AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) ANALYSIS OF GENETIC DIFFERENTIATION AMONG ASCOCHYTA BLIGHT RESISTANT ACCESSIONS OF FABA BEAN

Analisis AFLP (Amplified Fragment Length Polymorphism) *Terhadap Keeratan Genetik Antar Galur Tahan Kacang Babi Terhadap Bercak Ascochyta*

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ABSTRACT

Ascochyta blight caused by *Ascochyta fabae* is one of the most destructive diseases on faba bean. There was variation among putatively resistant faba bean accessions in their response to various isolates of *A. fabae*. The present study was conducted to investigate genetic similarities among the Ascochyta blight resistant faba bean accessions and to identify whether there was a relationship between the genetic similarity and genetic control of resistance to *A. fabae*, as well as between the genetic similarity and origin of the accessions. Amplified Fragment Length Polymorphism (AFLP) analysis was utilised to identify the genetic distance among 20 resistant and 2 susceptible accessions. Three primer combinations (*Pst*ACA-*Mse*CAG, *Pst*ACA-*Mse*CCA and *Ps* ACA-*Mse*CGA) revealed a high level of polymorphisms. The average genetic distance over all accessions was 0.34 with the pair-wise ranged from 0.09 to 0.53. The phylogenetic tree divided the 22 accessions into two major groups and several groups of two and three. The analysis was inconclusive when the genetic control of resistance to *A. fabae*, the region of origin and the source population were compared to the genetic distances among the accessions.

Key words : *Ascochyta fabae*, faba bean, resistance, AFLP analysis, genetic distances.

ABSTRAK

Bercak daun yang disebabkan oleh Ascochyta fabae merupakan salah satu kerusakan karena penyakit yang parah terhadap kacang babi (faba bean). Terdapat perbedaan respon diantara galur-galur yang tahan terhadap isolate A. fabae yang berbeda. Penelitian ini telah dilakukan dengan tujuan untuk mengkaji keeratan genetic diantara galur-galur kacang babi yang tahan terhadap isolate A. fabae dan untuk melihat apakah ada hubungan antara keeratan genetic dengan gen pengontrol ketahanan terhadap A. fabae, dan juga antara keeratan genetic dengan tempat asal dari galur-galur tersebut. Analisis AFLP (Amplified Fragment Length Polymorphisms) telah digunakan untuk mengidentifikasi keeratan genetic diantara 20 galur tahan dan 2 galur tidak tahan sebagai control. Tiga kombinasi primer ((PstACA-MseCAG, PstACA-MseCCA dan Ps ACA-MseCGA) menghasilkan polimorpi yang tinggi. Rata-rata keeratan genetic dari semua ke 22 galur adalah 0,34 dengan rentang pasangan dari 0,09 hingga 0,53. Pohon pilogenetik membagi 22 galur menjadi dua grup utama dan beberapa grup terdiri dari dua dan tiga galur. Hasil analisis menunjukkan bahwa keeratan hubungan antara sifat tahan terhadap A. fabae, asal dan populasi sumber tidak dapat disimpulkan dengan nyata.

Kata kunci : Ascochyta fabae, Kacang babi, Tahan, Analysis AFLP, Keeratan genetik

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INTRODUCTION

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)

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Table 1 Faba bean accessions used for the AFLP study of genetic similarity of *A. fabae* resistant accessions

Tabel 1. Galur-galur faba bean yang digunakan dalam analisa AFLP untuk keeratan genetik terhadap ketahanan pada A. fabae

Waite Acc.	ICARDA Acc[a] Name	Original population/ pedigree	Origin	Reaction to *A. fabae*	References
290	BPL 230 A884	ILB 142	Morocco	Resistant	1, 5
295	BPL 460 A888	ILB 284	Lebanon	Resistant	1, 3, 5
297	BPL 465 A889	ILB 285	Lebanon	Resistant	1, 5
299	BPL 471 A8710	ILB 287	Lebanon	Resistant	1, 2, 3, 5
303	BPL 472 A8713	ILB 287	Lebanon	Resistant	1, 3, 5
342	Giza-4	-	Egypt	Susceptible	3, 5
484	BPL 74 A883	ILB 37	Iraq	Resistant	1, 3, 5
496	BPL 365 A886	ILB 227	Morocco	Resistant	1, 5
508	BPL 818 A8815	ILB 549	Ethiopia	Resistant	1, 2, 3, 5
622	Ascot component		Naxos	Resistant	4
668	L 83120 A882	BPL 460* ILB 37	ICARDA	Resistant	1
674	L 83124 A8824	ILB 37* BPL 2485	ICARDA	Resistant	1
680	BPL 2485 A88302	ILB 37	Iraq	Resistant	1, 2, 3, 5
683	L 83114 B8826	ILB 938* BPL1390	ICARDA	Resistant	7
712	L 82003 B8834	BPL 472*BPL 261	ICARDA	Resistant	7
722	L 82009 B8837	A2* ILB 938	ICARDA	Resistant	7
948	Quasar		UK	Resistant	6
970	ILB 752		Sweden	Resistant	1, 2, 5
975	L 83125	BPL 460*BPL2485	ICARDA	Resistant	1
976	L 83136	ILB 37*BPL 460	ICARDA	Resistant	7
1046	BPL 646	ILB 382	UK, USSR	Resistant	1, 2, 3, 5
Icarus	BPL 710	L81-248571	Equador	Susceptible	4

[a]) The ICARDA accession number includes the selection number of the line provided to the Waite faba bean breeding program

1 Bond et al., 1994.
2 Rashid et al., 1991.
3 Hanounik and Robertson, 1989.
4 Ramsey et al., 1995.
5 Hanounik and Maliha, 1984.
6 Lockwood et al., 1985.
7 Robertson, L. pers comm.

To optimize crossing and selection programs, information about genetic variability is important. A high level of genetic variation between parents improves the efficiency and accuracy when constructing genetic maps. Further, it might be postulated that genetically distant genepools are more likely to include different genes, such as for disease resistance, than closely related genepools. Molecular markers which have been used to characterise plant genetic variability include isozymes (Koch and Kohler, 1991), Restriction Fragment Length Polymorphisms (RFLPs) (Manjarrez-Sandoval *et al*., 1997), Random Amplified Polymorphic DNAs (RAPDs) (Asante and Offei, 2003), simple sequence repeats (SSRs) (Tams *et al*., 2005), and Amplified Fragment Length Polymorphisms (AFLPs) (Levi and Thomas, 2007; Moon and Nicholson, 2007). Molecular marker technology has proven very useful in contributing to studies of genetic distance of several crops, including faba beans. Link *et a*l. (1995) utilised RAPD markers to classify germplasm and identify genetic distance among inbred faba bean lines, including European small-seeded lines, European large-seeded lines and Mediterranean lines.

The AFLP technique is based on the selective PCR amplification of restriction

fragments from a total digest of genomic DNA (Vos *et al*., 1995). Similar to RAPD analysis, AFLP assays need no prior sequence knowledge, but detect greater number of loci than that of RAPDs (Maughan *et al*., 1996). Vos *et al*. (1995) stated that AFLP is a combination of the reliability of the RFLP technique and the power of the PCR technique. Therefore, this technique is robust and reliable. AFLP markers have been used to measure genetic distance of several crops, such as watermelon (Levi and Thomas, 2007), tomato **(**Moon and Nicholson, 2007) triticale (Tams *et al*., 2005), coffee (Steiger *et al*., 2002), rice (Xiaonchuan *et al*., 2002), maize (Li *et al*., 2004), and sorghum (Menz *et al*., 2004). However, there has been no report on application of AFLP methodology to studying genetic distance in faba bean.

Ascochyta blight resistant faba bean accessions, and susceptible controls, were analysed by the AFLP technique with the aims to (1) identify the genetic similarities among the Ascochyta blight resistant faba bean accessions, (2) identify whether there was a relationship between genetic similarity and the genetic control of resistance to *A. fabae*, and (3) determine whether there was a relationship between genetic similarity and the origin of the accessions.

MATERIALS AND METHODS

Plant materials and DNA samples

The twenty-two accessions of faba bean used for the present study are listed in Table 1. Leaf samples of each accession were harvested from six plants grown in a glasshouse. These plants were grown from seed obtained from plants that had been selfed and progeny tested to confirm they were homozygous resistant to *A. fabae*. DNA extraction was carried out on leaf samples from individual plants and the extracts were combined to serve as the DNA sample of each accession.

DNA extraction

DNA was extracted as follows : the leaves were placed in a 2 ml Eppendorf® centrifuge tube, dipped in liquid nitrogen and ground to fine powder using a knitting needle. 750 ml of extraction buffer was added to the samples which were then homogenised by a quick vortex. 750 ml of phenol/chloroform/iso-amyl-alcohol (25:24:1) was added and mixed on a rotor for 30 min. The phases were separated by centrifuging at 5000 rpm for 5 min, and the supernatant was transferred to a new 1.5 ml Eppendorf® centrifuge tube. The phenol chloroform step was repeated (equal volumes of transferred supernatant and phenol chloroform). Following centrifuging, 650 µl of the upper phase was transferred to a new 1.5 ml tube to which 650 µl of chloroform was added and after mixing, the sample was centrifuged. 600 µl of supernatant was transferred to a new 1.5 ml tube. The DNA was precipitated by adding 60 µl 3 M Na-acetate pH 4.8 and 600 µl of isopropanol. The mixtures were centrifuged at 15,000 rpm for 15 min. The supernatant was discarded and the white DNA pellet was washed with 1 ml 70% ethanol. The ethanol was drained and the pellets were vacuum-dried. The DNA was resuspended in 50µl of TE buffer and stored at 4 ^{o}C.

Digestion of DNA

Three DNA samples (Acc 622, Acc 970 and Icarus) were digested separately using *Pst* 1, *Eco* R1 and *Dra* 1 (either Biolab® or Boehringer®) in order to identify the most appropriate digestion enzymes for further steps of the experiment. As all enzymes digested the DNA samples, *Eco* R1 (Biolab®) was initially used. However, in AFLP analysis, primer combinations based on the enzyme *Eco* R1 did not produce either any bands, clear bands or polymorphisms. Therefore, *Pst* 1(Boehringer®) was used as the alternative enzyme. DNA was digested at 37 ^{o}C for 4 hours using 2 µl *Pst* I, 1 µl BSA, 1 µl Spermidine, 1.2 µl 10x reaction buffer (supplied with the enzymes) and made up to a final reaction volume of 12 µl with sterile water.

Gel electrophoresis

Digested DNA was loaded onto a 1.5% agarose gel which was electrophoresed in 1 X TAE buffer for 2 hours at a current of 40 mA. Gels were stained in 10 µg ml^{-1} ethidium bromide for 30 min and checked for complete digestion under UV light.

Table 2 Sequences of adapters and primers used for AFLP analysis
Tabel 2. Urutan adapter dan primer yang digunakan pada analisis AFLP

Name	Sequence
Pst I adapter 1	5' - CTC GTA GAC TGC GTA CAT GCA - 3'
Pst I adapter 2	5' - TGT ACG CAG TCT AC - 3'
Mse I adapter 1	5' - GAC GAT GAG TCC TGA G - 3'
Mse I adapter 2	5' - TAC TCA GGA CTC AT - 3'
Pst I- 1 primer (A)	5' - GAC TGC GTA CAT GCA GA - 3'
Mse I- 1 primer (C)	5' - GAT GAG TCC TGA GTA AC - 3'
Pst I ACA	5' - GAC TGC GTA CAT GCA GAC A - 3'
Mse I CAG	5'- GAT GAG TCC TGA GTA ACA G - 3'
Mse I CCA	5' - GAT GAG TCC TGA GTA ACC A - 3'
Mse I CGA	5' - GAT GAG TCC TGA GTA ACG A - 3'

AFLP Analysis

The AFLP analysis was undertaken in the laboratory of Waite Campus, Adelaide University, using a method derived from Vos *et al*., (1995) with modifications suggested by Greg Penner (*pers. comm*..).

Preparation of template DNA

1μg of DNA was digested at 37^{o}C for 3 hours using 5 μl R-L buffer, 5 U *Mse* I, 5 U *Pst* I and made up to a final volume of 50 μl with sterile water. Stock solutions containing both *Mse* I adapters (Table 2) at 50 μM each, and of both *Pst* I adapters (Table 2) at 5 μM each were prepared. The stocks were heated at 90^{o}C for 3 min then the adapters were allowed to anneal at room temperature for more than 30 min. Ligation of adapters occurred by adding 1 μl *Mse* I adapters, 1 μl *Pst* I adapters, 1 μl 10 x R-L buffer, 1.2 μl 10 mM ATP, T4 DNA ligase (1U/μl) and 4.8 μl H_2O to the digested DNA sample (60 μl total volume), and incubated at 37 ^{o}C for 3 hours and then placed at 4 ^{o}C overnight. The next day the DNA was precipitated by adding 120 μl ethanol and 6 μl 4.8 mM sodium acetate (pH 4.8) and placed in liquid nitrogen for 5 min. The mixtures were centrifuged at 15,000 rpm for 15 min, the pellets were washed with 70% ethanol and then dried in a speed vac concentrator (Savant®) for 10 min. The DNA was resuspended in 60 μl 0.1 M TE .

Pre-amplification of prepared template was performed with complementary primers, using a single *Pst* I (*Pst* ACA) and three *Mse* I primers (*Mse* I CAG, *Mse* I CCA, *Mse* I CGA) (Table 2). This pre-amplification of DNA was prepared with 4 μl of the digested and ligased DNA mixed with 1μl 75ng/μl *Pst* I + 1 primer (Table 2), 1μl 75ng/μl of *Mse* I + 1 primer (Table 7.2), 4 μl 1.25 mM dNTPs, 2.5 μl 10 x Taq buffer, 1.5 μl 25 mM $MgCl_2$, 0.2 μl *Taq* polymerase (5U/μl) with sterile water added to a total volume of 25 μl. The PCR reaction conditions were : 20 cycles of 30 sec at 94 ^{o}C, 1 min at 56 ^{o}C , and 1 min at 72^{o}C. Following PCR, 100 μl H_2O was added to each sample. This sample served as the template DNA.

Selective Amplification of Template DNA

Only *Pst* I compatible primers were labelled. The primer was prepared by mixing 1μl 50 ng/μl primer 1, 1.5 μl ^{32}PγATP (10 Ci/ μl), 1 μl 10 x PNK buffer, 0.2 μl 10 U/μl T4 PNK, in a total volume of 10 μl. The mixtures were incubated at 37 ^{o}C for 30 - 60 min. Selective PCR was performed with 1μl of labelled primer *Pst* I, 0.5 μl (50 ng/μl) of unlabelled primer *Pst* I, 0.6 μl (50 ng/μl) *Mse* I, 2 μl 10 x *Taq* buffer, 1.2 μl 25 mM $MgCl_2$, 3.3 μl 1.25 mM d NTPs, 0.2 μl (5U/μl) *Taq* polymerase and 9.2 μl H_2O (total volume 18 μl). 2 μl template DNA was added to the reaction mixture. PCR conditions for the first cycle were: 30 sec at 94 ^{o}C, 30 sec at 65 ^{o}C, and 1 min at 72 ^{o}C. This was followed by 9 cycles over which the annealing temperature was decreased by 1 ^{o}C per cycle followed by 30 sec at 94 ^{o}C, 30 sec at 65 ^{o}C, and 1 min at 72 ^{o}C for 25 cycles.

Gel electrophoresis

The PCR product was mixed with 20 µl of gel loading buffer and denatured by incubation for 3 min at 90 °C. Gels were prepared by mixing 40 ml of sequencing gel (Sequagel-6®), 10 ml of complete buffer reagent (Sequagel®) and 450 µl 1% ammonium persulphate. Samples of 2 µl were loaded in 5x TBE per track and gels were run for 2 hours at 40 W, transferred to 3 MM chromatography paper and dried on a gel drier (Biopad®) for 45 min at 90 °C. Gels were exposed to X-ray film for about 2 days at room temperature.

Data analysis

Bands were scored as present (score 1) or absent (score 0) on autoradiographs. Monomorphic fragments were not included in the analysis, as suggested by Link *et al*. (1995). Estimates of divergence among genotypes were based on the probability that an amplified fragment from one accession was also present in another.

Table 3. Results of AFLP analysis of Acc 290, 622, 970 and Icarus using *Pst* I and *Eco* RI primers in combination with *Mse* I. Primer combinations that produced clear polymorphic band patterns and were selected for further testing are identified in bold print.

Tabel 3. Hasil analisis AFLP pada Acc 290, 622, 970 dan Icarus dengan menggunakan primer Pst I and Eco RI yang dikombinasikan dengan primer Mse I.. Kombinasi primer yang menghasilkan polimorpi yang jelas tertera dengan cetak tebal dan kombinasi tersebut yang digunakan dalam eksperimen ini.

	Pst I	*Eco* RI		
Mse I	ACA	AG	CAG	ACT
A	no band	no band	no polymorp	no polymorp
AC	no band	no band	no polymorp	no polymorp
ACA	no band	no band	no polymorp	no polymorp
ACT	not clear band	no band	no polymorp	no polymorp
C	no band	no band	no polymorp	no polymorp
CAA	not clear band	no polymorp	no polymorp	no polymorp
CAG	**polymorphic**	no polymorp	no polymorp	no polymorp
CAT	not clear band	no polymorp	no polymorp	no polymorp
CCA	**polymorphic**	no polymorp	no polymorp	no polymorp
CCT	no polymorp	no polymorp	no polymorp	no polymorp
CGA	**polymorphic**	no polymorp	no polymorp	no polymorp
CTA	no polymorp	no polymorp	no polymorp	no polymorp
CTG	no polymorp	no polymorp	no polymorp	no polymorp
G	no polymorp	no band	no polymorp	no polymorp
GAA	not clear band	no band	no polymorp	no polymorp
GAT	no band	no polymorp	no polymorp	no polymorp
GCC	low polymorphic	no polymorp	no polymorp	no polymorp
GTG	not clear band	no polymorp	no polymorp	no polymorp
TAA	no band	no polymorp	no polymorp	no polymorp
TG	no band	no polymorp	no polymorp	no polymorp
TGC	low polymorphic	no polymorp	no polymorp	no polymorp

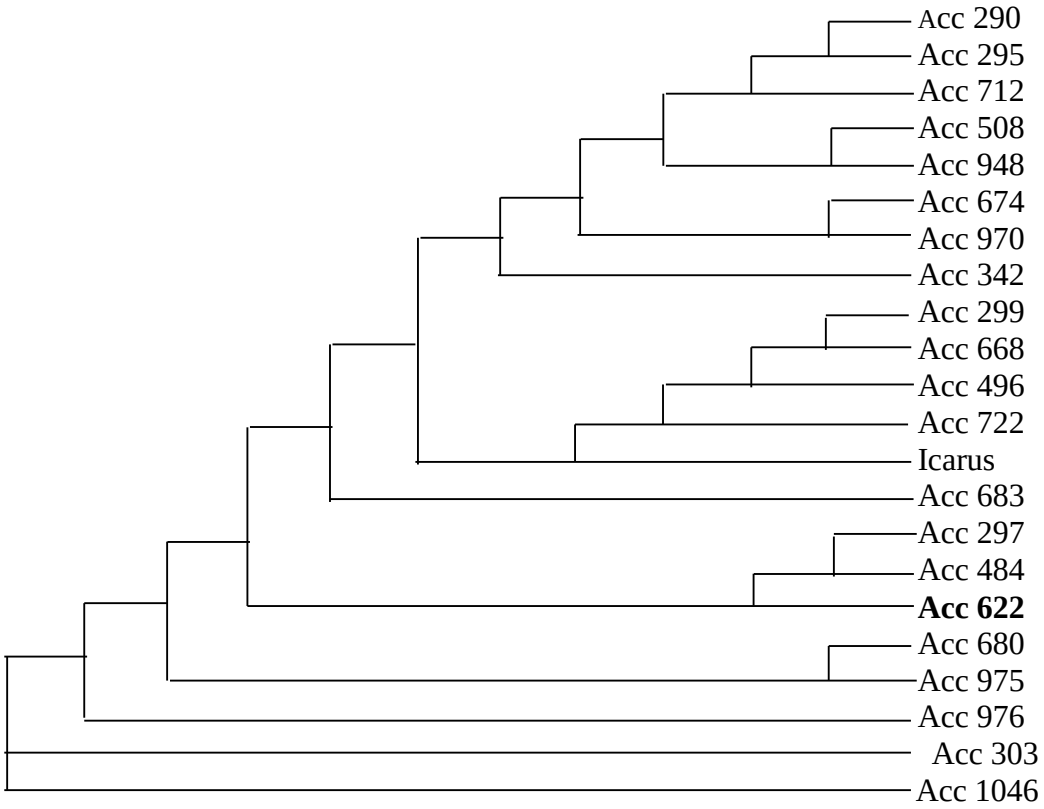

Figure 1. Dendogram of 22 faba bean accessions following AFLP analysis, using Acc 1046 as the outgroup

Gambar 1. *Dendogram 22 galur faba bean hasil dari analisis AFLP dengan memakai galur 1046 sebagai acuan (outgroup).*

The comparisons were calculated by means of Jaccard's formula (1908), according to the equation :

Genetic distance = $1-n_{xy}/(n_x+n_y-n_{xy})$,
where
n_{xy} is the number of bands common to line x and line y,
n_x is the total number of bands in line x,
n_y is the total number of bands in line y.

Genetic distances were used to construct a dendogram, displaying the hierarchical association among all accessions by the use of the average linkage as determined by the cluster procedure of Phylogenetic Analysis Using Parsimony (PAUP) version 3.11, ©Smithsonian Institution, 1993. Acc 1046 was selected as the outgroup to which other accessions were compared, since this accession had the largest average genetic distance (0.44).

RESULTS AND DISCUSSION

The preliminary results showed that 63 pairwise combinations of three *Eco* RI primers and twenty one *Mse* I primers tested against four DNA samples (Acc 290, 622, 970 and Icarus) either did not produce bands or bands were unclear or not polymorphic (Table 3). Alternative combinations of *Pst* I and *Mse* I primers were then used. Combinations of *Pst* ACA and 21 primers of *Mse* I were tested against four accessions (Acc 290, 622, 970 and Icarus). Three combinations revealed clear polymorphisms, and they were selected to analyse the full set of twenty two accessions.

A total of 176 bands were generated by the three AFLP primer combinations and 104 bands (62%) were polymorphic. The number of polymorphisms detected by individual primer pairs was 42, 23 and 39 for primers *Pst* ACA - *Mse* CAG, *Pst* ACA - *Mse* CCA and *Pst* ACA - *Mse* CGA, respectively. Figure 3 shows the DNA finger prints of 22 accessions after

PCR with the primer combination *Pst* ACA - *Mse* CAG.

The average genetic distance over all accessions was 0.34. The greatest distance of 0.53 was between Acc 297 and Acc 299 while the most similar (different at 9% of bands) were Acc 299 and Acc 668. The average genetic distance for individual accessions ranged from 0.30 for Acc 712 and Icarus to 0.44 for Acc 1046 (Table 4).

The dendrogram constructed on the basis of shared fragments divided the 22 accessions into two major groups, one of which comprised eight accessions (Acc 290, 295, 712, 508, 948, 674, 970 and 342) and the other group included five accessions (Acc 299, 668, 496, 722 and Icarus). The remaining accessions were either unpaired or as groups of two or three (Fig. 1). Acc 622 did not occur in either of the major groups but grouped with Acc 297 and Acc 484. When considered on a pairwise-basis most accessions had a genetic distance from Acc 622 of less than 0.40, while Acc 1046 was the most divergent with a value of 0.44 (Table 4).

The accessions included in the study originated from a wide range of countries, including the Middle East, North Africa, Northern Europe and Equador. There was no consistent trend between grouping on the dendogram (Fig. 1) and regions of origin. For example, four accessions (Acc 295, 297, 299 and 303) originated from Lebanon but they were evenly distributed across the dendogram and the genetic distance between each pair in this group of four accessions was greater than the average genetic distance over all accessions. Similarly, the two accessions of Moroccan origin (Acc 290 and 496) and the two Iraqi accessions (Acc 484 and 680) did not show a high degree of genetic similarity.

AFLP analysis using three primer combinations was used to construct a phylogenetic tree of 22 faba bean accessions resulting in two major groups of 8 and 5 accessions, and several groups of two or three accessions. The average genetic distance over all lines tested was 0.34, with the greatest distance among accessions being 0.53 (53%) and the closest distance being 0.09 (9%). These values are lower than those reported by Link *et al*. (1995) for genetic distances determined by RAPD analysis. They reported a range in genetic distance over 28 inbreed lines of faba bean, each represented by a single plant, of 0.306 - 0.646. One possible reason for the difference in range in genetic distance between the present study and that of Link *et al*. (1995) could be selection of germplasm tested. The objective of Link *et al*. (1995) study was to identify genetic distance between and within germplasm pools, thus entries were selected to represent a wide geographic range.

In the present study the major criterion for selection of entries was resistance to *A. fabae*, while two susceptible entries were included for comparison. This narrow focus could reasonably be expected to include a lower level of genetic diversity. In addition, the majority of accessions in the present study fall in the "Mediterranean" category, which Link et al. (1995) described as having a lower level of genetic diversity than European germplasm.

The number of AFLP primer pairs used to classify cultivars for purposes of determining genetic diversity differs between authors. Satoru *et al*. (2004) analysed 32 genotypes of tea with 5 primer combinations, which revealed a total number of 73 polymorphic amplified DNA fragments, and these classified the genotypes to three groups. Sharma *et al*. (1996) used a single primer combination to analyse 54 genotypes of *Lens*, and revealed 23 AFLPs from which a dendogram was constructed. Similarly, Zhu *et al*. (1998) concluded that one primer combination which can generate more than 30 polymorphic markers could be sufficient to classify 57 cultivated rice accessions, although as more markers were included in the analysis, there were fewer ambiguities.

There was little relationship between genetic similarity determined by AFLP analysis and region of origin. Although two accessions from northern Europe, Acc 948 and 970, fell into the same group, each Lebanese accession, Acc 295, 297, 299 or 303 occurred in a different group, as did the accessions from Morocco (Acc 290 and 496). This could indicate that there is as much diversity on a regional scale as across the Mediterranean basin, from where most accessions were derived. In addition, it could be a consequence of the criterion of selecting the entries, which resulted in a restricted gene pool tested.

Table 4. Similarity matrix of 22 accessions of faba bean as determined by AFLP analysis
Tabel 4. Matrik keeratan dari 22 galur faba bean berdasarkan hasil analisis AFLP.

Acc	295	297	299	303	342	484	496	508	622	668	674	680	683	712	722	948	970	975	976	1046	Icarus
290	0.33	0.35	0.45	0.43	0.34	0.36	0.39	0.35	**0.42**	0.39	0.46	0.35	0.30	0.28	0.38	0.36	0.42	0.40	0.33	0.40	0.37
295		0.46	0.38	0.44	0.39	0.42	0.28	0.34	**0.39**	0.35	0.39	0.40	0.31	0.32	0.35	0.35	0.39	0.32	0.44	0.47	0.29
297			0.53	0.36	0.38	0.35	0.53	0.45	**0.38**	0.49	0.46	0.34	0.39	0.44	0.43	0.44	0.46	0.44	0.38	0.40	0.45
299				0.48	0.26	0.47	0.15	0.33	**0.32**	0.09	0.24	0.36	0.32	0.33	0.20	0.28	0.33	0.39	0.35	0.49	0.29
303					0.33	0.46	0.48	0.43	**0.42**	0.41	0.47	0.36	0.39	0.44	0.39	0.39	0.35	0.44	0.32	0.40	0.39
342						0.38	0.29	0.30	**0.28**	0.20	0.28	0.29	0.18	0.25	0.23	0.29	0.30	0.36	0.21	0.41	0.25
484							0.46	0.42	**0.29**	0.41	0.43	0.41	0.34	0.37	0.43	0.45	0.40	0.37	0.38	0.51	0.41
496								0.35	**0.33**	0.12	0.25	0.37	0.28	0.31	0.20	0.32	0.32	0.34	0.36	0.46	0.24
508									**0.33**	0.30	0.35	0.37	0.37	0.32	0.34	0.29	0.35	0.37	0.36	0.40	0.30
622										**0.28**	**0.32**	**0.33**	**0.24**	**0.33**	**0.24**	**0.38**	**0.30**	**0.34**	**0.31**	**0.48**	**0.26**
668											0.22	0.29	0.25	0.25	0.14	0.30	0.27	0.33	0.28	0.43	0.22
674												0.38	0.26	0.33	0.26	0.30	0.28	0.40	0.35	0.47	0.31
680													0.31	0.35	0.34	0.33	0.36	0.32	0.27	0.38	0.31
683														0.27	0.23	0.31	0.30	0.31	0.23	0.50	0.23
712															0.27	0.31	0.31	0.42	0.30	0.39	0.22
722																0.32	0.26	0.35	0.32	0.46	0.17
948																	0.35	0.33	0.30	0.45	0.33
970																		0.41	0.33	0.39	0.32
975																			0.32	0.45	0.34
976																				0.44	0.35
1046																					0.50

If a greater proportion of lines from non-Mediterranean regions, such as northern Europe and China, had been included, the entries in the present study might form one major group among several. It is interesting to note that although the susceptible cultivar, Icarus, is derived from an Ecuadorean landrace, it groups with accessions of Mediterranean basin origin. This probably reflects the fact that the Spaniards introduced faba beans to South America in the post-Columbian era.

Accessions derived from the same source (ILB) did not necessarily fall within the same group. Acc 299 and 303 were both derived from ILB 287 but were genetically distant with a pair-wise genetic difference of 0.48, while Acc 484 and 680 derived from ILB 37 had a genetic distance of 0.41, higher than the average genetic distance overall accessions of 0.34. This indicated that there is a large degree of genetic variation within the landrace populations.

On the other hand, Acc 722 and Icarus were closely related with a genetic distance of 0.17. One parent of Acc 722 is ILB 938, the same population BPL 710 (source of Icarus) was derived from.

Acc 622, which was the standard accession for comparison of resistance genes in Chapter 6, did not fall within either the two major groups. Most accessions with different resistance genes to Acc 622 were in the one group (Acc 712, 948, 674 and 970) while Acc 299 was in the other major group (Figure 3). Despite the accessions with alternative resistance genes occurring in different groups to Acc 622, when examined on a pair-wise basis there did not appear to be a relationship between *A. fabae* resistance genes and genetic distance from Acc 622 (Table 4).

Acc 484 grouped with Acc 622 and the value of genetic distance was less than the average overall accessions (0.29). This is consistent with the previous screening result (preliminary testing) where it was concluded that both accessions have the same resistance gene. Acc 297, which was also considered to carry the same resistance gene as Acc 622 was also in the same group as Acc 622.

CONCLUSION

In conclusion, AFLP analysis offers a potential means of assessing genetic variations among accessions in faba bean. The analysis was inconclusive and this was possibly due to the large amount of genetic variation among accessions representing resistance to *A. fabae*, including accessions derived from the same origin. The high level of polymorphism displayed should enable the AFLP technique to be utilised in genetic mapping of faba beans. The results of genetic distance between pairs of accessions should enable selection of appropriate parents for mapping populations to maximise the level of genetic variation while including the specific traits to be mapped.

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