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Evaluation of Some Faba Bean Genotypes for Resistance to Chocolate Spot

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Nine faba bean lines were screened for resistance to chocolate spot, caused by *Botrytis fabae*, *in vivo*, using the detached leaf technique as well as under growth-chamber controlled conditions, and under field conditions during two seasons, *i.e.* 2006/2007 and 2007/2008, at Maryout Experimental Station.

There were a significant correlation between disease score index and diameter of lesions, using detached leaf assay. The disease progressed gradually by increasing the time of incubation. Genotype Aquadulce recorded the lowest lesion diameter during the tested time with average of 0.18 mm followed by line L8 with 0.25 mm. Genotypes showed different degrees of disease severity using detached leaf assay. Aquadulce and L8 were resistant genotypes 6 days after inoculation. Under growth chamber-controlled conditions, significant differences were detected among the nine faba bean genotypes against chocolate spot according to their Mass Disease Index (MDI). Most genotypes were susceptible (s) to moderately susceptible (MS), and the Spain genotype Aquadulce was moderately resistant (MR) to chocolate spot.

Under field conditions, during the two seasons, 2006-2007 and 2007-2008, the tested genotypes showed different degrees of reaction to chocolate spot which ranged from resistant to moderately susceptible according to their MDI. The check variety Giza 461 and the newly bred line NBL2 scored the lowest MDI (11.1) and classified as resistant, while the other genotypes were classified as moderately resistant to chocolate spot.

Aquadulce genotype recorded the highest values for 100/seed weight (88.7 g.). Line NBL3 recorded the highest values of number of pods /plant while line L8 was the lowest one. Among all genotypes, Line NBL4 exhibited the highest means for number of seeds/ pod (4.8) while line L3 recorded the maximum value for seed yield/plant (69.54 g).

DNA analysis of the tested genotypes showed that there is a specific molecular marker limited with disease resistance by using the primer 841. ISSR - PCR of eight primers discriminated the genotypes tested and the SPSS dendrogram classified them into two main clusters.

Keywords: *Botrytis fabae*, chocolate spot, disease resistance, faba bean, ISSR, molecular markers and similarity.

Faba bean is cultivated in more than 302,260 feddans of the total area cultivated with leguminous crops in Egypt. In spite of the high potential of this crop and the fact that cultural practices are very well mastered; yield is unstable from one year to another. This instability is attributed mainly to pests and diseases which greatly affect both yield and quality (Ibrahim and Nassib, 1979; and Tivoli *et al.*, 1990) particularly chocolate spot caused by *Botrytis fabae* and *B. cinerea* which has been for a long time one of the major limiting factors for development of winter types. Epidemic of this disease can cause severe yield losses (up to 100%), especially when favourable conditions prevail (Yi, 1986). However, fungicide application for control of fungal diseases has a public concern as a result of the hazard effect of fungicide on the environment (Dewaard *et al.*, 1993), induced systemic resistance by prior treatment with simple chemical substances against chocolate spot has been succeeded (Aly, 1989). At present, although genetic resistance to these pathogens generally provides partial protection, the use of resistant cultivars remains the major means to reduce yield losses (Tivoli *et al.*, 1992; Rahaem *et al.*, 2002 and Said *et al.*, 2004)

Biotechnology has been used as a tool to increase field crop productivity in the context of sustainable agriculture (Tecson, 2002). Molecular markers have been used for studying genetic diversity, genotypic identification and for marker assisted selection of major crops such as wheat, barley, canola and faba bean. Moreover, molecular markers such as RAPD and ISSR have recently shown excellent potentiality to assist selection (Afiah *et al.*, 2007a; Afiah *et al.*, 2008 and Torres *et al.*, 2010). The use of molecular markers can increase the efficiency of conventional plant breeding by identifying markers linked to the trait of interest that are difficult to evaluate and/or largely affected by the environment (Stuber, 1992; Semagn *et al.*, 2006 and Khalifallah *et al.*, 2004). So, there is a need to develop a rapid screening method to select the cultivars for chocolate spot resistance.

Tight linkage between molecular markers and genes for disease resistance can be of great benefit to breeding program by allowing the investigator to follow the DNA markers (PCR-based markers) through early generations rather than waiting for phenotypic expression of the resistance genes (Reddy *et al.*, 2002).

The objectives of the present work were to evaluate nine faba bean genotypes for resistance to chocolate spot caused by *Botrytis fabae*; identify new sources of resistance to chocolate spot; to find the most tolerant genotypes under natural infection conditions and to obtain reliable molecular genetic markers for chocolate spot.

Materials and Methods

The pathogen isolate:

Virulent isolate of *B. fabae*, obtained from collections of Plant Protect. Dept., Desert Res. Centre (Ismail, 2004), was used in this study. The fungus was maintained on PDA, transferred to faba bean leaf dextrose agar (FDA) medium (Tivoli *et al.*, 1986) and incubated at 20±2°C in a cycle of 12h darkness and 12h near ultraviolet light to induce sporulation. After 14 days of growth, the spore suspension was prepared in sterile distilled water and adjusted to 10^5 spore/ml.

Evaluation of faba bean genotypes for chocolate spot resistance:

a) Detached leaf technique:

Leaves of nine faba bean genotypes (Table 1) were cut from 4 week-old plants, surface sterilized by soaking in 2% sodium hypochlorite solution for 3 min, rinsed twice in distilled water and dried between filter paper before being placed abaxial surface uppermost in Petri dishes (15 cm in diameter) containing moistened filter paper. The cut ends of the petioles were wrapped with moisten tissue to prevent desiccation. Leaves were inoculated with 20 µL droplets of *B. fabae* (10^5 spore/ml) and incubated at 20°C with 12 h photoperiod provided by florescent Phillips cool white lamps. Lesions diameter were determined 24, 48 and 72 h. from inoculation as well as disease severity was measured 1, 2 and 6 days after inoculation using a 1–4 scale according to Hanounik (1986):

1. Highly resistant: No infection or very small flecks (1-25% necrosis).
2. Resistant: Necrotic flecks with few small lesions (>25-50% necrosis) and very poor sporulation.
3. Moderately resistant: Medium coalesced lesions (>50-75% necrosis) with intermediate sporulation.
4. Susceptible: Large coalesced lesions (>75-100% necrosis) with abundant sporulation.

Table 1. Names, pedigree and origin of the parental genotypes

Genotype name	Pedigree	Origin
G461	G3/ILB938	Egypt
L3	A2/ILB1179	ICARDA[a]
NBL1[b]	(A2/ILB1179)(ILB3879)04SEL-1	Egypt
NBL2	(A2/ILB1179)(ILB3879)04SEL-2	Egypt
NBL3	G461//A2/ILB1179	Egypt
NBL4	G716//A2/ILB1179[c]	Egypt
Aquadulce	ILB1266, kindly obtained from Darwish, I.H.[d]	Spain
L8	ILB3879	Canada
Nubariya-1	An individual plant selection from Rina Blanka	Egypt

a. ICARDA: International Centre for Agricultural Research in the Dry Areas.
b. NBL: Newly bred lines produced through Desert Research Centre Breeding Program. (Afiah and Abdel-Aziz, 2003 and Afiah *et al.*, 2007a).
c. G716:G461//842/83 x 50/455/83.
d. Agron. Dept., Fac. Agric., Shebin El-Kom, Menufiya University.

b) Growth –chamber conditions technique:

The nine faba bean genotypes were tested for chocolate spot resistance under greenhouse in order to confirm the results obtained in the field. 20 cm. diameter plastic pots, filled with sandy soil were used. Five plants were grown in each pot and four pots were used for each genotype. The experiment was carried out with a complete randomized design. Inoculation was done by spraying the fungal spore suspension (10^5 spore /ml) on the foliage with a high-volume sprayer on 4-week-old plants. The plants were then covered with plastic sheets for 48 h. to insure a high level of humidity and kept in the growth room at 20°C with 12h. photoperiod provided by florescent Phillips Cool white lamps.

Disease assessment:

Disease severity was recorded 2 weeks after inoculation for chocolate spot symptoms caused by *Botrytis fabae* on the foliage using a 0–9 scale according to Ding *et al.* (1993). The mass disease index (MDI) of genotypes in the field and greenhouse was determined (Ding *et al.*, 1993). MDI was calculated according to the following formula:

$$MDI= \{[(n1-0) + (n2-1) +(n3-3) + (n4-5) +(n5-7) +(n6-9)] / N -9\} - 100$$

Whereas: 0, 1, 3, 5, 7 and 9 are the disease severity levels on the leaves; *ni*: the number of plants having the same infection level; N: the total number of plants.

The response of the genotypes was expressed as the MDI values. Six resistance levels were used:

- HR (highly resistant), MDI= ranging between 0 and 2.0
- R (resistant),=MDI ranging between > 2.0–15.0
- MR (moderately resistant),=MDI ranging between >15.0–40.0
- MS (moderately susceptible),=MDI ranging between >40.0–60.0
- S (susceptible),=MDI ranging between > 60.0–80.0
- HS (highly susceptible),=MDI ranging between >80.0–100

c) Field experiments:

Field experiments were conducted during two growing seasons, *i.e.* 2006/2007 and 2007/2008 under natural infection conditions at Maryout Experimental Station to evaluate the nine faba been genotypes for chocolate spot resistance. The selected field area has a back history of severe infection with chocolate spot.

Randomized completely block design with four replications was used. The experimental unit consisted of five rows for each genotype. Each row was 3.5 m in length. Row spacing and distance between plants on rows were 50 cm. and 25 cm, respectively. Disease severity was recorded for chocolate spot symptoms on the foliage after the flowering stage using a 0–9 scale according to Ding *et al.* (1993). Seed yield/plant and its components, *i.e.* number of pods/plant, number of branches/plant, number of seeds/pod, and 100-seed weight were recorded for 10 plants of each genotype at harvest.

Detection of Molecular Markers associated with resistance to chocolate spot:

1. DNA preparation:

Genomic DNA from each genotype was isolated according to the method of Junhans and Metzlatt (1990).

2- ISSR-PCR analysis:

ISSR-PCR reactions were conducted according to Sharma *et al.*(1995) using eight primers, which were synthesized by Metabion Germany with the sequences shown in Table (2). The reaction conditions were optimized and mixtures were prepared (25 µl total volumes) consisting of the following: 1.0 µl dNTPs (10 mM), 1 µl Taq DNA polymerase (1U/1µl), 2.5 µl 10 X buffer, 3 µl MgCl2 (15 mM),

Table 2. List of ISSR primers; names and their nucleotide sequences

Primer name	Sequence	Primer name	Sequence
HB01	(CAA) 5	HB09	(GA)6 GG
HB02	(CAG) 5	17899A	(CA)6 AG
814	(CT) 8 TG	17899B	(CA)6 GG
17898B	(CA)6 GG	HB12	(CAC)3 GC

1.0 µl Primer (10mM), 1.0 µl Template DNA (50 ng/ µl) and 15.5 µl H2O up to 25 µl. Amplification was carried out in Stratgene Robocycler Gradient 96 which was programmed for 45 cycles as follows: Denaturation (one cycle) at 94°C for 2 minutes, followed by 30 cycles: as follows 94°C for 40 sec., 44°C for 45 sec., 72°C for 1 minute and 30 sec. and finally one cycle extension at 72°C for 20 minutes and 4°C (infinitive). Agarose Gel electrophoresis (1.2%) was used for resolving the PCR amplification products. The run was performed for one hour at 120 volt in Biometra submarine (40x20 cm). Bands were detected on UV-transilluminator and photographed by Biometra Bio Doc Analyze 2005.

Statistical analysis:

Recorded data were subjected to statistical analysis using the analysis of variance described by Snedecor and Cochran (1982). Means were separated using Duncan's multiple range test (Duncan, 1995).

Results and Discussion

Evaluation of faba bean genotypes to chocolate spot resistance, using detached-leaf technique:

Using the detached leaf, the diameters of chocolate spot lesions (Table 3) were increased gradually with progressing the incubation period. Genotype Aquadulce recorded the lowest lesion diameter with the average of 0.18 mm followed by line L8 with 0.25 mm. While genotype NBL4 recorded the highest lesion diameter, being 0.58 mm.

Faba bean genotypes showed different degrees of disease severity using detached leaf assay (Table 4) ranged from resistant to susceptible. Genotypes Aquadulce and L8 were (R) while the newly breed lines NBL1 and NBL2 were (MR) after 6 days from inoculation. It is worthy to mention that the susceptibility of most genotypes was increased by increasing the time exposed to the disease pressure under controlled conditions. This may be explained by the fact that older tissues are generally more susceptible to disease than younger ones (Deverall and Wood, 1961; Abou-Zaid, 1978). Moreover, detached-leaf within practically complete dominant systems, dominant alleles facilitate fungal penetration and inducing a hypersensitive response within the leaf (Jennifer *et al.*, 1979).

Table 3. Lesions diameter (mm) on detached-leaves of nine faba bean genotypes, artificially inoculated with *B. fabae* spores

Genotype	Lesion diameter (mm) after hours			
	24	48	72	Mean
G461	0.38	0.56	0.70	0.55
L3	0.39	0.53	0.63	0.52
NBL1	0.16	0.36	0.48	0.33
NBL2	0.20	0.44	0.58	0.41
NBL3	0.34	0.56	0.64	0.51
NBL4	0.47	0.54	0.74	0.58
Aquadulce	0.02	0.18	0.35	0.18
L8	0.09	0.28	0.37	0.25
Nubariya-1	0.05	0.41	0.44	0.30
L.S.D. 0.05	0.16	0.15	0.23	-

Table 4. Chocolate spot reactions on detached-leaves of nine faba bean genotypes (under laboratory conditions)

Genotype	Disease severity [a] after days			Reaction [b]
	1	2	6	
G461	2.0	3.0	4.0	S
L3	2.0	3.0	4.0	S
NBL1	1.0	2.0	3.0	MR
NBL2	1.0	3.0	3.0	MR
NBL3	2.0	3.0	4.0	S
NBL4	3.0	3.0	4.0	S
Aquadulce	1.0	1.0	2.0	R
L8	1.0	2.0	2.0	R
Nubariya-1	1.0	2.0	4.0	S
L.S.D. 0.05	0.01	0.01	0.87	-

[a] = Disease severity scored as a 1– 4 scale according to Hanounik (1986).
[b]= Reaction: 1-Highly resistant (HR), 2-Resistant (R), 3-Moderately resistant (MR) and 4- Susceptible (S)

Evaluation of faba bean genotypes under growth–chamber conditions

Significant differences were detected among the nine faba bean genotypes to chocolate spot according to their (MDI) under growth–chamber conditions (Table 5). However, most genotypes scored as S to MS for chocolate spot resistance and were significantly differed with the check genotype Giza 461 which was classified as HS one.

Table 5. Reaction of the nine faba bean genotypes to chocolate spot according to MDI values under growth–chamber conditions

Genotype	MDI [a]	Reaction [b]
G461	100.00	HS
L3	55.56	MS
NBL1	44.44	MS
NBL2	52.22	MS
NBL3	60.56	S
NBL4	67.78	S
Aquadulce	32.78	MR
L8	50.00	MS
Nubariya-1	53.89	MS
MEAN	57.47	-
L.S.D. 0.05	36.55	-

[a] MDI: Mass disease index on foliage, 7 days after inoculation.
[b] Reaction: (MR) Moderately resistant, (MS) Moderately susceptible, (S) Susceptible and (HS) Highly susceptible.

It is valuable to state that the Spain genotype Aquadulce scored as MR to chocolate spot. These results are in the same line with those recorded for the detached leaf assay (Table 5).

Evaluation of faba bean genotypes under field conditions:

Data presented in Table (6) show the means of MDI values of the nine genotypes grown during two winter seasons 2006/2007 and 2007/2008, in the field. Data are presented as means of two seasons because the reactions of all genotypes in both seasons were greatly similar. Data indicate that genotypes, L3, NBL1, NBL3, NBL4, Aquadulce, L8 and Nubariya-1 were classified as (MR) to chocolate spot. While, genotype Giza 461 and the newly bred line NBL2 scored the lowest (MDI) values and classified as (R).

It is valuable to mention that the check genotype Giza 461 was recorded as susceptible and highly susceptible when it was evaluated using the detached leaf assay and under growth-chamber conditions, respectively. The susceptibility to chocolate spot shown under growth-chamber conditions by different genotypes than in the field may be due to that conditions in the growth-chamber were more favourable to the disease. Also, the tolerance shown by some genotypes in the field could be broken under certain conditions of temperature and light, which may make these genotypes susceptible (Tivoli *et al.*, 1992; Rahaem *et al.*, 2002 and Said *et al.*, 2004). Moreover, a field trial mimics a natural infection and takes place more gradually and more slowly than under the controlled conditions of the green house, revealing more clearly the overall resistance of the plant, and the interaction of the pathogen with different plant organs at different stages of disease progression (Tivoli *et al.*, 1986).

Table 6. Reaction of nine faba bean genotypes to chocolate spot according to MDI values, under field conditions

Genotype	MDI [a]	Reaction [b]
G461	11.1	R
L3	33.3	MR
NBL1	25.9	MR
NBL2	11.1	R
NBL3	37.0	MR
NBL4	37.0	MR
Aquadulce	37.0	MR
L8	33.3	MR
Nubariya-1	25.9	MR
MEAN	28.39	
L.S.D. 0.05	11.10	

[a] MDI mass disease index on foliage after flowering.
[b] Reaction: (R) Resistant and (MR) Moderately resistant.

Coefficients of correlation between lesions diameter and disease severity on detached-leaves as well as MDI values recorded under growth-chamber and field experiments of the nine faba bean genotypes are indicated in Table (7). Data reveal that the disease severity values recorded under growth chamber conditions were significantly correlated with both lesions diameter and disease severity assessed on detached leaf. On the other hand, there was a poor correlation between disease severity recorded in the field and the most lesion diameter values as well as disease severity that determined either in the laboratory or in the growth-chamber.

Table 7. Coefficients of correlation between lesions diameter and disease severity on detached-leaves as well as MDI values recorded under growth-chamber and field experiments of the nine faba bean genotypes

Treatment	L.D.			D.S.			MDI-1
	24 h.	48 h.	72 h.	1 day	2 days	6 days	
L.D.-4 8h.	0.925**						
L.D.-72h	0.961**	0.958**					
D.S.-1 day	0.860**	0.721*	0.785*				
D.S.-2 days	0.885**	0.853**	0.838**	0.654			
D.S.-6 days	0.730*	0.747*	0.675*	0.703*	0.770*		
MDI-1	0.740*	0.799**	0.773*	0.542	0.665	0.748*	
MDI-2	-0.536	-0.745*	-0.638	-0.214	-0.596	-0.357	-0.546

L.D.= Lesion diameter, D.S.= Disease severity, MDI-1= Mass disease index of growth chamber experiment, MDI-2= Mass disease index of field experiment.

The differences between field and growth chamber tested leaves, or a possible interaction between genotype and environment may account for this lack of correlation (Harrison, 1981).

Data presented in Table (8) show the mean performance of yield and its attributes for the nine genotypes of faba bean during 2006/2007 and 2007/2008 seasons. The differences among genotypes for the studied growth parameters were highly significant except the No. of branches /plant which was not significant (Table 8). The results indicate that genotype Aquadulce recorded the highest values for 100/seed weight (88.7 g.) as well as genotypes G461 and NBL1 (62.4 and 79.3 g, respectively). For the number of pods /plant Line NBL3 recorded the highest values (43.3 pod) while line L8 recorded the lowest values (9.3 pod) and No. of branches /plant (3.7 branch).

Among all genotypes, Line NBL4 exhibited the highest means for the No. of seeds/ pod (4.8 seed). On the other hand, Line L3 exhibited the highest seed yield/plant (69.54 g), followed by Nubariya-1 (67.28 g).

Table 8. Mean performance of yield and its attributes for nine genotypes of faba bean during 2006/2007 and 2007/2008 seasons

Genotype	100/Seed * weight (g.)	No of pods/ plant	No. of Branches / plant	No of seeds / pod	Seed yield/ Plant (g.)
G461	62.4d **	26.0b-d	5.3a	3.50c-e	44.00de
L3	78.3b	16.5c-e	5.3a	4.17b	69.54a
NBL1	79.3b	22.0b-e	5.9a	3.83bc	48.44c-e
NBL2	74.0c	19.3b-c	5.3a	3.17de	48.07c-e
NBL3	65.7d	43.3a	5.3a	3.67b-d	55.74bc
NBL4	72.1c	27.8bc	5.3a	4.77a	56.62b
Aquadulce	88.7d	12.8de	5.3a	3.50c-e	50.59cd
L8	65.3a	9.3e	3.7a	3.00e	38.58e
Nubariya-1	73.7c	31.1ab	5.1a	2.40f	67.28ab
MEAN	73.29	23.13	5.19	3.56	53.21

* Each figure represents the mean of two seasons.
** Values followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test (P=0.05).

ISSR polymorphism in nine faba bean genotypes

For ISSR analysis, DNAs of the nine faba bean genotypes were subjected to PCR against eight primers (17898 B, 17899 A, 17899 B, 814, HB01, HB02, HB09 and HB12) as described in Table (9) and Figure (1). A total of 104 amplicons (amplified fragments) were generated by the eight primers in which 79 of them were polymorphic (75.9 %). The number of amplicons per primer varied from eight (17898 A) to twenty one (HB01). The size of the amplified fragments ranged from 185 bp (AF12) to 2100 bp (AF52). High number of monomorphic amplicons (eight) was scored for HB12 primer (Table 10).

Table 9. ISSR polymorphism in nine faba bean genotypes tested using ISSR-PCR with eight primers

bp	Amplicon	Primer	NBL4	L8	L3	NBL2	Nubariyal	Aquadulce	G461	NBL3	NBL1
1745	AF01	17898B	0	0	0	0	0	0	0	1	1
1345	AF02		0	0	0	0	0	0	0	1	1
1200	AF03		0	0	0	0	0	0	0	1	1
1083	AF04		0	0	0	1	0	0	0	1	1
900	AF05		0	0	0	0	0	0	0	0	0
686	AF06		0	1	0	1	0	1	1	1	1
621	AF07		1	1	0	1	0	0	0	1	1
556	AF08		1	1	0	1	0	0	1	1	1
539	AF09		0	0	0	0	0	1	0	0	0
501	AF10		0	1	0	1	0	0	1	1	1
437	AF11		0	1	0	1	0	0	0	1	1
185	AF12		0	0	1	0	1	0	0	0	0
1721	AF13	17899A	1	1	1	1	1	1	1	1	1
1055	AF14		1	1	1	1	1	1	1	1	1
965	AF15		1	1	1	1	1	1	1	1	1
741	AF16		1	1	1	1	1	1	1	1	1
544	AF17		1	1	1	1	1	1	1	1	1
476	AF18		1	1	1	1	1	1	0	0	0
350	AF19		0	1	1	0	0	0	0	1	0
300	AF20		0	0	0	1	0	1	0	0	0
1734	AF21	17899B	1	1	1	1	1	1	1	1	1
1335	AF22		1	1	1	1	1	1	1	1	1
1111	AF23		0	1	0	1	1	1	1	1	1
1056	AF24		1	0	1	0	0	0	0	0	0
945	AF25		1	1	1	1	1	1	1	1	1
784	AF26		0	0	0	1	1	0	1	0	0
749	AF27		1	0	0	0	1	0	0	0	1
671	AF28		0	1	1	1	0	0	1	0	0
573	AF29		1	1	1	1	1	1	1	1	1
470	AF30		1	0	0	0	0	0	0	0	0
453	AF31		0	1	1	1	1	1	1	1	1
270	AF32		0	1	0	0	0	0	1	1	1
2000	AF33	814	0	0	0	1	0	0	1	0	0
1950	AF34		1	0	0	0	0	0	0	0	0
1900	AF35		0	0	1	0	0	0	0	0	0
1600	AF36		1	0	0	0	0	0	0	0	0
1550	AF37		0	0	0	1	1	0	0	0	1
1300	AF38		1	0	0	0	0	0	0	0	0
1280	AF39		0	0	1	0	1	1	1	0	1
1090	AF40		0	1	1	0	1	1	1	1	1
948	AF41		1	0	1	1	1	1	1	1	1
845	AF42		1	0	0	0	0	0	0	0	0
800	AF43		0	1	0	0	1	1	1	0	1
727	AF44		0	1	0	0	1	1	0	1	1
700	AF45		1	0	1	1	0	0	1	0	0
620	AF46		1	1	1	1	1	1	1	1	1
510	AF47		1	1	1	1	1	1	1	1	1
430	AF48		0	0	0	0	0	0	1	0	0

Table 9. Continued:

20	AF49		1	1	1	1	1	1	1	1	1
390	AF50		0	1	0	0	0	0	0	0	0
350	AF51		1	1	1	1	1	1	1	1	1
2100	AF52		1	1	1	1	1	1	1	1	1
1650	AF53		1	0	1	0	1	0	1	1	1
1480	AF54		0	0	1	0	0	0	0	0	0
1380	AF55		0	0	0	1	1	0	0	1	1
1148	AF56		1	0	0	0	0	0	0	0	0
1103	AF57		0	0	1	1	0	0	1	1	1
995	AF58		0	0	0	0	1	1	0	0	0
962	AF59		1	1	1	1	1	1	1	1	1
891	AF60		0	0	0	1	0	0	0	0	0
810	AF61		0	0	1	0	1	0	0	0	0
780	AF62	HB01	1	1	0	1	0	1	1	1	1
660	AF63		1	1	0	0	1	1	1	1	1
640	AF64		0	0	1	1	1	1	1	1	1
570	AF65		1	1	0	0	0	0	0	0	0
560	AF66		1	0	0	1	1	1	1	1	1
490	AF67		1	0	0	1	1	1	1	0	0
470	AF68		0	0	0	0	1	1	0	0	0
430	AF69		0	0	1	1	1	1	1	1	1
405	AF70		0	1	0	0	0	0	0	0	0
390	AF71		0	0	1	0	1	1	1	0	0
300	AF72		1	0	0	1	1	1	0	0	0
1781	AF73		1	0	1	1	1	1	1	1	1
1009	AF74		1	0	1	1	1	1	0	1	1
891	AF75		0	1	0	0	0	0	0	0	0
855	AF76		1	0	1	1	1	1	1	1	1
770	AF77	HB02	0	0	0	0	0	0	0	1	1
694	AF78		0	0	0	0	0	0	0	0	1
687	AF79		1	1	1	1	1	1	1	1	1
572	AF80		1	1	1	1	1	1	1	1	1
460	AF81		1	0	0	0	0	0	0	0	0
2100	AF82		0	0	0	0	1	0	0	0	0
2000	AF83		0	0	0	0	1	0	0	0	0
1190	AF84		1	1	1	1	0	1	1	1	1
1044	AF85		0	0	0	0	1	0	0	0	0
1130	AF86		0	0	0	0	1	0	0	0	0
971	AF87		1	1	1	1	0	1	0	0	0
941	AF88	HB09	0	0	0	0	0	0	1	1	1
840	AF89		0	0	0	0	0	0	1	1	1
740	AF90		0	1	0	0	0	0	0	0	0
660	AF91		1	1	1	1	1	1	1	1	1
570	AF92		1	0	1	1	0	1	1	1	1
480	AF93		1	1	1	1	0	1	1	1	1
390	AF94		1	1	1	1	0	1	1	1	1
1317	AF95		1	1	1	1	1	1	1	1	1
1071	AF96	HB12	1	1	1	1	1	1	1	1	1
890	AF97		1	1	1	1	1	1	1	1	1
700	AF98		1	1	1	1	1	1	1	1	1

Table 9. Continued:

649	AF99		0	1	1	1	1	1	1	1	1
500	AF100		1	1	1	1	1	1	1	1	1
450	AF101		1	1	1	1	1	1	1	1	1
380	AF 102		1	1	1	1	1	1	1	1	1
300	AF103		1	1	1	1	1	1	1	1	1
270	AF104		0	1	1	1	1	1	1	1	1

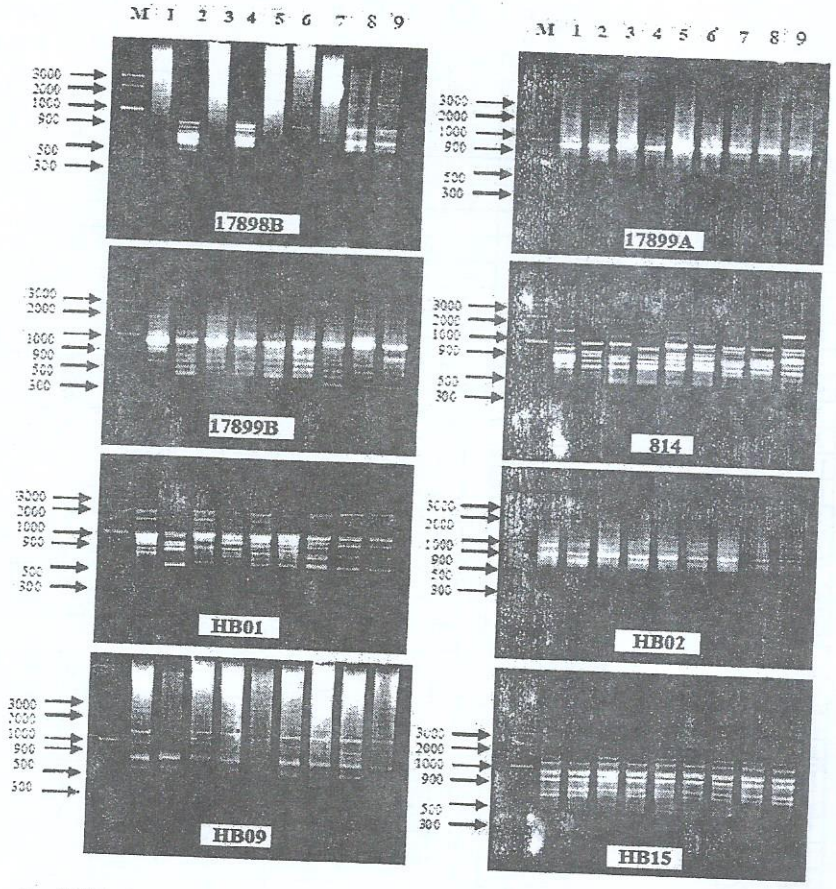

Fig. 1. ISSR fingerprints of the nine faba bean genotypes using eight primers. Lane M: is the standard DNA marker. Lanes 1-9: are Genotypes NBL4, L8, L3, NBL2, Nubariya-l, Aquadulces, G461, NBL3 and NBL1.

Primer 17898 B produced 12 bands in which fragment sizes ranged from 1745 to 185 bp, 12 of which were polymorphic (100% polymorphism). Primer 17899A produced 8 bands in which fragments sizes ranged from 1721 to 300 bp and 3 of them were polymorphic. Primer 17899B produced 12 bands with fragment sizes ranged from 1734 to 270 bp. Primer 814 yielded 21 bands with the fragment sizes of 2000 to 350 bp, 15 of them were polymorphic. While, the two primers HB01, and HB02 produced 21 and 9 bands with fragment sized 2100 to 300bp and 1781 to 460bp, respectively and primer HB09 produced 13 bands in which fragments sized ranged from 2100 to 390bp (92.3% polymorphism). Primer HB12 produced 10 bands in which fragment sized ranged from 1370 to 270bp, with lowest polymorphism (20.0%) among all primers. A total of 32 for genotypic unique bands were identified out of the polymorphic among the primers under study as shown in Table (10).

Table 10. Amplification results of the eight ISSR primers in nine faba bean genotypes tested using ISSR-PCR ISSR polymorphism

Primer code	TAF	PB	P%	Genotype																		TSM
				NBL4		L8		L3		NBL2		Nubariya-1		Aquadulce		G461		NBL3		NBL1		
				AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
17898B	12	12	100.0	2	0	5	0	1	0	6	1	1	0	2	1	3	0	9	0	9	0	2
17899A	8	3	37.5	6	0	7	0	7	0	7	0	6	0	7	0	5	0	6	0	5	0	0
17899B	12	9	75.0	6	3	8	0	7	0	8	0	8	0	6	0	9	0	7	0	8	0	3
814	19	15	78.9	10	4	8	2	9	1	8	0	10	0	9	0	11	0	7	0	10	0	8
HB01	21	19	90.5	10	1	6	1	9	1	11	1	14	0	12	0	11	0	10	0	10	0	4
HB02	9	7	77.8	6	1	3	3	5	0	5	0	5	0	5	0	4	0	6	0	7	1	5
HB09	13	12	92.3	6	0	6	1	6	0	6	0	5	7	6	0	7	1	7	0	7	0	8
HB12	10	2	20.0	8	2	10	0	10	0	10	0	10	0	10	0	10	0	10	0	10	0	2
Total	104	79	-	54	11	53	7	54	2	61	2	59	7	57	1	60	1	62	0	66	1	32

TAF= Total number of amplified fragments, PB = Polymorphic bands, P%= polymorphism percentage, AF= Amplified fragments / genotype, SM= Genotype- specific marker including either the presence or absence of a given band, TSM= Total number of specific markers.

The primer 17898B gave two positive markers (AF04 and AF09, respectively) for NBL2 and Aquadulce genotypes. The primer 17898B gave two negative bands (AF29 and AF31) and one positive unique band (AF30) for genotype NBL4. Primer 814 gave eight specific marker, four positive marker (AF34 AF36, AF38 and AF49, respectively) for line NBL4. While, the same primer gave one negative marker (AF41) and one positive marker (AF50) for genotype L8. Also, there were two positive markers (AF35 and AF48) for genotypes L3 and G461, respectively.

Meanwhile, primer HB01 gave four positive markers (AF54, AF56, AF60 and AF70) for genotypes L8, NBL4, L3, and NBL2, respectively. While, primer HB02 gave one positive marker (AF81) for genotype NBL4 and two markers for genotype L8, one negative marker (AF73) and one positive marker (AF75) respectively, as well as one positive marker (AF78) for genotype NBL1. For primer HB09, genotype L8 has one positive marker (AF90) will genotype Nubariya-1 has four positive markers (AF82, AF83, AF85 and AF86) and three negative markers (AF83 AF93 and AF94). While primer HB12 gave only two negative markers (AF99 and AF104) for genotype NBL4.

It is worthy to note that G461 and NBL2 were classified as resistant genotypes to chocolate spot under the field conditions (Table 6). Such superiority in mass disease index over the nine genotypes tested is correlated with the appearance of AF33 specific band of the ISSR primer. Therefore, based on the hypothesis that genotype with overall resistance may represent interesting sources of resistance, thus, NBL2 genotype could be merited inclusion in breeding programs (Tivoli *et al.*, 1992).

Based on ISSR marker polymorphisms, similarity matrix was developed by SPSS computer package (Table 11). The analysis was based on the number of markers that were differentiated between any given pair of genotypes.

Table 11. Similarity matrices for nine faba bean genotypes using eight primers based on ISSR analysis

Genotype	NBL4	L8	L3	NBL2	Nubariya-l	Aquadulce	G461	NBL3
L8	0.776							
L3	0.812	0.805						
NBL2	0.819	0.826	0.846					
Nubariya-l	0.745	0.738	0.832	0.798				
Aquadulce	0.819	0.839	0.872	0.878	0.890			
G461	0.798	0.846	0.878	0.884	0.832	0.896		
NBL3	0.776	0.852	0.832	0.865	0.783	0.852	0.896	
NBL1	0.761	0.826	0.805	0.852	0.812	0.852	0.896	0.969

The closest relationship was scored between the two genotypes; Line NBL1 and Line NBL3 followed by Nubariya-1 and Aquadulce (similarity of 0.969 and 0.890, respectively). It is worthy to note that, the closely related lines NBL4 and NBL3 shared in their ancestor line ILB1179 as shown in Table (1). On the other hand, the most distant relationship was scored between the check variety Nubariya-1 and each of Line 8 and Line NBL4 (similarity of 0.738 and 0.745), respectively.

Except genotypes L8 and NBL4 the dendrogram classified the other genotypes into two main clusters (Fig. 2). The first cluster was separated into two sub-clusters comprised 4 faba bean genotypes (NBL1, NBL3, G461 and NBL2), while the second sub-cluster, consisted of Nubariya 1, Aquadulce and L3.

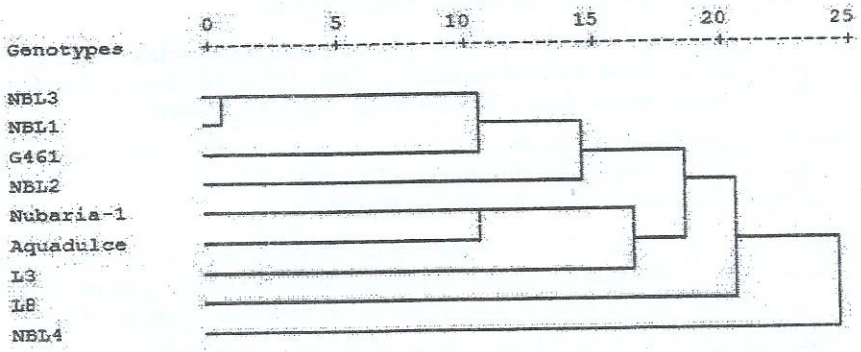

Fig. 2. Dendrogram of the nine faba bean genotypes using eight primers based on ISSR analysis.

This concept has been advocated by several investigators who stated that molecular markers have several advantages over the traditional phenotypic markers that were previously available to plant geneticists. They offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular marker linked to that trait (Afiah *et al.*, 2007 b and Torres *et al.*, 2010).

In summation, the results of this investigation provided some ISSR molecular markers associated either positively or negatively with faba bean genotypes productivity. They could be used to enhance breeding programs aimed to improve its disease tolerance by pyramiding genes controlling this polygenic character by the aid of marker-assisted selection. At least, the ISSR marker developed from this study can consequently be used in any further study to identify stress-tolerant genotypes in faba bean or any other field crop.

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تقييم بعض التراكيب الوراثية من الفول لمقاومة التبقع الشيكولاتى

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أجريت هذه الدراسة لتقيم تسعة تراكيب وراثية من الفول تشتمل على الصنف المحلى جيزة ٤٦١ والصنف الاسبانى Aquadulce وعدد من السلالات المرباة حديثا وتم التقييم تحت ظروف العدوى الصناعية بعزلة من الفطر *Botrytis fabae* باستخدام طريقة الأوراق المفصولة وكذلك تحت ظروف تحكم فى غرفة النمو فى درجات حرارة ٢٠ درجة مئوية وتبادل الإضاءة والظلام كل ١٢ ساعة. كما تم التقييم تحت ظروف الزراعة المطرية بمحطة بحوث مريوط التابعة لمركز بحوث الصحراء تحت ظروف العدوى الطبيعية فى حقل لة تاريخ مرضى سابق بالمسبب المرضى موضع الدراسة .

تم تسجيل أعراض المرض باستخدام الطرق القياسية حيث وجدت علاقة طردية معنوية بين كل من شدة الإصابة وقطر البقع على الأوراق المفصولة من هذه التراكيب حيث يزداد كل منهما بزيادة فترة التحضين. وقد أعطى الصنف الاسباني Aquadulce أقل قيمة لقطر البقع (١٨و٠مم) يليه السلالة L8 (٢٥و٠ مم) وكذلك حقق مقاومة للمرض على الأوراق المفصولة تحت ظروف المعمل ، فى حين سجلت باقي التراكيب مابين متوسط المقاومة إلى قابل للإصابة فى نفس التجربة. تحت ظروف التحكم فى غرفة النمو كان الصنف الاسباني Aquadulce متوسط المقاومة فى حين ظهر الصنف جيزة ٤٦١ متوسط القابلية للإصابة وذلك تبعا لقيمة الدليل التجميعي للمرض.

أظهرت التراكيب الوراثية التسعة درجات مختلفة من المقاومة للمرض تحت ظروف الحقل حيث تراوحت من مقاوم إلى متوسطة القابلية للمقاومة تبعا لقيمة الدليل التجميعى للمرض. حيث أظهر الصنف جيزة ٤٦١ والسلالة المرباه حديثا NBL2 أقل درجة لشدة الإصابة وأعلى مقاومة للمرض ، بينما صنفت باقي التراكيب الوراثية على أنها متوسطة المقاومة للمرض .

كانت هناك اختلافات معنوية بين تباين سلوك محصول النبات ومكوناته .حيث أعطى الصنف الاسباني Aquadulce أعلى قيمة لوزن ١٠٠ بذرة (٨٨,٧ جم) مقارنة بباقي التراكيب بينما أعطت السلالة NBL3 أعلى قيمة لعدد القرون في النبات بينما أعطت السلالة L8 اقل قيمة. وقد تفوقت السلالة NBL4 فى عدد البذور للقرن بينما أعطت السلالة L3 أعلى قيمة لمحصول البذور للنبات (٦٩,٥٠ جم).

أظهر تحليل نتائج التفريد الجزيئي للحامض النووى DNA للتراكيب الوراثية المختلفة وجود معلمات جزيئية لمقاومة المرض مع البادئ ٨١٤. وباستثناء السلالتين L8 و NBL4 أمكن تصنيف هذه التراكيب إلى مجموعتين رئيسيتين من خلال حساب معامل التشابه وتوزيع شجرة القرابة باستخدام تحليل SPSS وثمانية من البادئات بنظام ISSR –PCR .