Faba Bean (*Vicia faba* L.) Breeding for Resistance to Anthracnose (*Ascochyta fabae* Speg.) in the Czech Republic

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Abstract: In 2003–2005 faba bean accessions were evaluated in laboratory and field inoculation tests with a mixture of differently virulent isolates for susceptibility to anthracnose caused by the fungus *Ascochyta fabae* Speg. All tested commercial faba bean cultivars, both colour and white flowering, were found to be susceptible or highly susceptible. The highest level of resistance was found only in declared sources of resistance: 29H, L-8 and Petra. Segregation of F_2 plants derived from the cross of cultivar Merlin (susceptible, white flowering) with line Petra (resistant, colour flowering) was observed. The plants were moderately susceptible to highly susceptible. This finding indicates a multigenic character of resistance. Repeated selection cycles with the selection of resistant plants in F_2 and F_3 generations were performed. In the F_4 generation, colour flowering lines with a high level of resistance, nearly at the same level as in line Petra, were obtained. In comparison with the parental cultivar Merlin an increase in the resistance of selected white flowering lines was proved. Resistance will be increased/stabilized in further repeated selection cycles.

Keywords: faba bean; *Vicia faba*; *Ascochyta fabae*; anthracnose; sources of resistance; plant breeding

In addition to yield losses faba bean anthracnose causes serious problems in seed production due to the quality deterioration because of a high transfer of the pathogen to seeds (5–60%). Anthracnose causes the highest yield losses in *major* faba bean types (*Vicia faba* var. *major* Harz), Ti-forms (topless) and white flowering tannin-free faba bean types. The winter faba bean types are relatively more resistant than the spring ones (Breukelen 1985; Pritchard *et al*. 1989; Hanounik & Robertson 1989; Ondřej 1993).

Since 1980 permanent attention has been paid to searching for faba bean (*Vicia faba* L.) genetic sources of resistance to *Ascochyta fabae* Speg. (teleomorph *Didymella fabae* Jellis & Punithalingam) and to their utilization in resistance breeding in many countries: in England (Bond & Pope 1980; Jellis *et al*. 1984, 1991), Syria (Hanounik & Robertson 1989), Canada (Kharbanda & Bernier 1980; Rashid *et al*. 1991), France (Tivoli *et al*. 1986, 1992), Poland (Filipowicz 1983; Zakrzewska 1988), Spain (Avila *et al*. 2001; Silero *et al*. 2001) and in the Czech Republic (Ondřej 1990, 1993).

The existence of winter faba bean lines resistant to anthracnose was discovered in England (Bond & Pope 1980; Breukelen 1985; Jellis *et al*. 1984, 1991) and of *Vicia faba* var. *major* Harz in Syria (Hanounik & Robertson 1989). The resistant winter faba bean line 1B-18-1/3 was selected in England and used in breeding for winter faba bean resistance to anthracnose (cultivars Banner, Buldog and others). This line became the basis for the commercial cultivar Quasar. No spring faba bean

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type with resistance at the level of winter cultivars Banner and Quasar has been found. In inoculation tests with white flowering spring beans a relatively low degree of infestation was observed in cultivar Toret – harvested seed infestation in the range of 35–40% (Ondřej 1990, 1993).

Resistant lines of *Vicia faba* var. *major* were obtained in Syria. The lines BPL 471 and BPL 2485 were identified to be most resistant (Hanounik & Robertson 1989). These Syrian resistant lines from ICARDA were utilized in the Spanish breeding program to obtain resistant lines L-831818 and V-1220 (Rubiales 2006, personal communication).

In France, the selection of resistant sources of faba bean was targeted at the winter types. The selection resulted in line 29H. This line shows a high and stable resistance (at an immunity level) throughout the vegetation period. The high resistance of 29H was also proved in inoculation tests in Spain (Tivoli *et al*. 1986, 1992; Avila *et al*. 2001; Silero *et al*. 2001).

In the Czech Republic, a higher degree of resistance was confirmed in English winter faba bean cultivars Banner, Buldog and Qusar in inoculation tests in 1985–1991 (Ondřej 1990, 1993). The spring faba bean line SU-R5/13, originating from the hybrid population of Banner × SU-V3, was obtained after repeated selection cycles in 1986. The selection was carried out under an intense infection pressure of *A. fabae*. The line SU-R5/13 showed low yield losses (less than 5%) in comparison with the commercial spring faba bean cultivars (yield losses 30–70%). This line was selected repeatedly for resistance to co-occurring isolates of *A. fabae* originating from different growing localities (Rapotín, Chlumec nad Cidlinou and Temenice) in the Czech Republic. The obtained line named Petra differs from the line SU-R5/13 by its increased resistance to *A. fabae* and by a lower value of thousand seed weight (TSW). The line Petra has been utilized for the faba bean resistance breeding program. The faba bean resistance to *A. fabae* is partial (horizontal). There is a specific susceptibility range for each faba bean accession to the pathogen. The number of plants with improved resistance can gradually be increased by repeated selection cycles under an intense inoculation pressure (10^5–5×10^6 spores per ml). A certain optimal value of resistance can be attained merely by selection. It can contribute to the good shape, but it will not reach the necessary and high resistance level (Bond & Pope 1980; Kharbanda & Bernier 1980; Zakrzewska 1988, Ondřej 1990).

The isolates of *A. fabae* show virulence variability. From the highly resistant faba bean lines it is possible to isolate pathotypes with high and specific virulence overcoming the resistance of genetic sources. Repeated checks-up of the resistance level of genetic sources in field inoculation tests with various sets of isolates of *A. fabae* originating from the various growing localities are necessary and most desirable (Hanounik & Robertson 1989; Tivoli *et al*. 1992). By the application of RAPD marker technique the existence of 16 markers was detected that are associated with faba bean resistance to *A. fabae*. The markers were identified in 6 different linkage groups, most of them located on LG VIII on chromosome 3 (Avila *et al*. 2001).

The objectives of this research were: (1) to build up the collection of faba bean sources with declared resistance to *A. fabae*; (2) to evaluate and to compare the properties of resistance sources and to find out their suitability for breeding; (3) to extend genotypic and phenotypic diversity of resistance sources; (4) to evaluate the possibilities of increasing the resistance (or of obtaining the resistance) of white flowering faba bean types.

MATERIAL AND METHODS

Plant material

Resistant faba bean accessions originating from Spain (University of Cordoba): L-8 (*Vicia faba* var. *major* Harz); from France (INRA Rennes): 29H (winter faba bean, *Vicia faba* var. *minor* Harz) and from the Czech Republic: Petra (spring faba bean, *Vicia faba* var. *minor* Harz) and a selected set of control susceptible faba bean accessions from the collection of genetic resources (AGRITEC Šumperk) comprising Hobbit, Marcel, Baraca, Merlin, V-1085 and V-1117 were tested. The plants were evaluated individually, the acquired data were pooled and averaged.

Isolates and inoculum preparation

Isolates of *A. fabae* from localities Rapotín (RA), Chlumec nad Cidlinou (CH), Temenice (TE) and a reisolate from the line Petra (TE P/04) were used. The isolates of *A. fabae* (RA, CH, TE and TE/P04)

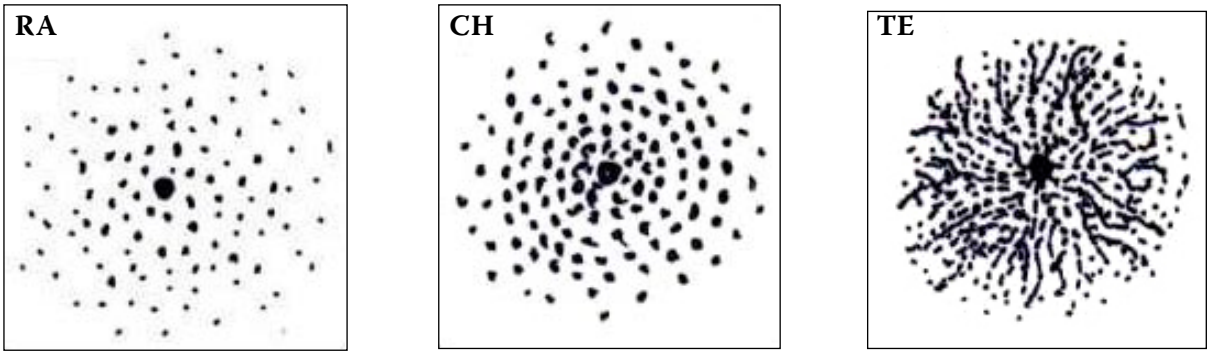

Figure 1. Pycnidium structure of *Ascochyta fabae* isolates on pea agar (RA – diffusional, CH – zonal, TE – radial); localities: RA – Rapotín, CH – Chlumec nad Cidlinou, TE – Šumperk-Temenice

differed in visual aspects of cultures, growth dynamics, medium colouring, pycnidium structure (zonal, radial, diffusional), pycnidium size and virulence (Figure 1, Table 1).

A. fabae was reisolated from infected seeds after harvesting. The reisolation revealed that all isolates used in the inoculation mixture participated in plant infection. The type CH (zonal pycnidium structure) was isolated from both susceptible accessions and resistant line at the same percentage (25–30%). The type RA (diffusional pycnidium structure) was mostly represented in susceptible accessions (70–80%). The more virulent types TE and TE/P04 (radial pycnidium structure) were isolated from the resistant line more often than from susceptible accessions (0–6%).

The inoculum was prepared according to Hanounik and Robertson (1989) by mixing pure cultures of the pathogen of the age 30 days that were grown on pea agar in Petri dishes (isolate mixture, each isolate was grown on 30 Petri dishes). The mixed suspension was diluted with water (titre 10^6 propagules/ml) for the total volume of 20 l of suspension for an area of 50 m^2.

Two inoculation methods were used for testing.

(a) Laboratory leaf drop test. Samples of leaves were taken from faba bean plants in the growth stage of flowering. The leaves were placed into Petri dishes on wet filter paper and injured by punctures (16–20 punctures per leaf). One drop of the inoculum was dropped on each leaf puncture. On day 4–6 after inoculation, the test was evaluated according to the average size of lesions. The degree of susceptibility corresponded to the size of lesions.

(b) Field test. Individual accessions were sown in 4 replications. Plants were inoculated by a suspension of *A. fabae* propagules at the flowering stage. A compressive hand sprayer was used for inoculation. The range scale 0–5 (0 = without symptoms, 1 = infection 1–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–89%, 5 = 90–100%) or percentage were used for the evaluation of plant and harvested seed susceptibility.

Breeding program

The line Petra – colour flowering (C), resistant (R) – and the cultivar Merlin – white flowering (W), highly susceptible (HS) – were crossed and

Table 1. Different characteristics of *Ascochyta fabae* isolates on pea agar

Ascochyta fabae isolate	Medium coloration	Pycnidium structure	Growth dynamics	Colony diameter on day 35 (cm)	Pycnidium size (mm)	Virulence	Necrosis size (mm)
RA	colourless	diffusional	increased	7.3	120–260	low	5.6
CH	fawn	zonal	medium	5.8	180–350	medium	6.2
TE	brown	radial	low	4.3	60–140	increased	8.7
TE/P04	swarthy	radial	low	4.0	60–140	increased	10.0

resistant progenies were selected in a two-stage procedure.

The first selection for resistance to *A. fabae* was carried out in the F_2 generation under field conditions. The plants with a score 1.0–2.5 (scale range 0–5) and with seed infection in the range of 0–15% were selected. The selection was repeated in the F_3 generation. The plants with a score lower than or equal to 2.0 and with seed infection in the range of 0–10% were selected. In the F_4 generation (2005) the source material and the hybrid population of F_2 and F_3 generations were compared.

Statistical evaluation

Field and *in vitro* trials were evaluated by analysis of variance and significance of simple contrasts with LSD test using the statistical program UNISTAT 4.53g (UNISTAT Ltd., London, UK) at $P > 0.05$ and $P > 0.01$ significance level.

RESULTS

The reactions of tested accessions to differently virulent isolates were evaluated under laboratory

Table 2. Evaluation of the susceptibility of faba bean accessions to isolates of *Ascochyta fabae* with different virulence (*in vitro*)

Line Cultivar	Isolate RA							Isolate CH						
	necrosis size (mm)					$P > 0.01$	$P > 0.05$	necrosis size (mm)					$P > 0.01$	$P > 0.05$
	observation				mean			observation				mean		
	1	2	3	4				1	2	3	4			
Petra	3.6	2.8	4.0	3.6	3.5	AB	BC	4.2	5.2	2.6	3.3	3.8	AB	ABC
L-8	2.8	2.5	3.5	3.5	3.1	AB	AB	3.2	3.0	2.8	4.0	3.3	AB	AB
29H	2.0	1.8	2.0	2.2	2.0	A	A	2.4	2.2	2.6	2.5	2.4	A	A
V 1117	4.3	5.0	3.6	4.4	4.3	B	BC	7.4	6.5	9.0	6.0	7.2	CD	D
V 1085	4.2	3.8	4.5	3.5	4.0	B	BC	4.5	3.6	5.2	4.8	4.5	AB	BC
Baraca	12.4	13.5	11.5	12.2	12.4	D	F	7.6	12.2	8.0	10.2	9.5	EF	E
Merlin	10.5	8.8	14.0	10.0	10.8	D	E	12.5	14.8	9.5	10.5	11.8	F	F
Marcel	7.4	5.2	7.7	6.5	6.7	C	D	7.0	10.2	7.0	11.0	8.8	DE	DE
Hobbit	4.8	3.8	5.2	4.2	4.5	B	C	4.8	4.5	6.6	4.2	5.0	BC	C
Line Cultivar	Isolate TE							Isolate TE/P04						
	necrosis size (mm)					$P > 0.01$	$P > 0.05$	necrosis size (mm)					$P > 0.01$	$P > 0.05$
	observation				mean			observation				mean		
	1	2	3	4				1	2	3	4			
Petra	5.2	5.6	3.8	4.6	4.8	A	A	5.0	4.5	5.6	6.0	5.3	AB	A
L-8	5.8	6.6	4.5	5.5	5.6	AB	A	5.0	7.0	5.2	4.0	5.3	AB	A
29H	3.8	2.5	4.0	3.0	3.3	A	A	2.9	3.6	3.8	3.5	3.5	A	A
V 1117	8.4	10.8	6.7	7.8	8.4	B	B	8.2	10.3	11.4	9.0	9.7	D	B
V 1085	8.4	9.5	7.8	8.8	8.6	B	B	9.4	12.0	10.5	8.4	10.1	D	B
Baraca	13.4	12.8	11.7	15.8	13.4	C	C	16.3	15.6	14.4	17.2	15.9	E	C
Merlin	14.2	15.0	12.3	16.2	14.4	C	C	17.4	16.2	15.8	16.1	16.4	E	C
Marcel	12.7	11.8	13.4	12.5	12.6	C	C	15.4	17.2	16.0	15.5	16.0	E	C
Hobbit	8.5	8.9	7.5	8.0	8.2	B	B	7.4	8.8	8.4	8.0	8.2	CD	B

Different letters indicate statistically different values at a $P > 0.05$ and $P > 0.01$ significance level according to Fisher LSD test

Table 3. Evaluation of the susceptibility of faba bean accessions to a mixture of isolates (RA, CH, TE, TE/P04) of *Ascochyta fabae* under field inoculation conditions; pooled data for individual plants in particular accessions; Šumperk 2005

Accession	Infection of leaves on day 20 after inoculation (0–5)*	Infection and destruction of growth apices (%)	Infection of pods (green maturity) (0–5)*	Infection of harvested seeds (%)
Petra	1.6	7.4	2.5	7.5
L-8	0.0	4.0	2.2	11.5
29H	0.0	5.3	2.0	4.2
V 1117	0.0	4.8	3.5	63.5
V 1085	1.2	15.5	3.8	40.0
Baraca	3.0	40.5	4.4	64.0
Merlin	3.0	66.4	4.6	53.5
Marcel	3.0	48.5	4.0	56.2
Hobbit	1.8	2.6	3.8	55.7

*Range scale 0–5: 0 – without symptoms; 1 – infection 1–10%; 2 – infection 11–25%; 3 – infection 26–50%; 4 – infection 51–89%; 5 – infection 90–100%

(*in vitro*) conditions (Table 2). The statistical analyses showed significant differences in resistance between accessions. The highest resistance to all isolates used was detected in lines 29H, L-8 and Petra. The susceptible control accessions (Hobbit, V-1085 and V-1117) were medium resistant. The cultivars Marcel, Baraca and Merlin were susceptible.

The results of laboratory tests were checked by using a mixture of isolates (RA, CH, TE and TE/P04) under field conditions (Table 3).

The lines L-8, 29H and Petra showed a high resistance level during the whole vegetation period. The control accessions (V-117, V-1085 and Hobbit) were characterised by the increased initial resistance of leaves and growth apices. However, their resistance decreased after blossom fall and they were susceptible in the maturation stage, analogously to cultivars Merlin, Mistral and Baraca (Table 3).

The differences in the size of lesions on the leaves (accessions L-8, Petra and Merlin) as well as the differences in the number of lesions per leaf and pycnidium number per lesion show the higher initial resistance of accession L-8 compared to Petra. The resistant accessions significantly decreased the infection pressure of anthracnose during the vegetation period (Table 4).

In 2002 the hybrid combination Petra × Merlin was crossed to obtain the progenies of white flowering faba bean with improved resistance to *A. fabae*. The selection for different phenotypes (colour flowering with black hilus, colour flowering with white hilus, white flowering with black hilus, white flowering with white hilus) with improved resistance to *A. fabae* was carried out under in-

Table 4. Evaluation of leaf reaction on day 30 after inoculation by a mixture of isolates (RA, CH, TE, TE/P04) of *Ascochyta fabae* under field conditions; Šumperk 2005

Accession	Necrosis number per leaf		Necrosis size (mm)		Pycnidium number per necrosis	
	range	mean	range	mean	range	mean
L-8	2–20	5.2	1–4	2.2	0–3	0.4
Petra	2–36	12.5	1–8	3.8	2–12	3.5
Merlin	10–45	22.0	4–16	9.6	8–110	34.3

Table 5. Comparative test; inoculation of the source hybrid population F_2 and selected lines F_3 and F_4 Merlin – highly susceptible (HS), white flowering (W) and Petra – resistant (R), colour flowering (C) by *Ascochyta fabae* under field conditions; WH – white hilum, BH – black hilum; Šumperk 2005

Accession, population, line	Frequency of plant distribution (%) in infection groups					
	0–5	6–10	11–15	16–20	21–30	31–60
Merlin HS (W, WH)	0.0	1.2	9.2	35.3	29.1	25.2
Petra R (C, BH)	51.4	26.3	17.1	3.9	1.3	0.0
Merlin × Petra F_2	12.0	17.5	37.3	18.6	9.3	5.3
Merlin × Petra F_3 (C)	12.7	25.6	25.1	13.3	13.3	10.0
Merlin × Petra F_3 (W)	1.4	2.2	18.2	39.0	29.0	10.2
Merlin × Petra F_4 (C, BH)	37.3	27.0	17.2	13.3	4.0	1.2
Merlin × Petra F_4 (C, WH)	0.0	2.6	3.6	38.3	37.4	18.1
Merlin × Petra F_4 (W, WH)	6.1	11.5	19.3	42.4	15.2	5.5

oculation conditions. The selected lines were sown and selection was repeated in the F_3 generation under field inoculation conditions. The colour flowering plants with seed infection in the range of 0–10% and white flowering ones with seed infection in the range of 0–15% were selected. In the F_4 generation (2005) the source material and the hybrid population of F_2 and F_3 generations were compared and selection efficiency was evaluated (Table 5).

In the colour flowering phenotype with black hilus (F_4) seed infection of up to 10% was found out in 64.3% of plants. In the colour flowering phenotype with white hilus (F_4) seed infection of up to 10% was found out in 2.6% of plants. In the white flowering phenotype with white hilus (F_4) seed infection of up to 10% was observed in 27.6% of plants. These results can be considered as a significant improvement of resistance. The resistance could be further increased and stabilized by further repeated selection cycles with stricter selection criteria (plant selection with seed infection lower than 5%).

DISCUSSION

In the present study, the occurrence of different virulent pathotypes of *A. fabae* and their variability in all culture parameters were proved in accordance with literature data (Kharbanda & Bernier 1980; Filipowicz 1983; Hanounik & Robertson 1989; Rashid *et al.* 1991; Tivoli & Maurin 1992).

In Syria, 4 different virulent pathotypes of *A. fabae* were detected. Inbred faba bean lines BPL 471 and BPL 2485 were resistant to all Syrian pathotypes. The line BPL 818 was resistant only to pathotype 1 and 3 (Hanounik & Robertson 1989). In Canada, the occurrence of ten different *A. fabae* pathotypes was detected, using a differential set of eight faba bean inbred lines (Rashid *et al.* 1991). Only three tested faba bean lines were resistant to all Canadian pathotypes (BPL 2485, 15025-2 and 14434-2).

In England, winter faba bean breeding for an increase in resistance to *A. fabae* was successful (Lockwood *et al.* 1985) and a number of inbred resistant lines was obtained. The highest resistance level of both leaves and pods was detected in the line IB 18-1/3. The resistant lines were used for the composition of synthetic cultivars (to prevent a decrease in the vitality of inbred lines): Maris Beagle, Buldog, Banner, Bourdon, Quasar. A higher level of resistance to *A. fabae* in the English cultivars Banner and Quasar was also proved in our field inoculation tests (Ondřej 1990, 1993). The cultivar Banner was used to cross a hybrid combination (Banner × SU-V3). Progenies were selected to obtain a spring faba bean type with higher resistance to a wide spectrum of *A. fabae* pathotypes from different production regions of the Czech Republic. The line SU-R5/13 proved to have the highest level of resistance. After reselection, new resistant lines were obtained and a new synthetic source Petra was made up. Petra differs from SU-R5/13 in lower TSW, improved earliness and enhanced vigour.

Literary data provide different information on the inheritance of *A. fabae* resistance. In Australia (Ramsey *et al*. 1995) the parental varieties Ascot × Icarus (resistant × susceptible) were crossed. The F_2 generation segregated 1:3 (resistant:susceptible). This fact showed that resistance is controlled by one recessive gene. In Canada Rashid *et al*. (1991) detected the existence of seven dominant genes of resistance, whilst in Australia Lawsawadsiri (1995) reported only one dominant gene in the line ILB 757. Other authors (Lockwood *et al*. 1985; Avila *et al*. 2001) suppose that faba bean resistance to *A. fabae* is multigenic and horizontal since in the F_2 generation continuous plant segregation from resistant to highly susceptible plants (with a very low and limited number of resistant ones) was found out. In the F_2 generation derived from the cross between lines Vf6 (resistant) and Vf136 (susceptible) segregation in the percentage proportion of resistant to medium susceptible to highly susceptible 1:14:23:62 was found out (Avila *et al*. 2001). The ascertained segregation of F_2 plants derived from the cross of cultivar Merlin with line Petra (Table 5) confirms that resistance is multigenic and horizontal.

Some resistant inbred lines show a higher resistance level of stems than leaves. Other lines are characterized by the high initial resistance of leaves and simultaneous susceptibility of stems and pods (Lockwood *et al*. 1985; Rashid & Bernier 1985). The lines 29H and L-8 show a high resistance level during the whole vegetation period. This fact provides evidence of the presence of more resistance genes with a different and specific expression. The until now detected occurrence of 16 markers that were identified on 6 different linkage groups in the resistant line Vf6 may not be definitive. The number of markers can be higher and different in the lines 29H and L-8 (Avila *et al*. 2001).

The utilisation of our donor Petra is very advantageous for the Czech faba bean resistance breeding program. Both the spring faba bean line Petra and commercial varieties have the same yield. The utilisation of the Spanish donor L-8 is not promising in Czech conditions. This faba bean type has a low growth habit, high TSW value and low yield. Similarly, the utilisation of the French donor 29H is not promising for the resistance breeding program because 29H is a winter type very susceptible to virus diseases, very late maturing in our conditions, producing low yield and of low seed quality.

Available sources of resistance to a wide spectrum of *A. fabae* pathotypes are known in winter faba bean (IB 1-18/3, 29H), colour flowering bean (L-8) and in colour flowering spring bean, whilst in white flowering bean there are not any known sources of resistance. There is a need for further intensive work to breed white flowering faba bean with a higher level of resistance to anthracnose.

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