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Gene Action and Molecular Markers Associated with *Orobanche* Resistance in Faba Bean (*Vici faba* L.)

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Abstract: A six-generation analysis was employed to estimate the genetic parameters for plant height, number of branches and pods/plant, 100-seed weight and seed yield/plant. The six generations were derived from the cross between Misr-1 (tolerant) x Giza-2 (susceptible) to *Orobanche*. The results indicated that F_1 exceeded its high performing parent for all studied traits except number of branches/plant. Inbreeding depression was positive and significant for all studied traits. Additive (d) gene effect was significant for all the studied traits except seed yield/plant; also, dominance gene effects (h) were significant for all the studied characters except for number of pods/plant and 100 seed weight. Two of the non-allelic gene interactions analysis (i) and (l), were significant for all characters except number of pods and 100-seed weight, while the (j) component was significant for seed yield/plant only. Bulked Segregant Analysis (BSA) with three molecular marker systems (RAPD, ISSR and SRAP) was performed to identify markers related to *Orobanche* tolerance. DNA was isolated from the two contrasting parents; F_1, two contrasting groups of F_2 plants and two contrasting groups of BC_1 (F_1 x Misr-1). Nine RAPD primers, ten ISSR primers and nine SRAP primers were used in this study. The results showed that six RAPD markers (OPA01 345 bp, OPA06 272 bp, OPA07 669bp, 385, 292 and OPB05 253 bp), six ISSR (HB 359 bp, 231 bp, HB08 521, 207 bp and HB11 509bp and 358 bp) and seven SRAP (SRAP1 354 bp, SRAP2 643bp and 200 bp, SRAP3 99 , 362, 169 bp and SRAP4 242 bp) were associated with *Orobanche* tolerance in faba bean These markers can be used in faba bean breeding for *Orobanche* resistance in marker-assisted selection programs.

Key words: Faba bean, gene action, *Orobanche* resistance, BSA.-molecular markers

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important sources of protein in Egypt and an essential source of livestock feed in the world. The acreages and seed yields vary with season and location. Among the constraints affecting this crop is its yield instability due to various biotic and a biotic limitations. For instance, losses of faba bean yield due to *Orobanche* parasitism may reach 100% (Zaitoun, 1990). In addition, control of *Orobanche* is not easy due to its production of huge amount of tiny, long-lived seeds. Different cultural practices as well as biological and chemical methods were suggested to control *Orobanche* and/or alleviate its effects. However, most of these methods are not satisfactory in controlling this parasite. The most feasible approach for controlling damage caused by *Orobanche* is evidently selection for genetically resistant cultivars of faba bean (Abdalla and Darwish, 1994, 1996, 1999, 2008; Khalil *et al*., 1994; Saber *et al*., 1999). Improving faba bean yield could be achieved through reducing cultivars susceptibility to various biotic stresses such as *Orobanche* parasite which is currently considered an important breeding objective. Such approach requires assessment of the different magnitude of the additive type of gene effects operating for tolerance to *Orobanche*. In this respect, Youssef (1999) reported that additive gene effects controlled the inheritance of number of branches/plant while dominance gene effects played the major role in the inheritance of seed yield/plant. Meanwhile, Attia *et al*. (2002) found that dominance effects played an important role in the inheritance for plant height; however, additive gene effects controlled the major portion of variation for 100-seed weight, number of pods per plant as well as seed yield/plant. In a six-generation analysis for seed yield and its components. Abul-Naas *et al*. (1991), El-Hady *et al*. (1997) and Bakheit *et al*. (2002) found that both additive and dominance gene effects were operating. Bakheit *et al*. (2010a) analyzed a diallel cross among seven faba bean cultivars and found that dominance gene effects played the major role for all studied traits.

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Cubero (1983) and Cubero and Fernandez (1991) and Abd El-Halim (1994) found that resistance/tolerance to *Orobanche* in faba bean is under major gene and polygenic control and indicated an additive–dominance model of polygenic system. Recently, different molecular markers (RFLPs, AFLP, SSR, TRAP, RAPDs, ISSR and SRAP) were used to identify markers related to *Orobanche* tolerance. Randomly amplified polymorphic DNA (RAPD) is based on the PCR amplification of random DNA segments that behave as dominant alleles (Williams *et al*., 1990). Inter-Simple Sequence Repeats (ISSR) technique is a new type of DNA markers based on PCR. This technique amplifies DNA directly using microsatallite sequences (Fang and Roose, 1997). Sequence-related Amplified Polymorphism (SRAP) is an efficient molecular technique with the markers behave as co-dominant and more reproducible than RAPDs (Li and Quiros, 2001). In recent years biochemical and molecular approaches were more reliable to identify markers associate with resistance to *Orobanche* in faba bean. Many researchers such as Gutierrez and Kuti (2001), Roman *et al*. (2002), Rubiales (2003), Khalil *et al*. (2004), Abd El-Maksoud *et al*. (2006), Rispail *et al*. (2007), Zeid *et al*. (2009), Abo El-kheir *et al*. (2010) and El-Sayed *et al*. (2013) found biochemical and molecular markers associate with resistance/tolerance to *Orobanche* in faba bean. Bulked Segregant Analysis (BSA), is highly efficient methods used to identify markers linked to a gene of interest because it can detect a small percentage of polymorphisms in F_2 individual progeny tests. The use of BSA in combination with PCR-based molecular markers (RAPD, ISSR and SRAP) has proven to be a very powerful technique for identifying markers tightly linked to/or co-segregating with, genes underlying monogenic traits (Agrama and Moussa, 1996; Cho *et al*., 1996; Nakamura *et al*., 2001; Rostoks *et al*., 2002; Shen *et al*., 2003; Avila *et al*., 2003; Diaz-Ruiz *et al*., 2010; Torres *et al*., 2010; El-Sayed *et al*., 2013).

This work was carried out for:- (1) determining the relative importance of the additive dominance and epistatic effects in the inheritance of some agronomic traits using the six-populations (P_1, P_2, F_1, F_2, BC_1 and BC_2) model of *Orobanche* tolerant x *Orobanche* susceptible faba bean cross (*Vicia faba* L.) and (2) identifying the molecular markers for *Orobanche* tolerance in faba bean using Bulked Segregant Analysis (BSA) with three molecular marker systems (RAPD, ISSR and SRAP).

MATERIALS AND METHODS

The investigation was carried out at the Agricultural Experimental Farm of Assiut university, Egypt during the three successive seasons of 2008/2009, 2009/2010 and 2010/2011. In the first season, a cross was established between the two local varieties Misr-1(P_1) and Giza-2 (P_2) which have been tested for *Orobanche* resistance in different locations and environments by many authors. The parental cultivar, the new variety Misr-1 is highly tolerant to *Orobanche* as reported by Abd El-Maksoud and Hamada (2007), Massoud (2008), Haridy (2009) and Bakheit *et al*. (2010b). The second parental cultivar Giza-2 is highly susceptible to *Orobanche* as reported by Abd El-Halim (1994), Abdalla (2003), Haridy (2009) and Bakheit *et al*. (2010a). In the second season the F_1 was backcrossed to each of its respective parents to produce BC_1 ($P_1 \times F_1$) and BC_2 ($P_2 \times F_1$). Also the F_1 plants were selfed to produce F_2 seeds.

Six population experiment: In third season, the six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) were field grown in a randomized complete block design with three replications on 3th November, 2010 in a heavily infested field with broomrape at the Experimental farm of Assiut University. Plots within replicates consisted of ridges 3-4 m long and 60 cm width with 20 cm between hills on one side of the ridge with one plant/hill. Each plot consisted of 14 ridges (two ridges for each of P_1, P_2, F_1, BC_1 and BC_2 and 4 ridges for F_2). The recommended agricultural practices for faba bean production were applied at the proper time. Data were collected on 30 individual plants for each of P_1, P_2, F_1, BC_1 and BC_2 and 60 individual plants for F_2 in each replication. At harvest, plant height (cm), number of branches/plant, number of pods/plant, 100-seed weight and seed yield/plant (g) were recorded for each plant.

The scaling tests of Jinks and Jones (1958) were applied as, $A=2\overline{BC_1}-\overline{P_2}-\overline{F_1}$, $A=2\overline{BC_2}-\overline{P_2}-\overline{F_1}$ and $C=4\overline{F_2}-2\overline{F_1}-\overline{P_1}-\overline{P_2}$ to test the validity of an additive-dominance model. The Joint Scaling test was extended to fit the 6-parameter model as outlined by Jinks and Jones (1958). These parameters, are the mean, (m); the pooled additive effects, d; the pooled dominance effects, h; pooled the additive x additive effects (i), the pooled additive x dominance epistatic effects, (j); and the pooled dominance x dominance epistatic effects, (l). Heterosis and % inbreeding depression were calculated for the studied traits according to Bhatt (1971).

Molecular markers

Isolation of genomic DNA: Total genomic DNA was isolated from young leaves of faba bean plants using CTAB protocol for plants (Murray and Thompson, 1980) with some modifications. RNA was removed from the DNA preparation by adding 10 μL of RNAase

(10 mg mL^{-1}) and then incubated for 30 min at 37°C. DNA sample concentration was quantified by using a spectrophotometer.

Bulk segregant analysis (BSA): In order to perform BSA for identification of marker closely linked to *Orobanche* tolerance, DNA isolated from P_1, P_2, F_1 and four DNA bulks were prepared from equimolar of DNA from two extreme groups of 30 individual F_2 plants (15 tolerant plants and 15 sensitive plants) and two extreme groups of 30 individual BC_1 (15 tolerant plants and 15 sensitive plants) according to agro-physiological traits.

RAPD, ISSR analysis: Nine ten-mer random oligonucleotide (RAPD) primers, ten Inter-simple sequence repeat (ISSR) primers and nine SRAP primers (Table 1), obtained from (Metabion International AG), were used in this investigation to identify markers related to *Orobanche* tolerance in faba bean. The reaction conditions were optimized and mixtures (25 μL total volume) were composed of 11.0 μL dH_2O, 3.0 μL 10X reaction buffer, 3.0 μL dNTP's mix, 1.0 μL primer for (RAPD and/or ISSR) and 2.0 μL for (SRAP), 4.0 μL $MgCl_2$, 0.3 μL Taq DNA polymerase and [1 μL Template DNA (25 ng μL^{-1}) for RAPD and 2.0 μL of genomic DNA (50 ng μL^{-1}) for ISSR)]. Amplification condition were carried out in a TECHNE thermocycler (Model FTGEN5D, TECHNE, Cambridge Ltd, Duxford and Cambridge, U.K.) with the following specification: Initial denaturation for 5 min at 94°C (1st step), 40 cycles of 1 min at 94°C, 1 min at 34°C for RAPD primers, 40 °C for ISSR or 35 °C for SRAP primers and 2 min at 72°C (2nd step), 10 min at 72°C, then followed by a final hold at 4°C.

Amplification products were separated by horizontal gel electrophoresis unit using 1.4% (for RAPD analysis), 2% for (ISSR analysis) or 2.5% for (SRAP analysis)agarose gel. Electrophoresis was carried out under constant voltage of around 80V for approximately 3-3.5 h. The banding patterns were visualized on a transilluminator (Ultra-Violet Product, Upland, CA, USA).

Data analyses: The banding patterns obtained from RAPD, ISSR and SRAP were scored as present (1) or absent (0), each of which was treated as independent character regardless of its intensity.

Table 1: Code and sequence of primers that gave polymorphism

Primer codes	Sequence (5' to 3')
RAPD	
OPA01	5'-CAG GCC CTT C-3'
OPA06	5'-GGT CCC TGA C-3'
OPA07	5'-GAA ACG GGT G-3'
OPA08	5'-GTGACGTAGG-3'
OPB05	5'- TGCGCCCTTC -3'
ISSR	
HB	5'-CAC ACA CAC ACA AC -3'
HB08	5'-GAG AGA GAG AGA GG -3'
HB13	5'- GAG GAG GAG GC -3'
HB11	5'- GTG TGT GTG TGT CC -3'
HB12	5'-CCA CCA CCA GC-3'
SRAP	
SRAP1	5'–GAC TGC GTA CGA ATT AAT–3'
	5'–TGA GTC CAA ACC GGA AG–3'
SRAP2	5'–GAC TGC GTA CGA ATT AAC–3'
	5'–TGA GTC CAA ACC GG AGC–3'
SRAP3	5'–GAC TGC GTA CGA ATT TGC–3'
	5'–TGA GTC CAA ACC GGA AT–3'
SRAP4	5'–GAC TGC GTA CGA ATT TGC–3'
	5'–TGA GTC CAA ACC GGA CC–3'

RESULTS

Mean performance: Before applying the biometrical analysis, a "t" test was performed in order to ascertain the differences between the two parental genotypes for each the studied characters which proved to by significant and valid to analyze the types of gene effects.

Means and standard errors of the six generation (P_1, P_2, F_1, F_2, BC_1 and BC_2) of the faba bean cross for the studied characters are given in Table 2. The F_1 mean exceeded that of its high performing parent (HP) for plant height, number of pods/plant, 100-seed weight and seeds yield/plant. The dominance effect as indicated by [h] value was positive for all traits. The backcross population's means were mid-way between the F_1 and their respective parental genotypes for all studied traits except BC_2 for number of branches/plant.

Table 2: Mean performance±standard error for the six populations of the faba bean cross (Misr 1 x Giza 2) for the studied traits

Characters populations	Plant height (cm)	No. of branches/plant	No. of pods/plant	100 seed weight (g)	Seed yield/plant (g)
$\overline{P1}$ (Misr 1)	92.7±1.25	5.07±0.19	30.2±0.87	66.0±1.10	57.6±1.20
$\overline{P2}$ (Giza 2)	86.8±1.11	3.43±0.18	22.5±0.64	62.3±1.30	42.1±0.92
$\overline{F1}$	96.8±1.38	4.93±0.16	32.5±0.84	75.5±1.24	64.2±1.57
$\overline{BC1}$ (F1×P1)	97.0±0.89	5.23±0.20	29.5±0.80	70.8±1.28	60.4±1.51
$\overline{BC2}$ (F1×P2)	91.7±1.08	4.73±0.18	25.4±0.66	67.7±1.37	59.0±1.53
$\overline{F2}$	89.2±1.05	4.47±0.16	27.9±0.58	67.8±1.21	49.3±1.18
Heterosis (%)					
Mid-parent	7.89±0.57**	16.08±0.054**	23.34±1.29**	17.71±2.00**	28.74±3.35**
Better parents	4.50±0.66**	-2.63±0.063**	7.62±1.49**	14.44±3.17**	11.46±3.87
% Inbreeding depression					
$\overline{F1}-\overline{F2}/\overline{F1}$	7.92±3.01*	9.46±0.05**	14.15±1.04**	10.24±3.00**	23.21±3.88**
$h=\overline{F1}-1/2\left(\overline{P1}+\overline{P2}\right)$	7.08	0.68	6.15	11.35	14.35

Estimates of % heterosis (Table 3) revealed positive and significant heterosis effects over mid-and better parent in plant height (7.85 and 4.24), number of pods/plant (23.34 and 7.62), 100 seed weight (15.03 and 14.39) and seed yield/plant (28.79 and 11.46). However, for number of branches/plant the % heterosis relative to the better parent was negative (-2.76). Inbreeding depression % was positive and significant for plant height (7.85), number of branches/plant (9.33), number of pods/plant (14.15), 100 seed weight (10.20) and seed yield/plant (23.21).

Assessment of the component of genetic variance: Non-allelic gene interaction was operating for all characters since the A, B and C scaling test (Table 3) indicated that at least one of the scaling test values was significant except for 100 seed weight. Additive gene effects [d] were significant for all the studied characters except for seed yield/plant. Also, dominance gene effects [h] were significant for all the studied characters except for number of pods/plant and 100 seed weight. With respect to epistatic interactions the results indicated that [i] effects (additive x additive) and [l] effects (dominance x dominance) were significant for plant height, number of branches/plant and seed yield/plant. While, type [j] effects (additive x dominance) was significant for seed yield/plant only.

Molecular genetic analysis: The bulked segregant analysis (BSA) was adopted in this investigation (Michelmore *et al*., 1991) to detect markers for *Orobanche* tolerance in faba bean. BSA identifies markers linked to a molecular trait of interest in the segregating F_2 and BC populations generated from the hybrid between the two contrasting genotypes (resistance and sensitive in this study). four DNA bulks from the most two contrasting F_2 and BC_1 groups were used along with their parents and F_1 plants to develop RAPD, ISSR and SRAP markers associated with *Orobanche* tolerant.

RAPD markers: Out of nine RAPD primers screened for polymorphism between (Misr-1 and Giza-2), five RAPD primers (55.56%), with polymorphic bands were suitable to differentiate between the two parents. Five RAPD primers amplified a total of 38 DNA bands from two parents with an average of 7.6 bands/primer and ranged in size from 996 bp (OPA06) to 172 bp (OPA01) (Table 4, Fig. 1). Of the 38 bands obtained, 20 bands (52.63 %) were polymorphic. fifteen of bands polymorphisms were arbitrary; however, five bands were found to be useful markers related to *Orobanche* tolerant as shown in Table 4.

The OPA01 primer, generated one strong polymorphic fragments at 345 bp which was presented only in F_1, tolerant F2 bulk, tolerant BC_1 bulk and Misr-1 (resistant parent) whereas missing in sensitive F_2 and BC_1 bulks and Giza-2 (sensitive parent), as shown in Fig 1. In addition primer OPA06, produced a strong polymorphic band at 272 bp that was present only in the F_1, tolerant F_2 bulk, tolerant BC_1 bulks and Misr-1 (resistant parent), but

Table 3: Scaling tests, gene effects and components of genetic variance in faba bean cross (Misr 1 x Giza 2) for the studied traits

Characters estimation	Plant height (cm)	No. of branches/plant	No. of pods/plant	100 seed weight (g)	Seed yield/plant (g)
Scaling test					
A	4.50± 2.573	0.47±0.473	-3.70±2.010	0.07±3.051	-1.00±3.60
B	-0.33±2.799	1.10±0.432*	-4.20±1.682*	-2.40±3.281	11.67±3.56**
C	-16.50±5.296**	-0.50±0.776	-6.10±3.056*	-8.20±5.684	-30.93±5.88**
Gene effects					
Mean [m]	89.17±1.25**	4.47±0.19**	27.90±0.87**	58.30±6.17**	49.30±1.25**
Additive [d]	5.33±1.11**	0.50±0.18**	4.10±0.64**	1.83±0.85*	1.40±1.11
Dominance [h]	27.75±1.38**	2.75±0.16**	4.35±0.84	20.77±15.09	55.93±5.30**
Additive xadditive [i]	20.67±1.05**	2.07±0.16*	-1.80±0.58	-	41.60±5.05**
Additive x Dominance [j]	2.42±0.89	-0.32±0.20	0.25±0.80	-	-6.33±1.63**
Dominance x Dominance [l]	-24.83±1.08**	-3.63±0.18**	9.70±0.65	-	-52.27±7.71**

Table 4: DNA polymorphism among contrasting parents (Misr-1 and Giza-2), F_1, two contrasting groups of F_2 and two contrasting groups of BC_1 bulk with RAPD and ISSR markers

Primers	bp	P_1	P_2	F_1	F_2^R	BC_1^R	F_2^S	BC_1^S
RAPD	381	1	0	0	1	1	1	1
OPA01	345	1	0	1	1	1	0	0
	302	1	1	1	1	0	1	1
	266	1	1	1	1	1	1	1
	242	1	1	1	1	1	1	1
	215	1	0	1	1	1	1	0
	172	1	1	1	1	1	1	1
	996	1	1	1	1	0	1	1
OPA06	654	1	0	0	1	1	0	1
	537	1	1	1	1	1	1	1
	425	0	1	1	1	1	1	1
	380	1	1	1	1	1	1	1
	339	1	0	1	1	1	1	1

Table 4: Continue

Primers	bp	P_1	P_2	F_1	F_2^R	BC_1^R	F_2^S	BC_1^S
	309	1	1	1	1	1	1	1
	272	1	0	1	1	1	0	0
	669	1	0	1	1	1	0	0
	582	1	1	1	1	1	1	1
OPA07	514	1	1	1	1	1	1	1
	434	1	0	1	1	0	0	1
	385	1	0	1	1	1	0	0
	342	1	1	1	1	1	1	1
	292	0	1	1	0	0	1	1
	260	1	1	1	1	1	1	1
	233	1	0	0	1	0	1	1
	563	1	1	1	1	1	1	1
OPB05	411	1	0	1	1	1	1	1
	296	1	1	1	1	1	1	1
	253	0	1	1	0	0	1	1
	230	1	1	1	1	1	1	1
	192	1	1	1	1	1	1	1
	176	0	1	1	1	1	1	1
	382	0	1	0	0	0	0	0
	343	1	1	1	1	1	1	1
OPA08	306	1	1	1	1	1	1	1
	265	1	1	1	1	1	1	1
	244	1	0	1	1	1	1	1
	215	1	1	1	1	1	1	1
	173	1	1	1	1	0	1	1
	816	1	1	1	1	1	1	1
	680	1	1	1	1	0	1	1
HB	559	1	1	1	1	1	1	1
	484	1	1	1	1	1	1	1
	426	1	1	1	1	1	1	1
	359	1	0	1	1	1	0	0
	319	1	1	1	1	1	1	1
	282	1	1	1	1	1	1	1
	260	1	1	1	1	1	1	1
	231	1	0	1	1	1	0	0
	208	1	1	1	0	1	0	1
	182	1	0	0	0	0	0	0
	521	1	0	1	1	1	0	0
	443	1	0	1	0	1	1	0
HB08	415	1	1	1	1	1	1	1
	392	1	1	1	1	1	1	1
	357	1	1	1	1	1	1	1
	325	1	1	1	1	1	1	1
	290	1	1	1	1	1	1	1
	264	1	1	1	1	1	1	1
	256	1	1	1	1	1	1	1
	235	1	1	1	1	1	1	1
	207	0	1	1	0	0	1	1
	436	1	0	1	1	0	1	1
	393	1	1	1	1	1	1	1
	360	1	0	1	1	1	1	1
HB12	293	1	1	1	1	1	1	1
	272	0	1	1	1	1	1	1
	240	1	1	1	1	0	0	1
	654	1	1	1	1	1	1	1
	548	1	1	1	1	1	1	1
	509	0	1	1	0	0	1	1
HB11	419	1	1	1	1	1	1	1
	382	1	1	1	1	1	1	1
	358	0	1	1	0	0	1	1
	327	1	1	1	1	1	1	1
	251	1	1	1	1	1	1	1
	714	1	1	1	1	1	1	1
	602	1	0	1	1	1	1	1
HB13	498	1	1	1	1	1	1	1
	438	1	0	1	1	1	1	1
	388	0	1	1	1	1	1	1
	327	1	0	1	1	1	1	0
	277	1	1	1	1	1	1	1

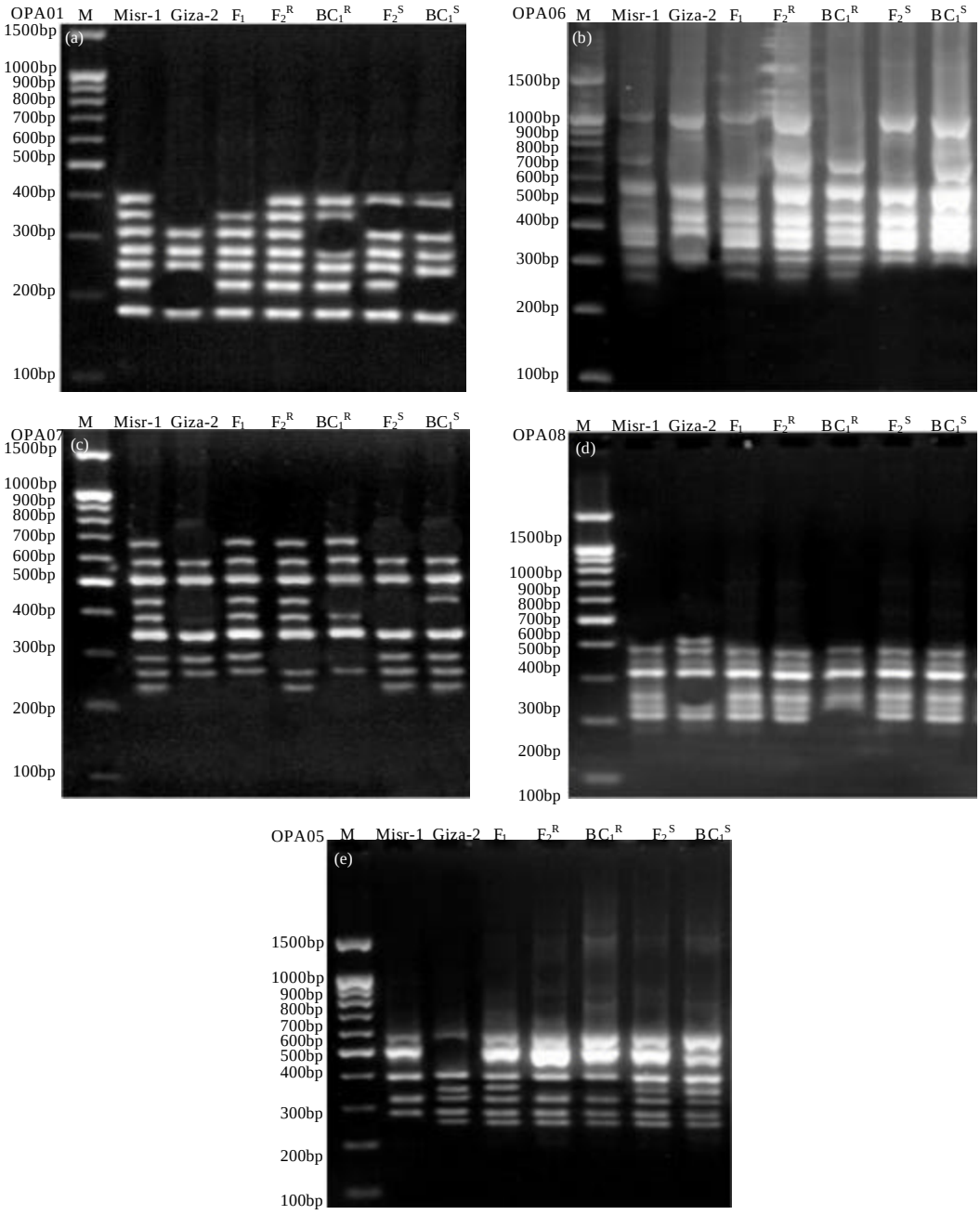

Fig. 1(a-e): RAPD profiles for the tolerant parent (Misr-1), sensitive parent (Giza-2), their F_1, tolerant F_2 bulk (F_2^R) and tolerant BC_1 bulk (BC_1^R), sensitive F_2 bulk (F_2^S) and sensitive BC_1 bulk (BC_1^S) using five ten-mer primers

not in the sensitive F_2, BC_1 bulked DNA and Giza-2 (sensitive parent). Primer OPA07, produced two strong polymorphic bands, bands at 669 bp and 385 bp were present only in the F_1, tolerant DNA bulked and Misr-1 (resistant parent), whereas band at 292 bp was present only in the sensitive F_2 and BC_1 bulked DNA and Giza-2 (sensitive parent). Also, primer OPB05, produced a strong polymorphic band at 253 bp that was present only in the F_1, sensitive F_2 and BC_1 bulked DNA and Giza-2 (sensitive parent), but not in the tolerant F_2 and BC_1 bulked DNA and Misr-1 (resistant parent). Primer OPA08 seemed to be the only one to generate no *Orobanche* tolerant-related markers but produced unique band at 382 bp for Giza-2 (sensitive parent). These polymorphic markers; viz, The OPA01 345 bp, OPA06 272 bp, OPA07 669 bp , 385 bp and 292 bp and OPB05 253 bp, were further used to check their

linkage to *orobanche* tolerant genes, using a segregating F_2 and BC populations, derived from the cross between the tolerant parent (Misr-1) and the sensitive parent (Giza-2).

ISSR marker: Out of ten ISSR primers screened for polymorphisms between the two tested parents, five ISSR primers (50%) with polymorphic bands were suitably recognized to differentiate between the two parents. The five ISSR primers were used to screen DNA bulks of F_1, F_2, BC_1 and their parents (Misr-1 and Giza-2). A total of 44 DNA fragments were obtained from genotypes with an average 8.8 bands/primer that ranged in size from 816 bp (HB) to 182 bp (HB), (Table 4 and Fig. 2). Of the 44 bands

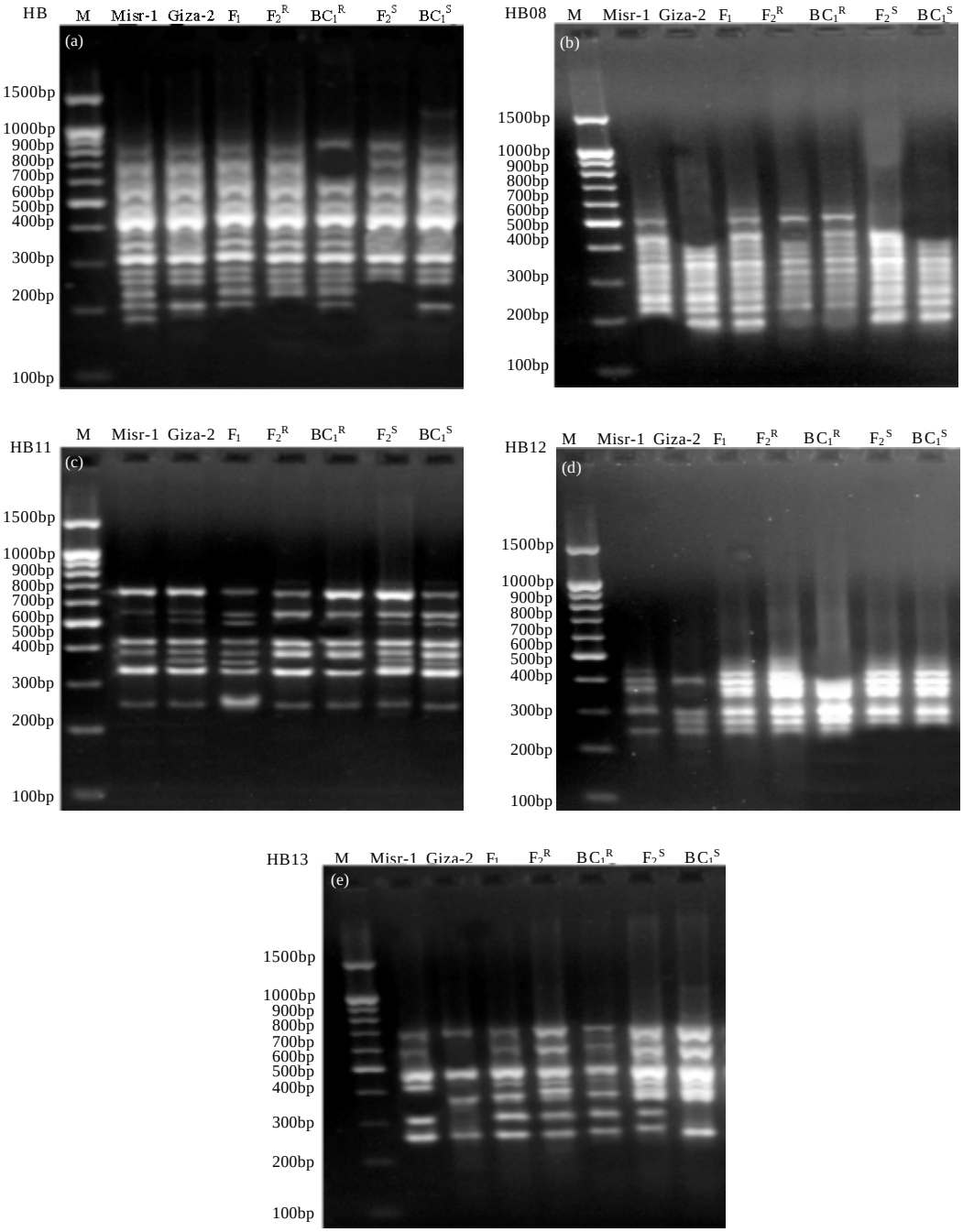

Fig. 2(a-e): ISSR profiles for the tolerant parent (Misr-1), sensitive parent (Giza-2), their F_1, tolerant F_2 bulk (F_2^R) and tolerant BC_1 bulk (BC_1^R), sensitive F_2 bulk (F_2^S) and sensitive BC_1 bulk (BC_1^S) using five primers

obtained, 18 (40.91%) were polymorphic (Table 4, Fig. 2). Of these five ISSR primers, the HB primer generated two polymorphic fragments at 359 bp and 231 bp which were present only in the tolerant genotypes (Misr-1, F_1 and DNA bulk for F_2 and BC_1) whereas, these fragments not found in sensitive genotypes (F_2 and BC_1 bulk and Giza-2), These bands may be considered as specific markers for *Orobanche* tolerance (Fig. 2). HB primer generated polymorphic unique band at 182 bp in the tolerant parent (Misr-1). In addition, HB08 primer generated a strong marker (band at 521 bp) only in the tolerant parent, F_1 plants and tolerant F_2 and BC_1 bulks. In contrast, in the sensitive genotypes, HB08 primer could amplify fragment with molecular size 207 bp; such fragments are specific for sensitive genotypes. The primer HB11, generated polymorphic fragments at 509 bp and 358 bp which were present only in the sensitive parent (Giza-2) and the sensitive DNA from F_2 and BC_1 bulks and were missing in the tolerant genotypes. Primers HB12 and HB13 generated high percentage polymorphic fragments (50 % and 57.14%) between the two parents but with no molecular markers related to *Orobanche* tolerance.

SRAP markers screening: Among the nine SRAP primers screened, four primers (SRAP1, SRAP2, SRAP3 and SRAP4) were polymorphic between the sensitive and resistant parents. The four primers generated a total of 41 DNA fragments among the tested genotypes with an average of 10.25 bands/primer which ranged in size from 997 bp (SRAP3) to 89 bp (SRAP4) (Table 5, Fig. 3). The total number of polymorphic markers and the percentage of polymorphism were 23 and 56.1%, respectively (Table 5).

Table 5: DNA polymorphism among contrasting parents (Misr-1 and Giza-2), F_1, two contrasting groups of F_2 and two contrasting groups of BC_1 bulk with SRAP marker

Primers	bp	P_1	P_2	F_1	F_2^R	BC_1^R	F_2^S	BC_1^S
SRAP1	546	1	1	1	0	1	1	0
	457	1	1	1	1	1	1	1
	390	1	1	1	1	1	1	1
	354	1	0	1	1	1	0	0
	311	1	1	1	0	0	1	1
	270	0	1	1	1	1	1	1
	235	1	0	1	1	1	1	1
	188	1	1	1	1	1	1	1
	140	1	1	1	1	1	0	1
	709	1	1	1	1	1	1	1
	643	0	1	1	0	0	1	1
	502	1	1	1	1	1	0	1
SRAP2	447	1	0	1	0	0	1	0
	391	1	1	1	1	1	1	1
	357	1	1	1	1	1	1	1
	311	1	0	1	1	1	1	1
	278	0	1	1	0	1	1	1
	254	1	1	1	1	1	1	1
	239	1	1	1	1	1	1	1
	223	1	0	0	0	0	0	0
	200	1	0	1	1	1	0	0
	997	1	0	1	1	1	0	0
	493	0	1	1	1	1	1	1
	420	1	1	1	1	1	1	1
	362	1	0	1	1	1	0	0
SRAP3	324	1	1	1	1	1	1	1
	282	1	1	1	1	1	1	1
	246	1	1	1	1	1	1	1
	226	1	1	1	1	1	1	1
	208	1	0	1	1	1	1	1
	169	0	1	1	0	0	1	1
	547	1	1	1	1	1	1	1
	464	1	0	1	1	1	1	1
SRAP4	344	1	1	1	1	1	1	1
	309	1	1	1	1	1	1	1
	269	1	1	1	1	1	1	1
	242	1	0	1	1	1	0	0
	173	1	0	1	1	1	1	1
	146	1	1	1	1	1	0	0
	117	0	1	1	1	1	1	1
	89	1	1	1	1	1	1	1

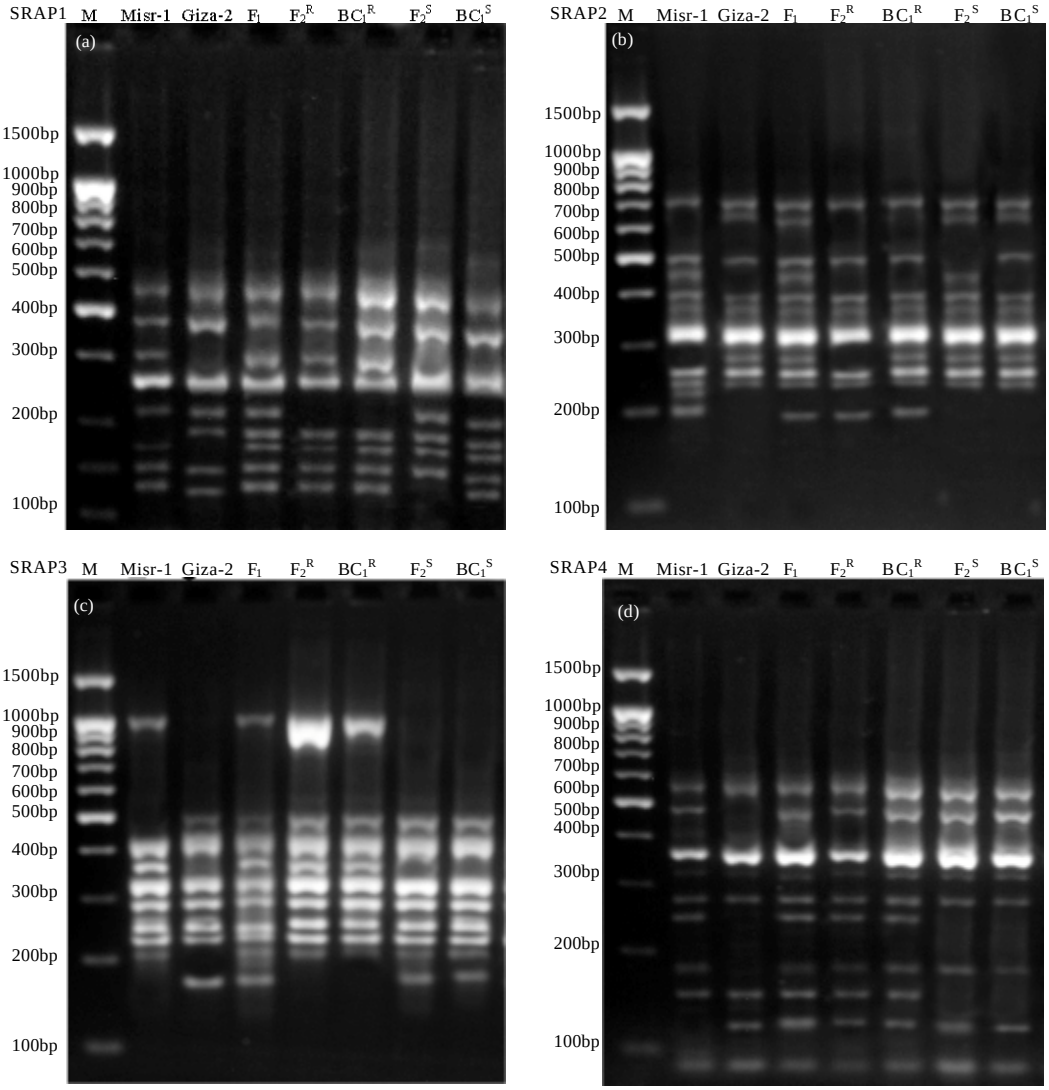

Fig. 3(a-d): SRAP profiles for the tolerant parent (Misr-1), sensitive parent (Giza-2), their F_1, tolerant F_2 bulk (F_2^R) and tolerant BC_1 bulk (BC_1^R), sensitive F_2 bulk (F_2^S) and sensitive BC_1 bulk (BC_1^S) using four primers

Of these four SRAP primers, the primer SRAP1 generated one polymorphic fragment at 354 bp which was present only in the F_1, tolerant DNA bulked F_2 and BC_1 and tolerant parent (Misr-1) but was missing in the sensitive parent (Giza-2) and the sensitive F_2 and BC_1. In addition, primer SRAP2 generated a strong band at 643 bp in the sensitive genotypes but was missing in the tolerant genotypes, while a band at 200 bp was generated only in the tolerant genotypes. The SRAP3 primer, generated a polymorphic fragment at 362 bp which was present only in the tolerant parent, F_1 and tolerant bulked F_2 and BC_1. On the other hand polymorphic fragment at 169 bp amplified only in the sensitive F_2 and BC_1 bulks and the sensitive parent (Giza-2). Primers SRAP4 produced a strong polymorphic band at 242 bp that was present only in the F_1, tolerant F_2 and BC_1 bulks and Misr-1 (resistant parent), but not in the sensitive F_2 and BC_1 bulked DNA and Giza-2 (sensitive parent).

DISCUSSION

The fact that the of F_1 hybrid exceeded its high performing parent (HP) for plant height, number of pods/plant, 100 seed weight and seed yield/plant, provide evidence for the presence of over-dominance and/or epistasis gene effects. Meanwhile, the other studied

characters showed partial to complete dominance. Also these results indicated that the two parental genotypes of this cross were greatly different in genes controlling these characters. The dominance effect [h] was positive for all studied characters indicating heterotic effect and that the dominant increasing alleles were more frequent than the decreasing recessive ones in the genetic constitution of the studied parental genotypes. The presence of positive and significant heterosis over the mid-and the better parent in most of the studied traits suggests that dominance and/or over dominance gene effects were acting towards taller plants, greater number of pods/plant, heavy 100 seed weight and out yield/plant. Meanwhile, the negative and significant heterosis obtained indicated that dominant genes were acting toward less number of branches/plant. These results are in agreement with these reported by El-Hosary (1982), Abul-Naas *et al.* (1991), Helal (1997) and Bakheit *et al.* (2002). The positive % inbreeding depressions for all characters confirm the role of dominance and non-allelic gene interaction as the scaling tests A, B and C showed. The six-parameter model as proposed by Mather and Jinks (1977) indicated the significance of the different types of non-allelic gene interaction which is in harmony with those obtained by Abul-Naas *et al.* (1991) and Bakheit *et al.* (2002). Separation of the component of non-allelic interaction indicated significant values for simple additive [d] effects for all traits and dominance effect [h] for plant height, number of branches/plant and seed yield/plant. The magnitude of dominance [h] was greater than the additive [d] effect except for number of pods/plant indicating the importance of dominance gene effects. Similar results were reported by Abul-Naas *et al.* (1991), El-Hady *et al.* (1997) and Bakheit *et al.* (2002).

Bulk segregant analysis was firstly reported by Michelmore *et al.* (1991) to identify RAPD markers tightly linked to genes for resistance to lettuce downy mildew. In this study, screening molecular markers related to Oronanche resistance in faba bean was employed in a segregating population (Misr-1 X Giza-2). The plant populations were genotyped by RAPD, ISSR and SRAP markers. The present results support the idea that BSA can provide fast detection of molecular markers linked to genes of interest. Using BSA combining RAPD analysis, four specific bands (OPA01 345 bp, OPA06 272 bp, OPA07 669bp and 292 bp) were found in tolerant genotypes these bands could be considered as positive markers for *Orobanche* tolerance. While, two fragments (OPA07 292 bp and OPB05 253 bp) were tightly linked to *Orobanche* sensitive genotypes. These results confirmed the useful application of RAPDs-PCR with BSA analysis which agreed with El-Sayed *et al.* (2013) who used six RAPD primers to detect markers for *Orobanche* tolerance and found that four RAPD markers were tightly linked to *Orobanche* tolerance. Gutierrez and Kuti (2001) used RAPD markers to differentiate between 10 faba bean genotypes that showed resistance and susceptibility to *Orobanche crenata*. They observed distinct polymorphic DNA bands corresponding to either resistance or susceptibility in 15 tested primers. Fifteen ISSRs primers were used to obtain ISSRs markers for blast disease by Fahmy *et al.* (2006) and they reported that ISSRs are considered good molecular markers for blast disease, especially when comparing parents, F1 and F2 individual plants. In this study we used five ISSR primers to identify marker linked to *Orobanche* tolerant. Three positive markers [359 bp and 231 bp (HB primer) and 521 bp (HB08)] and three negative markers [207 bp (HB08) and 509 bp abd 358 bp (HB11)] were linked to *Orobanche* tolerant/sensitive in faba bean genotypes (tolerant and sensitive parents, F_1, tolerant and sensitive F_2 and BC_1 bulk). Our results in agreement with El-Sayed *et al.* (2013) who used four ISSR primers to detected markers related to *Orobanche* tolerant and found that positive and negative marker tightly linked to *Orobanche* tolerant in faba bean sensitive and tolerant parent, their subsequent F_1 and DNA bulks of the tolerant and sensitive groups of F_2 and BC_1 segregating populations. These results confirmed the useful application of ISSRs-PCR analysis to detect the genetic variability between sensitive and tolerant genotypes which agree with El-Sayed *et al.* (2013) who demonstrated that ISSR markers are a valuable method for detecting genetic variability among faba bean varieties and for rapidly identifying tolerant and sensitive genotypes to *Orobanche*. Furthermore, Ghareeb (2006) indicated some molecular markers for burchid resistant in faba bean for parents, F_1 and F_2 individual genotypes. Also the results indicated seven SRAP markers (SRAP1 354 bp, SRAP2 643 bp and 200 bp, SRAP3 99, 362, 169 bp and SRAP4 242 bp) were linked to the *Orobanche* tolerant in faba bean. The use of molecular markers can increase the efficiency of conventional plant breeding by identifying markers linked to the trait of interest which are difficult to evaluate and/or are largely affected by the environment. Hence, there is a need to develop a rapid screening method to select the disease resistance individuals. Tight linkage between molecular markers and genes for disease resistance can be of great benefit to *Orobanche* resistance breeding programs by allowing the investigator to follow the DNA markers (PCR-based markers) through early generations rather than waiting for phenotypic expression of the resistant genes. Molecular markers that are closely linked with target genes present a useful tool in plant breeding since they can help to

detect the tolerant genes of interest without the need of carrying out field evaluations. Also, it allows for screening big number of breeding materials at early growth stages and in short time. The present study indicated that RAPD, ISSR and SRAP markers, combined with bulked segregant analysis, could be used to identify molecular markers linked to the *Orobanche* resistance in faba bean. Once these markers are identified, they can be used in faba bean breeding programs as a selection tool in early generations.

CONCLUSION

The bulk segregant analysis method provided a rapid and simple alternative technique to identify six RAPD markers, six ISSR and seven SRAP linked to *Orobanche* resistance in faba bean. These markers in this study can be regarded as markers tightly linked to a quantitative character and it can be used in molecular-assisted breeding procedures. molecular-assisted breeding of *Orobanche* resistance in faba bean plants cab identify the *Orobanche* resistance of plants in the early stage of breeding, increasing selection veracity and efficiency and can accelerate reasonable and rapid utilization of resistance gene, shortening the breeding cycle.

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