 14434-2) were resistant to all 10 isolates, and one line (15035-1) was resistant to nine. Many lines were resistant to at least four isolates. When evaluated in the field, all inbred lines were homogeneous and expressed infection-type reactions identical to their reactions observed under controlled growth room conditions.

Development of host differentials and race identification. The use of two isolates in sequence on the same plant did not have any pronounced effect on the infection-type reactions. Eight homozygous S_5 inbred lines with good vigor and seed yield, and representing groups of inbred lines with similar reaction types to a number of isolates, were chosen as host differentials. The eight inbred lines

Table 1. Fava bean populations with plants resistant to isolates A and Y^1 of *Ascochyta fabae* in 3 yr of field testing

Resistant plants[a] (%)	1981[b] A MSP	1981[b] A OPP	1981[b] Y^1 MSP	1981[b] Y^1 OPP	1982[c] A	1982[c] Y^1	1982[c] A & Y^1	1983[d] A	1983[d] Y^1	1983[c] A	1983[c] Y^1	1983[c] A & Y^1
80–100	11	0	5	0	3	20	3	5	6	13	5	2
60–79	12	3	4	3	10	14	5	4	3	9	10	10
40–59	14	17	7	3	7	11	7	7	7	7	16	9
20–39	15	59	9	7	13	2	11	3	5	4	2	10
1–19	4	66	2	6	14	2	14	3	0	1	0	2
0	9	90	0	26	3	1	10	1	1	0	1	11
Total	65	235	27	45	50	50	50	23	22	34	34	44

[a] Resistance was based on infection-type reaction (scored on a scale of 0–5). Plants with scores of 0–2 (0 = no infection, 1 = flecking or bronzing, 2 = localized lesions <2 mm in diameter with no pycnidia) were classed as resistant.
[b] Mass-selected populations (MSP) previously tested to *A. fabae* and open-pollinated populations (OPP) tested for the first time.
[c] Mass-selected populations sequentially tested to A and Y^1 in the same trial.
[d] Mass-selected populations tested to A and Y^1 in separate trials.

Table 2. Reactions of eight fava bean host differential lines and race differentiation in *Ascochyta fabae*

Inbred lines[a]	Isolate/race A/1	D/1	E/1	B/2	X/3	Y/3	C/4	X^1/5	Y^1/6	Z/7
15025-2	R[b]	R	R	R	R	R	R	R	R	R
15035-1	R	R	R	R	R	R	S	R	R	R
15041-2	R	R	R	R	R	R	S	R	R	S
ACK-1-21	S	S	S	R	R	R	R	R	R	R
ACK-1-9	S	S	S	R	R	R	R	R	S	S
ACK-2-2	S	S	S	R	R	R	R	S	R	S
ERF-3-14	S	S	S	R	S	S	S	S	S	S
HF-7-2	S	S	S	S	S	S	S	S	S	S

[a] Sources: 15025-2, unknown origin in Middle East; 15035-1, Ethiopia; 15041-2, ICARDA, Middle East; ACK-1-21, ACK-1-9, and ACK-2-2, cv. Ackerperle, Germany; ERF-3-14, cv. Erfordia, Germany; and HF-7-2, cv. Herz Freya, Germany.
[b] R = resistant, S = susceptible. Resistance was based on infection-type reaction: 0 = no infection, 1 = flecking or bronzing, 2 = localized lesions <2 mm in diameter with no pycnidia, 3 = localized lesions 2–3 mm in diameter with pycnidia, 4 = spreading lesions 3–5 mm in diameter with pycnidia, and 5 = coalescing lesions >5 mm in diameter with pycnidia. Plants with scores of 0–2 were classed as resistant.

used for race differentiation reacted uniformly and consistently to a given isolate pair in all tests. The scores for each line and isolate combination from the three tests were combined (Table 2).

Seven virulence groups are proposed for the 10 isolates (Table 2). The most virulent isolates (A, D, and E) can be grouped into race 1 and the least virulent (X and Y), into race 3. The remaining five isolates and races are: isolate B, race 2, isolate C, race 4; isolate X^1, race 5; isolate Y^1, race 6; and isolate Z, race 7.

DISCUSSION

The procedure for evaluating fava bean populations for resistance to *A. fabae* in the field was effective. Symptoms on susceptible cultivars were typical of those induced by natural infection. The use of infection-type reactions for rating allowed selection of resistant plants. Certain genotypes expressed resistant reactions (infection type 0–2) on stems only, suggesting that resistance in the leaves and stems may be under different genetic control in these genotypes. Mass selection was effective in improving the homogeneity of several populations for resistance to two isolates of the fungus. Similar results were obtained in selecting for rust resistance in heterogeneous fava bean cultivars and accessions (19). Since fava bean is a partially cross-pollinated crop, populations with 80–100% or 60–80% of plants resistant to *A. fabae* should prove useful as gene pools for population improvement through recurrent selection programs.

The number of populations with a high percentage of plants resistant to isolate A or to isolate Y^1, or to both, varied slightly from year to year. This may have been due to the influence of environmental differences on the infection process and disease development. The greater number of populations with >80% plants resistant to isolate Y^1 than to isolate A in 1982 vs. 1981 (Table 1) reflects the level of improvement obtained as a result of three cycles of mass selection. Many populations resistant to isolate Y^1 were the result of 3–4 yr of testing and selection, whereas populations with resistance to isolate A were the result of only 2 yr of testing and selection. This could also be due in part to the fact that isolate A is more virulent than isolate Y^1 (15).

The high number of populations (22 of 34) with >60% plants resistant to isolate A in the sequential inoculation in 1983 in comparison with the number of populations (nine of 23) with similar resistance in separate trials with individual isolates (Table 1) may be due in part to the fact that temperature was very high (20 C nights and 30 C days) at the time of inoculation with isolate A in 1983.

The variability in the field tests, both within and among fava bean populations, for reaction to individual isolates of *A. fabae* was also observed among single plant selections and their progenies in the growth room evaluation. However, four cycles of testing and selfing under controlled conditions were sufficient to allow identification of several lines that were homogeneous for resistance to one or more of the 10 isolates. The data suggest that resistance to *A. fabae* is race-specific. The fact that some lines were resistant to more than one isolate may be due to the presence of several genes for resistance in these lines (6,18). Genetic analyses of resistance to the specific isolates in many of the inbred lines developed in this study will be reported elsewhere.

Sequential inoculation proved effective in selection of inbred lines with resistance to two isolates, notwithstanding the slightly less severe reaction observed when some isolates were used second in the sequence.

The high degree of variability for virulence observed in the population of *A. fabae* in this study confirms previous reports on physiological specialization (15) and race differentiation (11). Isolates used in this study originated from different regions of the world (1,15), and this is probably why more races were differentiated than previously (11). These findings indicate that breeding programs, in their efforts to incorporate resistance to *A. fabae*, need to establish the race composition of the fungus in all areas where the resistant cultivars are to be used.

The set of eight inbred lines allowed differentiation of seven groups of isolates (races) based on infection-type reactions that could be readily classed as susceptible or resistant. This set of differentials should prove useful to national and international programs for preliminary screening and differentiation of races of *A. fabae* in regions of the world where the fungus is common.

The present study was conducted concurrently with that of Hanounik and Robertson (11), but the results cannot be compared. Although inbred lines 14434-2, 15035-1, and BPL2485 originated from the same open-pollinated accessions as those used in Syria, they were exposed to different isolates and could be carrying different genes for resistance. In view of the heterogeneity observed in fava bean populations evaluated in this study, further tests using inbred lines and common isolates are required to clarify the relationship among different races of *A. fabae*.

The infection-type reactions of the inbred lines with resistance under growth room conditions were identical to their reactions under a range of environmental conditions encountered over 3 yr of field evaluation. This indicates that fava bean germ plasm or segregating populations could be evaluated under both conditions.

A limited quantity of seed of the host differentials is available upon request from the University of Manitoba or from ICARDA.

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LITERATURE CITED

1. Ali, F. H. 1985. Evaluation of the components of resistance to *Ascochyta fabae* Speg. on faba beans (*Vicia faba* L.) and the effect of temperature on mycelial growth of ten isolates of *A. fabae*. M.Sc. thesis. University of Manitoba, Winnipeg, Canada. 89 pp.
2. Bernier, C. C. 1975. Diseases of pulse crops and their control. Pages 439-454 in: Oilseed and Pulse Crops in Western Canada—A Symposium. J. T. Harapiak, ed. Western Co-operative Fertilizers Ltd., Calgary, Alta., Canada.
3. Bernier, C. C. 1980. Fungicidal control of Ascochyta blight of faba beans. FABIS Newsl. 2:43.
4. Blotnicka, K. 1981. The most important fungus diseases of broad bean (*Vicia faba minor*) and their occurrence in Poland. (Abstr.). Faba Bean Abstr. Commonw. Agric. Bur. 1:3.
5. Bond, D. A., and Pope, M. 1980. *Ascochyta fabae* on winter beans (*Vicia faba*): Pathogen spread and variation in host resistance. Plant Pathol. 29:59-65.
6. Ellingboe, A. H. 1981. Changing concepts of host pathogen genetics. Annu. Rev. Phytopathol. 19:125-143.
7. Filipowicz, A. 1893. The pathogenicity of isolates of *Ascochyta fabae* Speg. on horse bean (*Vicia faba* L. var. *minor* Harz.). (Abstr.). Faba Bean Abstr. Commonw. Agric. Bur. 4:47.
8. Gaunt, R. E., and Liew, R. S. S. 1981. Control strategies for *Ascochyta fabae* in New Zealand field and broad bean crops. Seed Sci. Technol. 9:707-715.
9. Hampton, J. G. 1980. The significance of *Ascochyta fabae* in broad beans in the Manawatu, and methods for its control. N.Z. J. Exp. Agric. 8:305-308.
10. Hanounik, S. 1980. Effect of chemical treatments and host genotypes on disease severity/yield relationships of Ascochyta blight in faba beans. FABIS Newsl. 2:50.
11. Hanounik, S. B., and Robertson, L. D. 1989. Resistance in *Vicia faba* germ plasm to blight caused by *Ascochyta fabae*. Plant Dis. 73:202-205.
12. Hewett, P. D. 1973. The field behaviour of seed-borne *Ascochyta fabae* and disease control in field beans. Ann. Appl. Biol. 74:287-295.
13. Jellis, G. J., Lockwood, G., and Aubury, R. G. 1984. Resistance to Ascochyta blight (*Ascochyta fabae*) in a winter-hardy line of faba bean (*Vicia faba equina*). FABIS Newsl. 10:27-29.
14. Kharbanda, P. D., and Bernier, C. C. 1979. Effectiveness of seed and foliar application of fungicides to control Ascochyta blight of fababeans. Can. J. Plant Sci. 59:661-666.
15. Kharbanda, P. D., and Bernier, C. C. 1980. Cultural and pathogenic variability among isolates of *Ascochyta fabae*. Can. J. Plant. Pathol. 2:139-142.
16. Krupinsky, J. M., and Scharen, A. L. 1983. A high humidity incubation chamber for foliar pathogens. Plant Dis. 67:84-86.
17. Lockwood, G., Jellis, G. J., and Aubury, R. G. 1985. Genotypic influences on the incidence of infection by *Ascochyta fabae* in winter-hardy faba beans (*Vicia faba*). Plant Pathol. 34:341-346.
18. Nelson, R. R. 1978. Genetics of horizontal resistance to plant diseases. Annu. Rev. Phytopathol. 16:359-378.
19. Rashid, K. Y., and Bernier, C. C. 1984. Evaluation of resistance in *Vicia faba* to two

isolates of the rust fungus *Uromyces viciae-fabae* from Manitoba. Plant Dis. 67:16-18.
20. Rashid, K. Y., and Bernier, C. C. 1985. Race identification in *Ascochyta fabae*. (Abstr.). Can. J. Plant Pathol. 7:448.
21. Rashid, K. Y., and Bernier, C. C. 1986. The genetics of resistance in *Vicia faba* to two races of *Uromyces viciae-fabae* from Manitoba. Can. J. Plant Pathol. 8:317-322.
22. Wallen, V. R., and Galway, D. A. 1977. Studies on the biology and control of *Ascochyta fabae* on faba bean. Can. Plant Dis. Surv. 57:31-35.
23. Yu, T. F. 1947. Ascochyta blight and leaf and pod spot of broad bean in China. Phytopathology 37:207-214.