VARIATION IN THE RESISTANCE OF FABA BEAN (*Vicia faba* L.) TO ASCOCHYTA BLIGHT

*VARIASI KETAHANAN KACANG BABI (*Vicia faba *L.) TERHADAP JAMUR BERCAK ASCOCHYTA*

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ABSTRACT

Faba bean (*Vicia faba* L.) is important grain legumes in the world as it is a source of protein in the human diet, used as animal feed and for crop rotation to break disease cycles of cereals. Faba bean is adversely affected by a fungal disease Ascochyta blight (*Ascochyta fabae*) which limit productivity and production in some countries. There was variation among putatively resistant accessions in their response to various isolates of *A. fabae*. The present study was conducted to identify variation in the resistance of several faba bean accessions to isolates of *A. fabae* and variability of virulence among the isolates. The experiment was conducted in two stages using two different seed samples, namely bulk samples and single plant derived pure lines. Eight accessions tested were Acc299, 303, 342, 508, 680, 948, 970 and Ascot, while eight isolates used included 252/92, A26, 166/92, 493/92, 219/92, 260/92, 331/91 and 526/92. The reaction of pure lines of Acc 303, 680, 948, 970 and 622 or Ascot were resistant to all isolates tested, while Acc 299 and 508 were resistant to several isolates, but not all. The pathogenic variability of isolates of *A. fabae* was apparent. This is based on the fact that some isolates (331/91 and 526/92) caused more disease on all accessions while other isolates caused disease on either most, or only a few accessions. Isolate 331/91 resulted in the greatest discrimination between resistant and susceptible accessions and for this reason it was suggested to select for genetic studies in the resistance of faba bean (*Vicia faba* L.) to Ascochyta blight.

Key words : *Ascochyta fabae*, faba bean, resistance, virulence, variability

ABSTRAK

Kacang babi (Vicia faba L.) *adalah kacang-kacang yang penting di dunia karena merupakan sumber makanan yang mengandung protein, dapat digunakan sebagai makanan ternak dan sebagai tanaman untuk pergiliran tanam serealia dalam usaha memotong siklus keberadaan penyakit. Kacang babi rentan terhadap serangan jamur bercak daun Ascochyta* (Ascochyta fabae) *yang dapat menurunkan produktivitas dan hasil tanaman di beberapa negara. Terdapat perbedaan respon yang nyata diantara galur-galur yang tahan terhadap isolat A. fabae.*

Penelitian ini telah dilakukan dengan tujuan untuk mengidentifikasi vasiasi ketahanan beberapa aksesi kacang babi terhadap beberapa isolat A. fabae *dan variabilitas virulensi diantara isolat-isolat tersebut. Percobaan dilakukan dua tahap dengan menggunakan sampel benih yang berbeda, yaitu sampel bulk dan tanaman tunggal berasal dari galur murni. Delapan galur yang di uji antara lain Acc299, 303, 342, 508, 680, 948, 970 dan Ascot, sedangkan delapan isolat yang digunakan antara lain adalah 252/92, A26, 166/92, 493/92, 219/92, 260/92, 331/91 and 526/92. Reaksi dari beberapa galur murni (Acc 303, 680, 948, 970 dan 622 atau Ascot) adalah tahan terhadap semua isolat yang diujikan, sementara Acc 299 dan 508 hanya tahan terhadap beberapa isolat saja, dan tidak terhadap semua isolat yang diujikan. Variabilitas virulensi dari isolat-isolat A. fabae tampak nyata mengingat dua isolat (331/91 and 526/92) menyebabkan infeksi terhadap semua genotipe sementara isolat yang lain menimbulkan penyakit hanya terhadap beberapa genotipe saja. Isolat 331/91 menunjukkan diskriminasi yang paling tinggi antara penampakan rentan dan tahan dari aksesi-aksesi, sehingga isolat tersebut dapat disarankan untuk dapat digunakan di dalam pengujian lebih lanjut untuk ketahanan kacang babi (*Vicia faba L.) terhadap Ascochyta fabae.

Kata kunci : Ascochyta fabae, *kacang babi, tahan, virulen, variabilitas*

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the oldest and the seventh most important grain legumes in the world (Ricciardi, et al., 2001; Rees et al., 2000). In the Middle East it is a major source of protein in the human diet, whereas in Europe and other developing countries it is used primarily as animal feed (Turpin et al., 2003). Faba bean is an attractive crop for farmers because of its potential to produce high grain yields in dry environments (Loss and Siddique, 1997), for rotation benefits derived from breaking disease cycles of cereals (Felton, et al., 1998), and for adding significant amounts of fixed nitrogen (N) to soils of declining fertility (Rochester, et al., 1998).

Faba bean is adversely affected by numerous fungal diseases that vary in incidence and severity from region to region. The most known disease is Ascochyta blight (*Ascochyta fabae*) which limit faba bean productivity and production in countries in the Mediterranean regions, China, Latin America, China, Ethiopia, and Australia (ICARDA Press Releases, 2008). This pathogen infects aerial parts of plants: leaves, stems and pods (Zakrzewska, 2004), and the infection may lead to the whole pod becoming necrotic and may cause loss of yield and also reduce the quality of faba beans through seed infection (Torres, *et al*., 2006). *Ascochyta fabae* is particularly prevalent under wet and cooler conditions and was reported that infection levels on a highly susceptible cultivar were severe when inoculated with *A. fabae*, and yield decreased by 90% compared with that of plants treated with the fungicide mancozeb (Mcvicar et al., 2005; Sillero et al., 2001).

Genetic differences in the reactions of faba beans to *A. fabae* have been reported. Kharrat *et al*. (2006) found that several lines carry either minor or major resistance genes to *A. fabae*, and resistance might be controlled by one or more resistance gene(s) (Lawsawadsiri, 1995). A number of studies have shown that there is large variation among genotypes of faba bean in the response to infection by a range of isolates of *A. fabae* (Ondrej, and Hunady, 2007). A high degree of variability for virulence of *A. fabae* has also been identified, as well as a differential interaction between isolates of *A. fabae* and lines of faba beans (Hanounik and Robertson, 1989).

Resistant lines showed variable reaction to isolates of *Ascochyta fabae* from different regions of the world (Hanounik and Robertson, 1989). For example, accessions BPL 472 and BPL 818, which were resistant to *A. fabae* when tested in many countries, were susceptible to French isolates. This indicated that these accessions have specific resistance compared with several other lines which were consistently resistant in all countries tested (Hanounik and Robertson, 1989). The pathogen causing Ascochyta blight has been ascribed *Ascochyta fabae* Speg., which infects aerial parts of plants: leaves, stems and pods (Zakrzewska, 2004). The infection may lead to the whole pod becoming necrotic and may cause loss of yield and also reduce the quality of faba beans through seed infection (Torres, *et al*., 2006). Hanounik (1980) reported that infection levels on a highly susceptible cultivar were severe when inoculated with *A. fabae*, and yield decreased by 90% compared with that of plants treated with the fungicide Mancozeb. Genetic differences in the reactions of faba beans to *A. fabae* have been reported. Kharrat *et al*. (2006) found that several lines carry either minor or major resistance genes to *A. fabae*, and resistance might be controlled by one or more resistance gene(s) (Lawsawadsiri, 1995).

The experiments reported aimed to identify variation in the resistance of several faba bean accessions, most of which had been tested in many countries, to isolates of *A. fabae*. The variability of virulence among isolates of *A. fabae* collected from several regions in Australia was also studied.

METHODS

Trial 1

The experiment was conducted in two stages using two different seed samples, namely bulk samples and single plant derived pure lines. The bulk samples were used rather than the pure lines which had not been developed. A series of eight experiments were conducted based on eight isolates of *A. fabae* collected from different regions in Australia. The eight isolates were A37, A26, 201/92, 166/92, 219/92, 260/92, 331/91 and 526/92. Each individual isolate was tested against eight accessions (299, 303, 342, 508, 680, 948, 970 and Ascot) which were selected on the basis of the known range of resistance reported by several authors. Four accessions (299, 303, 508 and 680) were tested in several countries by Hanounik and Robertson (1989). Acc 948 and 970 were resistant lines that originated from Europe (Lockwood et al. 1985). Ascot was the resistant cultivar adapted to the Australian environment reported by Ramsey et al. (1995), while Acc 342 (Giza-4) was selected as the susceptible control, since this accession was used in the host differential set studies by Hanounik and Robertson (1989). These seed of all accessions used in this experiment was obtained from bulk samples maintained by the Waite faba bean breeding program.

The experiment was arranged as a series of randomised complete block designs to determine the reaction of each accession separately for each isolate. This enabled the effect of each isolate on a range of accessions to be determined but not the interaction between isolates and accessions. The experiment was conducted as a series of randomised complete blocks, rather than a split plot design, to prevent cross-infection by the various isolates if all were included in the one block. The experiment contained four replications, each of which was four plants.

Seeds were sown in plastic punnet in trays using Recycled Soil (RS) soil. The trays were placed in plastic covered humidity chambers (cabinets) within a glass house. The cabinet had fold-down flaps at the front and these were left open during initial establishment, but closed following inoculation to maintain high humidity. The cabinets contained automated overhead misters which were programmed to operate at up to six times a day with overhead misting for 1 minute, twice per day to maintain humidity. The temperature of the glasshouse was maintained at or below 20 ^oC by evaporative air-conditioning.

Isolates of *Ascochyta fabae* were supplied by South Australian Research and Development Institute (SARDI), and represented isolates collected from different faba bean growing areas in Australia. Single spore cultures were stored in sterile water at 4 ^oC. Eight isolates were used for the experiment on the interaction between isolates and genotypes. Isolates were subcultured on 2% PDA and incubated at 25 ^oC for two weeks. Pycnidiospores were collected by flooding the surface of the agar with sterile RO (Reverse Osmosis) water and scraping with a glass rod. The spore suspension was then poured onto a new petri dish containing 2% agar and covered by autoclaved cellophane. The cultures were incubated at 25 ^oC.

Four weeks after sowing plants in the individual experiments were inoculated separately with the eight isolates. Pycnidiospores were harvested as prepared above, and the concentration was adjusted to 3-5 x 10^5 spores/ml using a Neubauer© haemocytometer. The inoculum was applied to plants with 2-3 drops of Tween-20 per litre. . Rating was conducted when the symptoms were spread evenly across the trial (approximately 14 days after inoculation) and the symptoms on the susceptible controls were either 7 or 9. The classifications of resistant and susceptible follow Hanounik and Robertson (1989) : 1 = highly resistant, 3 = resistant, 5 = moderately resistant, 7 = susceptible, and 9 = highly susceptible.

Trial 2

The second trial was carried out using seeds collected from pure resistant selections. Using the same procedure as above (the first trial), eight accessions (Acc299, 303, 342, 508, 680, 948, 970 and 622) were tested with eight isolates (252/92, A26, 166/92, 493/92, 219/92, 260/92, 331/91 and 526/92). In this experiment the cultivar Ascot, which is a composite of two selections, was replaced by Acc 622, one of the accessions from which Ascot was derived. The isolates A37 and 201/92 used in the first trial were not included in the second trial, due to contamination during multiplication and insufficient production of spores. The trials were arranged as above. Data from the series of experiments were individually analysed by analysis of variance calculated using Genstat version 3.

RESULTS AND DISCUSSIONS

Trial 1

Symptoms of disease developed 14 days after inoculation, particularly on Acc 342 and Acc 299. Lesions caused stems of susceptible plants to break, while symptoms did not develop on leaves of resistant plants. The eight accessions in each series of experiments showed variation in reaction to the eight isolates (Table 1).

Four accessions (Acc 303, 948, 970 and Ascot) displayed resistance to all isolates tested as the mean disease score was less than 3 of a 1-9 rating scale for all isolates. Although the reactions of Acc 508 and 680 to five isolates were resistant with score less than 3, these accessions had scores more than 3 in reaction to three isolates (260/92, 331/91 and 526/92). Acc 299 appeared susceptible as it produced symptoms greater than 3 to all isolates tested, and was significantly more susceptible than the susceptible control (Acc 342) when tested with isolates 166/92 and 219/92 (LSD 5% = 1.1 and 0.9, respectively).

The virulence among isolates to eight accessions varied between experiments in the series. The overall average virulence of all individual isolates to all accession tested is presented in Figure 1. Three isolates, 260/92, 331/91 and 526/92, produced severe symptoms on Acc 299 and Acc 342, and these isolates also caused symptoms on Acc 303, 948, 970 and Ascot, whereas less symptoms developed on these accessions when inoculated with the other isolates (Table 1).

Table 1. Disease scores of eight accessions inoculated with eight different isolates of *A. fabae*, using seeds collected from bulk samples

Accession	Disease score following inoculation with isolate							
	A37	A26	201/92	166/92	219/92	260/92	331/92	526/92
Acc 299	3.6	4.4	3.6	4.6	5.8	5.1	5.5	4.8
Acc 303	2.1	1.1	1.5	2.0	1.0	2.0	2.3	2.1
Acc 342	4.2	3.3	3.8	2.4	2.0	4.6	5.8	6.8
Acc 508	2.6	2.0	2.0	2.6	1.0	3.9	4.1	3.1
Acc 680	2.5	2.0	1.9	1.9	1.9	3.8	3.5	3.3
Acc 948	1.0	1.0	1.0	1.0	1.0	1.3	1.4	1.1
Acc 970	1.0	1.3	1.1	1.6	1.3	2.5	1.9	2.9
Ascot	1.3	1.5	1.3	1.5	1.3	1.6	2.4	1.9
LSD 5%	1.6	1.5	1.3	1.1	0.8	1.7	1.7	1.3

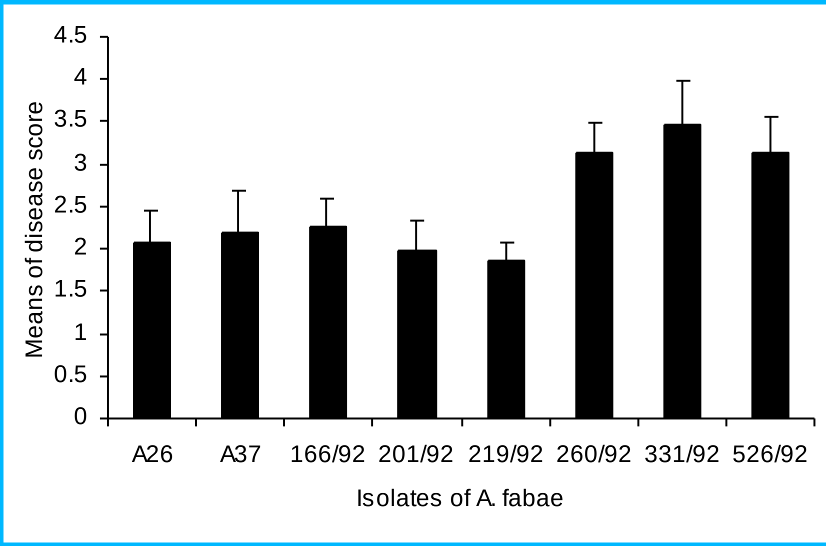

Figure 1. Variation in the virulence of eight isolates of *A. fabae* on eight faba bean accessions, using seeds collected from bulk samples. The bars represent the standard error of the mean

Trial 2

Similar to the results of the first test, the reaction of the eight accessions from seeds derived from single self-pollinated plants varied when inoculated individually with eight isolates of *A. fabae* (Table 2). Acc 303, 948, 970 and 622 (substituted for Ascot) were resistant with scores less than 3. Acc 680 and 508 produced disease scores less than 3 to all isolates tested, with the sole exception of Acc 508 with isolate 331/91 which produced symptoms with a score higher than 3. Although having a score more than 3 in reaction to several isolates, Acc 299 was significantly more resistant to isolates A26, 166/92 and 219/92 compared to Acc 342 (Table 2).

As in the first trial, three isolates, 260/92, 331/91, and 526/92, produced the most severe symptoms and the mean score for 331/91 was greater than 3, while the other isolates were less virulent with the mean of symptoms less than 3 (Figure 2).

Table 2. Disease scores of eight accessions inoculated with eight different isolates of *A. fabae*, using seeds of pure lines

Accession	Disease score following inoculation with isolate							
	252/92	A26	493/92	166/92	219/92	260/92	331/92	526/92
Acc 299	3.0	2.4	3.4	1.4	1.9	4.5	5.4	3.9
Acc 303	1.0	1.0	1.3	1.4	1.0	2.1	2.6	1.5
Acc 342	3.6	4.8	3.8	4.1	2.5	3.6	4.9	4.5
Acc 508	2.2	1.8	2.0	1.5	1.0	1.8	4.9	2.5
Acc 680	1.3	1.4	2.4	1.4	1.3	2.5	2.9	2.6
Acc 948	1.0	1.4	1.0	1.0	1.0	1.4	2.4	1.0
Acc 970	1.0	1.1	1.0	1.0	1.0	1.4	1.6	1.0
Ascot	1.3	1.0	1.0	1.4	1.0	1.1	1.9	1.6
LSD 5%	1.5	1.2	1.6	0.9	0.6	1.5	1.6	1.7

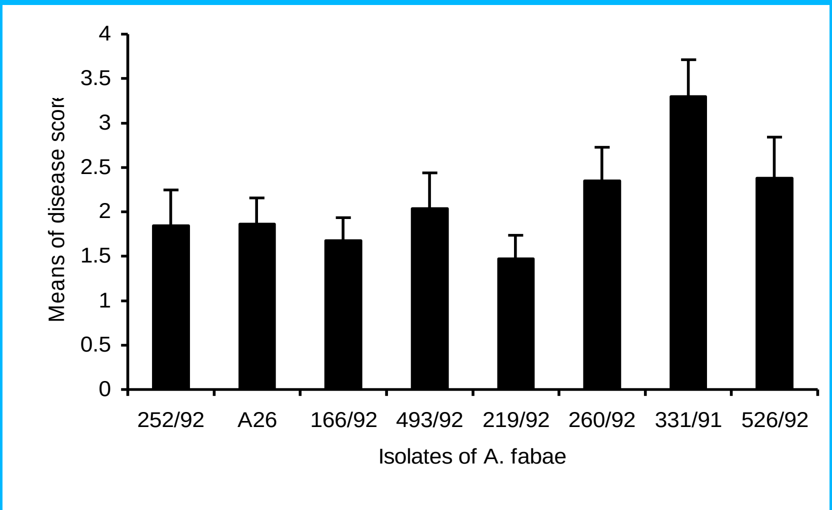

Figure 2 Variation in the virulence of eight isolates of *A. fabae* on eight faba bean accessions, using seeds collected from pure lines. The bars represent the standard error of the mean

These experiments demonstrated that lines developed by self-pollination and progeny testing were more homogeneous in resistance than the original accessions, particularly Acc 299, 508 and 680. Acc 303, 622 (Ascot), 948 and 970 appeared to be homogeneous for resistance to all isolates tested either using pure lines or bulk samples. As the bulk samples of several accessions were derived from several cycles of selfing and single plant selection (J. Paull, pers. com.), it is probable that these accessions were homozygous in resistance to *A. fabae*.

The two trials suggested that the reaction of pure lines of Acc 303, 680, 948, 970 and 622 or Ascot were resistant to all isolates tested, while Acc 299 and 508 were resistant to several isolates, but not all. Similar variation has been indicated in several reports. Hanounik and Robertson (1989) reported the results of testing a number of faba bean lines in six geographically diverse countries. Several of these lines were included in the present study. BPL 2485 (Acc 680) was identified as resistant in the six countries and was considered to have a broad resistance to *A. fabae*. BPL 472 (Acc 303) and BPL 818 (Acc 508) were resistant in five countries, but susceptible in France. Lawsawadsiri (1995) also identified variation in the reaction of Acc 508 to a range of isolates of *A. fabae* and it was classified as resistant to seven Australian isolates, but it was susceptible to isolate A26. In the present trials, both the bulk population and a pure line of this accession were resistant to A26, but not to isolate 331/91. Acc 299 (BPL 471) was also studied by Lawsawadsiri (1995) and, as with the present trials, Acc 299 was susceptible to isolate A26. However, Hanounik and Robertson (1989) reported BPL 471 (Acc 299) was resistant in six countries.

Resistance of Ascot to a mixed inoculum of several isolates of *A. fabae* was reported by Ramsey et al.

(1995) in two trials with ratings of 1.3 and 0.27 respectively on a 0-5 rating scale. Resistance of Acc 970 to isolate A26 was reported to be homogeneous when tested by Lawsawadsiri

(1995), with a mean score of 0.1 on a 0-5 rating scale. The resistance of Quasar (Acc 948) was assessed by Jellis and Vassie (1995) who reported it had a mean score of 2.4 with the range of individual plants of 1-5 on a 1-9 rating scale.

Although it is not possible to identify a race structure of isolates of *A. fabae* in these trials, as the experimental design was not appropriate to investigate the differential interaction between accessions and isolates, the pathogenic variability in Australian isolates of *A. fabae* was apparent. This is based on the fact that some isolates caused more disease on all accessions (331/91 and 526/92), while other isolates caused disease on either most, or only a few accessions. This indicated that the virulence of isolates depended on the resistant host used. For example, seven accessions were resistant to isolate 219/92 but the bulk of Acc 299 developed severe symptoms in reaction to this isolate. In regard to this accession, Hanounik and Robertson (1989) demonstrated that only 4% of isolates of *A. fabae* inoculum IA induced susceptible reactions on BPL 471 (Acc 299), but 20% of isolates induced susceptible reactions on ILB 1814. For inoculum IB, 8% and 84% of isolates induced susceptible reactions on BPL 471 and ILB 1814, respectively.

It was concluded that pathogenic variability of *A. fabae* was high, while variation was also observed within and between several accessions. Isolate 331/91 resulted in the greatest discrimination between resistant and susceptible accessions and for this reason it was suggested to select it for genetic studies in the resistance of faba bean (*Vicia faba* L.) to Ascochyta blight.

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