GENETIC IDENTIFICATION OF SOME EGYPTIAN VICIA FABA L. CULTIVARS AND THEIR RELATION TO OROBANCHE CRENATA L. TOLERANCE

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ABSTRACT

Ten Egyptian faba bean cultivars were assessed for Orobanche tolerance in a field trial during the winter season 2002-2003. Two traits related to Orobanche infection were estimated i.e., number of spikes/plant and weight of Orobanche stems/plant. Statistical analysis revealed differences among the studied cultivars in relation to Orobanche tolerance/susceptibility. The results confirmed that Giza 843 and Misr1 were the most tolerant cultivars, while Giza 643, Giza716 and Giza717 were the most susceptible ones. SDS-PAGE and RAPD analysis were used to study the genetic relationships among the ten faba bean cultivars and to detect some molecular genetic markers for Orobanche tolerance /susceptibility. Both SDS-PAGE and RAPD analysis revealed distinctive bands that successfully determined tolerant from susceptible bean cultivars. Data analyses for both SDS-PAGE and RAPD-PCR were used to construct a dendrogram showing the genetic relationships among the faba bean cultivars and fitted well with their pedigrees.

INTRODUCTION

Broad bean (Vicia faba L.) is one of the most important winter crops in Egypt. It is consumed by human and animal as a leguminous food with high protein content (22-30%). Plants frequently encounter external stress conditions that badly affect growth, development and productivity. Stress can be either abiotic or biotic. Biotic stresses

include the exposure of plants to infection with different organisms such as bacteria, virus, fungi, insects and parasitic weeds. Some of these biotic stress causal agents can cause severe damage in different crops. Parasitic weeds are serious problem in many agricultural production systems. Unlike normal weeds that merely compete with the crop plants for nutrition and harbor diseases, root parasitic weeds damage the crops by attaching their own roots to the roots of the crop plant and taking their nutrition and water. They are especially hard to control because they can not be treated as a separate plant and because they inflict much damage before emerging above the ground (Verkleij and Kuijper, 2000).

Genetic resistance of Orobanche was found to be associated with polygenes and showed complete dominance over susceptibility (Cuberu, 1973). Number of Orobanche stems per plant (or per m2) appeared to be the most stable resistance expression and the most suitable for use in screening (Boorsma, 1980). Moreover, additive model with no dominance for the genetic control of resistance to O. crenata L. was also proposed (Cubero and Hernandez, 1991). The inheritance of parasitism is not simple; and that recessive quantitative genes conferring resistance/tolerance were involved (Darwish et al., 1999).

Evolutionary relationships were efficiently investigated using SDS-PAGE patterns of seed protein. Protein banding pattern was efficiently used to identify different genotypes (Haider et al., 2001; El-Fiky et al., 2002). Electrophoretic protein banding patterns of Egyptian V. faba cultivars and some of their induced mutants were used to speculate the genetic system controlling resistance vs. susceptibility to O. crenata at a biochemical level, which showed a unique banding pattern for each suggesting that resistance to O. crenata in V. faba is controlled by a polygenic system (Hussein et al., 2000). Some protein markers were found to be related to some economic traits that are very valuable in crop breeding (Sayed, 2005; El-Sayed, 2006).

RAPD markers are easily generated by PCR, useful for the rapid generation of phylogenetic relationship data and have been used for bulked sergegant analysis in cereals and other numerous plant species (Rafalski and Tingey, 1993; Jones et al., 1997; Rashed et al., 2006; Fahmy et al., 2007; Abdel-Tawab et al., 2008).The advantages and disadvantages of particular molecular markers need to be assessed for

each breeding program as the cost and speed of the analysis are of primary importance. Haider et al. (2001) concluded that RAPDs-system is useful for classification of germplasm and identification of divergent heterotic groups in faba bean (El-Sayed, 2006). However, DNA markers provide a unique opportunity to monitor introgression of genes of resistance in breeding programmes (Filho et al., 1999).

The objectives of this study were to assess the Orobanche tolerance of ten Egyptian faba bean cultivars, to study the genetic diversity and relationships among these cultivars and to detect some molecular genetic markers related to Orobanche tolerance in faba bean.

MATERIALS AND METHODS

This investigation was carried out in the laboratories of the Genetics Department, Faculty of Agriculture, Ain Shams University, and Cell Research Department, Field Crops Research Institute, ARC, Giza, Egypt. Ten faba bean (V. faba L.) cultivars were supplied by Leguminous Crops Research Department, ARC, Egypt. Table (1) shows the cultivars with their pedigrees and the planting zones in Egypt.

Table (1): List of the ten faba been cultivars, their pedigrees and planting zone in Egypt

Code No.	Cultivar name	Pedigree*	Planting zone in Egypt **
1	Giza 3 (G3)	G.1X NB 19	North Delta
2	Giza 40 (G40)	Single plant selection from R40	Upper Egypt and New valley
3	Giza 429 (G429)	Single plant selection from 402	Upper Egypt
4	Giza 461 (G461)	G. 3 X ILB 938	North Delta
5	Giza 643 (G643)	249/801/80	South Delta
6	Giza 674 (G674)	F. 402 X BPL 582	Middle and Upper Egypt
7	Giza 716 (G716)	461/842/83 X 503/ 453/83	North Delta
8	Giza 717 (G717)	503/453/83 X ILB 938	North Delta
9	Giza 843 (G843)	561/276/85 X 461/845/83	North Delta and Middle Egypt
10	Misr 1 (M1)	G.3 X 123 A/45/76	All Egypt

*&** Data were obtained from Leguminous Crops Section, ARC.

I- Orobanche tolerance assessment

A field experiment was performed in Giza experimental farm, ARC, to evaluate the tolerance of the ten faba bean cultivars infected with Orobanche sown in sick plots (plots heavily infected with O. crenata seeds). A randomized complete block design with three replications was adopted. Data were recorded for the number and weight of Orobanche/faba plant as well as four yield-related traits for faba bean; plant height, plant dry weight, number of branches/plant and 100-seed weight. Statistical analyses were performed using LSD method (Duncan, 1995).

II- Molecular genetic studies

1- SDS-protein electrophoresis (Water-soluble proteins)

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to detect the protein banding patterns of the ten faba cultivars including the highly tolerant and susceptible cultivars for Orobanche crenata. Protein fractionation was performed on vertical slab (16.5 cm x 18.5 cm x 0.2 cm, Hoefer E600, Amersham Pharmacia biotech) according to the method of Laemmli (1970) as modified by Studier (1973). Gels were stained with Comassie brilliant blue; photographed and scanned with Bio-Rad video densitometer Model 620 USA, at a wavelength of 577. Software data analysis for Bio-Rad Model 620 densitometer and computer were used.

2- Randomly amplified polymorphic DNA (RAPD)

Five seeds from each faba bean cultivar were germinated in plastic pots for 2 weeks. DNA isolation from plant tissues was done using DNA-easy plant Mini Kit (QIAGEN) according to Tao et al. (1993). PCR reaction was conducted using ten arbitrary 10-mer primers; their universal names and sequence are illustrated in Table (2). The amplification was carried out according to Williams et al. (1990) in a DNA thermocycler (MWG-BIO TECH Primuse) programmed as follows: One cycle at 94oC for 2 min, 45 cycles each of 94oC for1 min; 35oC for 1:30 min; and 72oC for 2 min and one cycle of 72oC for 5 min, then 4oC infinite. Gels were photographed and scanned with Bio-Rad Video Densitometer Model 620 USA, at a wave length of 577, the software instruments for data analysis,

densitometer and computer were used. Bands were considered identical when having the same molecular size.

Table (2): List of the ten primer names and their nucleotide sequences using RAPD-PCR

Primer code	Sequence →	Primer code	Sequence →
OP-A07	5′GAAACGGGTG 3′	OP-F04	5′ GGTGATCAGG 3′
OP-B14	5′ TCCGCTCTGG 3′	OP-F05	5′ CCGAATTCCC 3′
OP-B17	5′ AGGGAACGAG 3′	OP-F06	5′ GGGAATTCGG 3′
OP-B20	5′ GGACCCTTAC 3′	OP-F08	5′ GGGATATCGG 3′
OP-F01	5′ ACGGATCCTG 3′	OP-F09	5′ CCAAGCTTCC 3′

3- Genetic relationships among faba bean cultivars

Cluster analysis (similarity index) based on combined analysis of both SDS-PAGE and RAPD was calculated using un-weighted pair group method analysis to deduce a dendrogram showing the genetic relationships among the studied faba bean cultivars.

4-Molecular genetic markers related to Orobanche tolerance / susceptibility

The densitometric analysis for both SDS-PAGE and RAPD combined data related to Orobanche tolerance assessments were used to detect molecular genetic markers for Orobanche tolerance in faba bean.

RESULTS AND DISCUSSION

I. Orobanche tolerance assessment in faba bean

A field experiment was conducted using ten Egyptian faba bean (V. faba L.) cultivars sown in sick plots (plots heavily infected with O. crenata). Two traits that directly related to Orobanche infection, i.e., number and weight of Orobanche spikes/plant were estimated to assess differences in Orobanche tolerance/susceptibility of the ten V. faba L. cultivars. Table (3) presents the obtained data for the studied traits which showed significant differences among cultivars. Significant differences were observed among some cultivars for number of Orobanche spikes/plant. G674, G843, and M1 cultivars

showed the lowest mean values for number of Orobanche spikes/plant, while G717, G716 and G643 had the highest values. Statistical analysis for weight of Orobanche spikes/plant showed highly significant differences among cultivars. M1 and G843 exhibited the lowest mean values for this trait, while the highest mean values were recorded for G717, G643 and G716.

Table (3): Means of two Orobanche-related traits and four yield-related traits in the ten faba bean cultivars

Cultivar name	No. *Orobanche* spikes/plant	Weight of *Orobanche*/ plant (g)	No. of branches /plant	Plant height (cm)	100-Seed weight (g)	Plant dry weight (g)
G3	1.32	69.17	3.87	80.43	14.55	170.00
G40	1.27	87.00	3.47	80.57	14.08	200.00
G429	0.90	72.83	3.77	80.77	15.57	197.00
G461	1.37	51.83	3.57	68.80	15.92	115.00
G643	2.74	133.00	4.00	80.27	16.30	226.00
G674	0.57	71.83	4.13	83.60	16.19	170.00
G716	3.33	111.00	3.77	69.37	16.65	240.00
G717	3.77	143.70	3.37	81.73	17.02	165.00
G843	0.58	23.83	3.83	81.30	16.75	170.00
M1	0.60	26.67	4.20	78.83	15.08	230.00
L.S.D. at 5%	1.16	69.17	0.96	19.81	0.47	52.39

Therefore, the three cultivars; G674, G843, and M1 can be considered as Orobanche tolerant genotypes, while G643, G716 and G717 were the most susceptible cultivars. These results agreed with Abdalla and Darwish (1998) and Ghalwash (2003) who reported that number and weight of Orobanche spikes/plant increased in the susceptible cultivars and vise versa. In addition, it is well known that stress sensitivity or resistance depends on the species, the genotype and the developmental stage of the plant.

Means of the four yield-related traits; branches no./plant, plant height (cm), 100-seed weight (g) and plant dry weight (g); for the ten faba cultivars under Orobanche infection are shown in Table (4). Non-

significant differences were observed for number of branches/plant and plant heights, while 100-seed weight (g) and plant dry weight traits showed little significant differences. From data analysis (Table 4); M1 and G843, which were considered as the most tolerant cultivars, exhibited good performance for most yield-related traits. On the other hand, G717 which was considered as the most susceptible genotype and G716 and G643 that were moderately susceptible cultivars did not show good results for these yield-related traits. However, Abdalla and Darwish (1994) found that more parasite infestation are usually accompanied by reduced 100-seed weight, relatively lower host growth, low flowers and pods and highly affected yield of pods on plants.

II- Molecular genetic studies

1- Identification based on SDS-protein

The electrophoretic banding patterns of proteins extracted from the seeds of the ten faba bean cultivars are shown in Figure (1) and their densitometric analysis are illustrated in Table (4). The presence or absence of bands was marked with (1) or (0), respectively. The results of SDS-PAGE revealed a total number of 32 bands with molecular weights (MW) ranging from about 11.00 to 91.00 kDa, which were not necessarily present in all cultivars. Data showed 16 common bands (monomorphic), while the remaining 16 bands were polymorphic with 50% polymorphism.

The ten cultivars showed different patterns in presence of bands (Figure 1 and Table 4). There was no resemblance between any two cultivars and each cultivar was characterized by a unique fingerprint. At the same time, there was a marker band(s) for some cultivars such as band 2 at MW of 78 kDa in G716, bands 3, 6 and 10 at 74, 62 and 54 kDa, respectively, in G643 and G716 cultivars, band 11 at MW of 51 kDa for cultivar M1, band 20 at 29 kDa for G674 and G717 cultivars, band 23 at MW of 20 kDa for G643 and M1 cultivars, band 27 at 16 kDa for G461 and G674 and band 29 at 14 kDa for G461 and G643. However, there were some negative markers such as the absence of band 7 at 60 kDa for G717, band 19 at 32 kDa for G3 and G461 and band 22 at 27 kDa for G461 (Table 4). The densitometric analysis of the SDS-protein banding patterns was found to be useful in the identification of the faba bean cultivars.

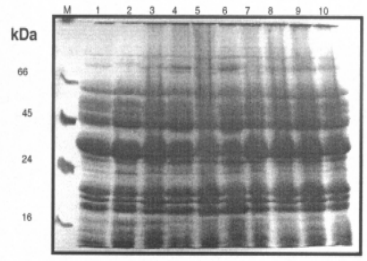

Figure (1): SDS-protein banding patterns for the seeds of the ten faba bean cultivars

M= Protein marker 1= Giza 3 2= Giza 40 3= Giza 429 4=Giza 461 5= Giza 643 6= Giza 674 7= Giza 716 8= Giza 717 9= Giza 843 10= Misr 1

Table (4): Densitometric analysis for SDS-seed storage protein (water soluble fraction) in the ten faba bean cultivars

Band No.	MW (kDa)	G3	G40	G429	G461	G643	G674	G716	G717	G843	M1
1	91	1	1	1	1	1	1	1	1	1	1
2	78	0	0	0	0	0	0	1	0	0	0
3	74	0	0	0	0	1	0	1	0	0	0
4	72	1	1	0	1	1	0	1	1	1	1
5	66	1	1	1	1	1	1	1	1	1	1
6	62	0	0	0	0	1	0	1	0	0	0
7	60	1	1	1	1	1	1	1	0	1	1
8	59	1	1	1	1	1	1	1	1	1	1
9	55	0	0	1	1	1	1	1	0	0	0
10	54	0	0	0	0	1	0	1	0	0	0
11	51	0	0	0	0	0	0	0	0	0	1
12	50	1	1	1	1	1	1	1	1	1	1
13	48	1	1	1	1	1	1	1	1	1	1
14	46	1	1	1	1	1	1	1	1	1	1
15	45	1	1	1	1	1	1	1	1	1	1
16	36	0	0	0	0	1	0	1	0	1	0
17	35	1	1	1	1	1	1	1	1	1	1
18	33	1	1	1	1	1	1	1	1	1	1
19	32	0	1	1	0	1	1	1	1	1	1
20	29	0	0	0	0	0	1	0	1	0	0
21	28	1	1	1	1	1	1	1	1	1	1
22	27	1	1	1	0	1	1	1	1	1	1
23	20	0	0	0	0	1	0	0	0	0	1
24	19	1	1	1	1	1	1	1	1	1	1
25	18	1	1	1	1	1	1	1	1	1	1
26	17	1	1	1	1	1	1	1	1	1	1
27	16	0	0	0	1	0	1	0	0	0	0
28	15	1	1	1	1	1	1	1	1	1	1
29	14	0	0	0	1	1	0	0	0	0	0
30	13	1	1	1	0	0	0	1	1	1	1
31	12	1	1	1	1	1	1	1	1	1	1
32	11	1	1	1	1	1	1	1	1	1	1
Total		20	21	21	21	27	22	27	21	21	22

1= Band presence 0= Band absence

Table (5): Number of amplified fragments and specific markers of the ten faba bean cultivars based on RAPD-PCR analysis with 10-random primers

Primers			Cultivars																				
			G.3		G40		G429		G461		G 643		G674		G716		G717		G843		M1		
	TAF	PB	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	TSM
OP-A07	5	-	5	-	5	-	5	-	5	-	5	-	5	-	5	-	5	-	5	-	5	-	-
OP-B14	15	10	9	1	11	-	8	-	11	-	12	-	10	-	8	1	11	-	12	-	12	-	2
OP-B17	19	18	12	-	11	-	12	-	12	-	11	-	8	2	14	1	11	1	15	2	12	-	6
OP-B20	15	13	9	2	8	-	8	-	10	1	8	-	8	-	9	-	9	-	8	-	10	-	3
OP-F01	11	8	9	-	8	-	6	-	6	-	4	-	7	-	6	-	4	-	4	-	8	2	2
OP-F04	6	4	3	-	3	2	5	1	3	-	3	-	3	-	3	-	3	-	3	-	4	-	3
OP-F05	5	3	5	-	5	-	3	-	2	-	2	-	5	-	2	-	3	-	3	-	4	-	-
OP-F06	12	9	9	-	5	-	9	-	4	1	4	-	4	-	9	1	4	-	9	-	7	-	2
OP-F08	6	4	4	-	4	-	3	-	5	-	3	-	5	-	5	1	3	-	3	-	5	-	1
OP-F09	4	3	4	-	3	-	3	-	4	-	3	-	4	-	3	-	3	-	3	-	3	-	-
Total	98	72	69	3	63	2	62	1	62	2	55	-	59	2	64	4	56	1	65	2	70	2	19

TAF= Total amplified fragments, PB = Polymorphic bands, AF = Amplified fragments, SM = Specific markers including either the presence or absence of a band, TSM = Total number of specific markers

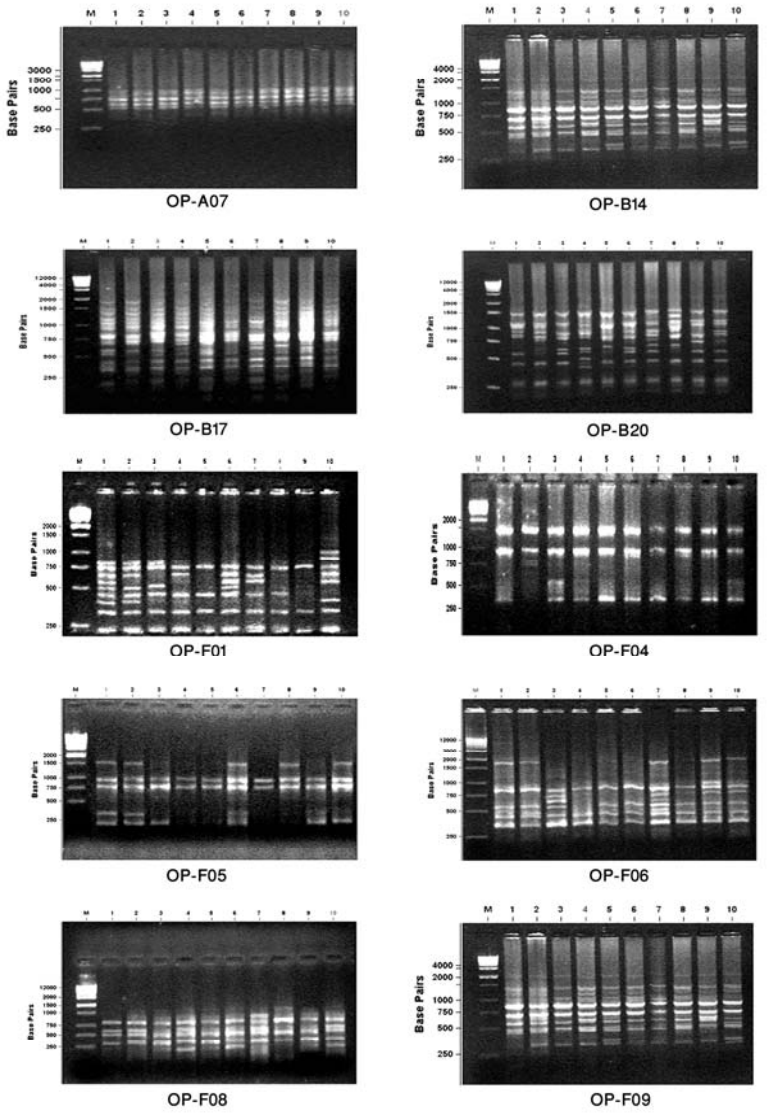

Figure (2): RAPD banding patterns generated by ten- random primers.

M= DNA marker 1= Giza 3 2= Giza 40 3= Giza 429
4= Giza 461 5= Giza 643 6= Giza 674 7= Giza 716
8= Giza 717 9= Giza 843 10= Misr 1

These results confirmed that SDS-protein PAGE is a highly successful technique in cultivar identification which coincides with Hughes and Murphy (1983), Goodrich et al. (1985), Abdel-Tawab et al. (1993), Potokina and Eggi (1997). Jaramillo et al. (1999) reported that SDS-PAGE was widely used to separate proteins, directly related to genetic background and can be used to certify the genetic makeup of wild, cultivars, or newly derived cereal plants.

2- Identification based on RAPD analysis

RAPD-PCR was used to analyze the genetic diversity of the ten faba bean cultivars. Ten-arbitrary random primers (Table 2) were used to determine RAPD polymorphism of the ten Egyptian faba bean cultivars. The amplified fragments and specific markers for Orbanche tolerance are shown in Figure (2) and Table (5). All the ten primers successfully amplified DNA fragments for all genotypes. A total number of 98 fragments were visualized across the ten investigated genotypes. Band number ranged from 4 fragments (primer OP-F09) to 19 (primer OP-B17) across cultivars. Polymorphism mean across the 10 primers was 69.4% (Figure 2 and Table 5). One primer (OP-A07), only, showed no polymorphic differences among cultivars, while primers OP-F08 and OP-F09 exhibited low polymorphism. On the other hand, primers OP-B17, OP-B20, OP-F06 and OP-F01 exhibited high polymorphic differences and were useful in faba bean cultivar identification.

RAPD-PCR analysis requires neither cloning nor sequencing of DNA, and it can detect several loci simultaneously. Short 10-mer random primers are frequently used to amplify DNA and usually detect polymorphism. Instead of being simple, in-expensive and fast, RAPDs reproducibility are sufficiently problematic for polygenetic studies unless great care is made to ensure stringent annealing conditions (Hash and Bramel-Cox, 2000). The previous results are in agreement with Link et al. (1995) and El-Sayed (2006) who reported that RAPDs data are useful for classification of germplasm and identification of divergent heterotic group in faba bean.

3- Genetic relationships among faba bean cultivars

Cluster analysis based on combined SDS-PAGE and RAPD-PCR data as shown in Table (6) was carried out using UPGMA computer program. The highest similarity index recorded was 0.91 between G3 and G40 cultivars, while the lowest similarity index (0.76) was observed between G674 and M1 cultivars. Dendrogram for the genetic relationships among the ten cultivars is shown in Figure (3). The cultivars were separated into three clusters. Cluster one included G3, G40, G429, G843 and M1; cluster 2 included G461, G643, G674 and G716, while cluster 3 contained only G717. Within the first cluster, 3 sub-clusters were obtained; one contained two faba bean cultivars (G3 and G40), the second contained two faba bean cultivars (G429 and G843), and the third had one faba bean cultivar (M1).

Table (6): Similarity indices (pairwise comparison) among the ten faba bean cultivars based on SDS-protein and RAPD-PCR data

Cultivar	G3	G40	G429	G461	G643	G674	G716	G717	G843	M1
G40	0.91									
G429	0.84	0.83								
G461	0.82	0.81	0.81							
G643	0.79	0.83	0.86	0.87						
G674	0.80	0.80	0.82	0.84	0.85					
G716	0.81	0.80	0.83	0.82	0.85	0.80				
G717	0.77	0.81	0.80	0.79	0.82	0.80	0.77			
G843	0.82	0.83	0.87	0.78	0.82	0.77	0.81	0.81		
M1	0.82	0.84	0.80	0.78	0.80	0.76	0.81	0.77	0.86	---

The second cluster was divided into three subclusters, the first contained two faba bean cultivars (G461 and G643), while the other two subclusters contained one faba bean cultivar each, i.e., G674 and G716, respectively. The study of genetic diversity using protein electrophoresis and RAPD-PCR analysis seemed to be powerful means and could discriminate among the ten faba bean cultivars. Bagheri et al. (1995) and Hoey et al. (1996) found that RAPDs could

be used in clarifying genetic relationships within a species and also it could be useful in cultivar identification in Pisum sativum (L) that both protein and RAPD-PCR results appear to play an important role in the differentiation among different cultivars.

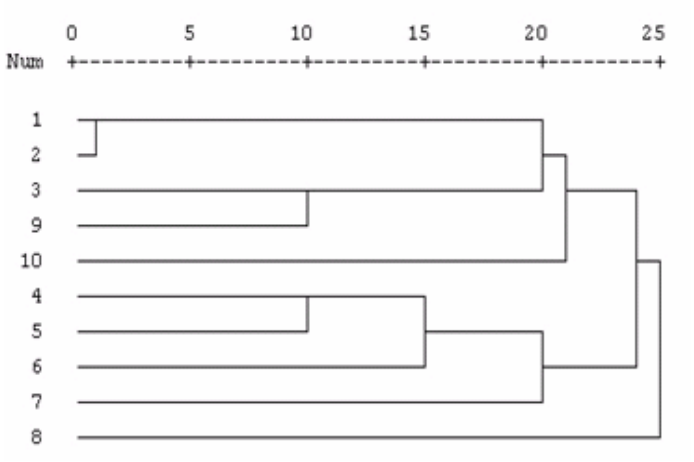

Figure (3): Dendrogram for the genetic relationships among the ten faba bean cultivars based on SDS-protein and RAPD-PCR data.

1= Giza 3 2= Giza 40 3= Giza 429 4= Giza 461 5= Giza 643
6=Giza 674 7= Giza 716 8= Giza 717 9=Giza 843 10= Misr 1

4-Molecular markers related to Orobanche tolerance/ susceptibility

Molecular genetic markers for Orobanche tolerance based on SDS-protein and RAPD-PCR are grouped in Table (7). SDS-protein pattern analysis of the ten faba bean cultivars (Figure 1 and Table 3) exhibited some markers for Orobanche tolerance (Table 7). The presence of band 11 with 51 kDa in the tolerant cultivar M1 may be considered as a protein marker for Orobanche tolerance because it appeared as gene expression when this cultivar was sown under Orobanche stress. However, band number 2 with MW 78 kDa was present only in the susceptible cultivar G716, whereas, it was absent in the other cultivars. At the same time, bands number 3 and 6 with MWs 74 and 62 kDa, respectively, appeared only in the susceptible cultivars; G643 and G716 which could be used as protein markers (Figure 1 and Table 7) for the susceptibility of cultivars to Orobanche.

Dorr et al. (1994) revealed that resistance to Orobanche in sunflower was expressed after penetration of host roots. Concurrent to induced stress tolerance, protein metabolism of the cells undergoes changes in terms of acquiring specific proteins which either not detected (inducible) or present in low amounts (constitutive) in non-stressed cells. Stress proteins may be involved in avoiding stress, in repairing damage or in protecting the cellular machinery (Ingram and Batles, 1996, Singla et al., 1997; Grover et al., 1998). In general, the results obtained from SDS-protein electrophoresis support the conclusions reached by biometrical analysis on the importance and usefulness of SDS technique for determining molecular marker bands to be used in Vicia faba improvement programs. This conclusion is in agreement with those of Sayed (2005) and El-Sayed (2006) who found molecular markers to some important traits in alfalfa and faba beans, respectively.

Table (7): Molecular genetic markers for Orobanche tolerance based on SDS-protein and RAPD-PCR

Protein or primer	Cultivar	Molecular marker
Protein	Misr 1 (T) Giza 716 (S) Giza 643 (S)	+ P_{11} (51 kDa) + P_2 (78 kDa), + P_3 (74 kDa), + P_6 (62 kDa) + P_3 (74 kDa), + P_6 (62 kDa)
Primer OP-B14	Giza 716 (S) Giza 717 (S)	-2298 bp, -1373 bp, -1373 bp,
Primer OP-B17	Giza 843 (T) Giza 716 (S) Giza 717 (S)	+2135 bp, -1252 bp -839 bp -649 bp,
Primer OP-F01	Misr 1 (T) Misr 1 (T), Giza 843 (T)	+1080 bp, +933 bp -443 bp
Primer OP-F06	Giza 716 (S)	+1356 bp
Primer OP-F08	Giza 716 (S)	+956 bp,

T = Tolerant cultivar S = Susceptible cultivar + = Positive marker - = Negative marker

RAPD analysis was used, also, to detect molecular genetic markers for Orobanche tolerance (Figure 2 and Table 5). Ninteen RAPD markers were obtained as shown in Table (7); five for Orobanche tolerance using primers; OP-B17, OP-F01, OP-F06 and OP-F08. At the same time, seven markers were detected for Orobanche susceptibility with primers; OP-B14, OP-B17 and OP-F01. Hussein et al. (2000) showed that DNA fingerprints from faba beans

tolerant to broomrape gave unique DNA bands compared to other varieties using RAPDs.

Finally, molecular genetic markers based on SDS-protein and RAPD-PCR confirmed their importance in marker assisted selection (MAS) to select the most Orobanche-tolerant cultivars instead of long breeding programs, and to study the genetic relationships among faba cultivars which agreed with many authors (Williams et al., 1990; Quiros et al., 1991; Fahmy et al., 2006; Abdel-Tawab et al., 2008).

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التعرف الوراثي لبعض أصناف الفول البلدي المصري وعلاقتهم بتحمل الهالوك

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تم تقييم عشرة أصناف من الفول البلدي المصري من حيث تحملها لنبات الهالوك في تجربة حقلية بمركز البحوث الزراعية. قدرت صفتين مرتبطتين بعدوى الهالوك وهما عدد السنابل ووزن سيقان الهالوك لنبات الفول. وقد أظهرت التحليلات الإحصائية وجود إختلافات بين أصناف الفول المدروسة طبقا لدرجة تحملها أو حساسيتها، فكانت الأصناف جيزة 843 ومصر1 هي الأكثر تحملا بينما كانت الأصناف جيزة 643، جيزة 716 وجيزة 717 هي الأكثر حساسية. وقد استخدم تحليل البروتين بطرقة الهجرة الكهربائية بواسطة الـ SDS وتحليل المادة الوراثية باستخدام طريقة البادئات العشوائية بجهاز تفاعل البلمرة المتسلسل RAPD-PCR لدراسة العلاقات الوراثية بين أصناف الفول العشرة، وكذلك تحديد بعض الدلائل الوراثية الجزيئية للتحمل أو للحساسية للإصابة بالهالوك. وقد أظهرت هذه التحاليل حزم مميزة نجحت في تحديد العلاقات الوراثية بين أصناف الفول العشرة وعمل رسم للعلاقات بين هذه الأصناف (Dendrogram)، وكذلك إيجاد الدلائل الجزيئية الهامة التي نجحت في التمييز بين الأصناف المتحملة والحساسة للهالوك.