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GENETIC INTERACTIONS AND RELATIONSHIP AMONG FABA BEAN (*VICIA FABA* L.) AND *OROBANCHE* ASSOCIATED BY ISSR MOLECULAR MARKERS

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Abstract

This study aimed to detect variations between four faba bean cultivars by using hybridization between minor, equine, major types. In addition, studying the detected variations on molecular markers level using ISSR and determine the genetic similarity between faba bean plants and its parasitic weed *O. crenata*. Five different ISSR primers were used and general results showed that the total amplified fragments (TAF) that induced in the three crosses using all primers were 301 bands (with an average 20.07 per primer) which distributed as 214 polymorphic bands (PB), 90 unique bands (UB) and finally 1 monomorphic band (MB). The molecular similarity between faba bean host and *Orobanche* parasite may be indicate some kind of complementary genes system.

Key words: Faba bean, *Orobanche crenata,* Molecular Markers, ISSR Markers, Genetic Polymorphism, Genetic variability and similarity.

Introduction

Faba bean (*Vicia faba* L.) has a great role in human nutrition as a major source of protein. The crop is generally included in the crop rotation with other leguminous crops to keep soil fertile and productive through nitrogen fixation. Moreover, faba bean is known to suffer from narrow genetic base and useful variability. Therefore hybridization offers good possibilities for widening the genetic base, studying the nature of genetic systems controlling the inheritance of traits and/or transfer of characters/genes from genotype to another.

Broomrape (*Orobanche crenata*) is a parasitic plant on the host faba bean. The genetic nature of broomrape resistance is not that clear till now and requires more studies on Egyptian faba bean genotypes. Sources of resistance to broomrape are scarce and of complex nature. However, several tolerant cultivars were released to farmers in Egypt from Agriculture Research Center (ARC) and Cairo University. An acceptable level of resistance was found in Vf1071, an inbred line selected from the Egyptian *cv*. Giza 402 in Southern Spain (Sillero *et al.,* 1996). This line has been used in breeding programs to develop the well-adapted, high yielding *cv*. *Baraca* (Cubero and Moreno, 1999)

O. crenata is an annual parasitic weed that causes heavy losses to its host crop faba bean. Determining the genetic diversity in *Orobanche germplasm* is a preliminary crucial step in faba bean breeding programs. It helps in identifying liable criteria of host tolerance. So that, Abdalla *et al.*, (2016) studied the genetic diversity and genetic relationships among and within *Orobanche* collected from two divergent locations in Egypt (Giza and Sids) by using five different ISSR primers. However, the results showed that there were 73 fragments with an average of 14.6 fragment / primer were detected and the polymorphism percentage ranged from 0.86-0.94

Developing cultivars that resist biotic stresses is one of the major goals of breeding programs of faba bean. Molecular markers can help these programs by tagging the important traits, helping in screening the genotypes

and selecting them throughout the course of breeding programs (Gillanders *et al.,* 2002 and *Gadalla et al.,* 2012), ISSR is a popular marker system, owing to its ability to detect polymorphisms without requiring the sequence information necessary for primer design. The main advantage of ISSRs is that no sequence data for primer construction are needed. This is mostly dominant marker. It is widely used for characterization of genetic relatedness among populations (Tomar *et al*., 2014).

ISSR markers are suitable for investigating genetic diversity among *O. aegyptiaca* genetic groups and able to discriminate between individuals (Abedi *et al*., 2014). ISSR markers have several benefits over other techniques: first, it is known to be able to discriminate between closely related genotypes (Fang and Roose, 1997 and Hodkinson *et al.,* 2002) and second, it can detect polymorphisms without any previous knowledge of the crop's DNA sequence. ISSR markers are quick and easy to handle and more informative for the evaluation of genetic diversity (Korbin *et al.,* 2002 and Rakoczy and Bolibok, 2004).

The objectives of this study were to:

1. Explore the variability obtained from crossing divergent faba bean genotypes in tolerance to *Orbanche*.

2. Select good tolerant combinations from segregating generations.

3. Determine the genetic similarity between faba bean plants and its parasitic weed *O. crenata* using ISSR molecular markers.

Materials and Methods

The present investigation was conducted during the three growing seasons: 2015/16, 2016/17 and 2017/18, at Gemmeiza Research Station, ARC, Egypt, in two different locations. Molecular studies were carried out at Genetics Department, Faculty of Agriculture, Zagazig University, Egypt.

Four widely diverse faba bean genotypes namely Nubaria 1 (P_1), Giza 843 (P_2), Camiliena (P_3) and Cairo 33 (P_4) were used as parents in this study (Table 1). Crossing was carried out among the four faba bean genotypes by hand under insect free cage during 2015/16 season using Cairo 33 only as a female plant. In 2016/17, the parental genotypes were planted again under insect free cage and re-hybridized to secure more F_1 hybrid seeds. The F_2 seeds were obtained from the F_1 plants raised under cages. In 2017/18, the four parents and each of 3 F_1's and 3 F_2's were planted in open naturally *Orobanche*-infested field. A randomized complete blocks design with three replications was used. Each entry was represented by one row in parents and F_1's and four rows in F_2's. Each row was 2.5 m long, 50 cm between rows and seeds were sown individually at 20 cm distance.

DNA Isolation

Thirty four plants from faba bean (F_2 crosses and parents) and attached *Orobanche* were individually collected from the naturally *Orobanche*-infested field during the growing season of 2017/2018 and prepared for molecular assay.

The genomic DNA was isolated from 1g of young leaves of faba bean plants and shoot tip of *Orobanche* spikes. Plant samples were ground to a fine powder in liquid nitrogen and extracted using Biospin plant genomic DNA extraction kit (Bio Basic Inc. Kit Leading Supplier and Manufactures of Life Science Products and services, Canada). DNA quality was checked using 1.0% agarose gel electrophoresis.

ISSR amplification

ISSR amplification reactions were carried out in 15 µl volume containing 1µl DNA (40 ng), 7.5µl Master Mix (Gene Direx one PCR TM), 1µl template DNA and 1µl primer. Five different primers were tested (Table 2). The amplification reaction consisted of an initial denaturation step at 94°C for 7 min, followed by 35 cycles of 30 sec. at 94°C (denaturation), 45 sec. at 52°C (annealing) and 2 min at 72°C (extension) followed by a final extension step at 72°C for 5 min. Amplification products were electrophoresed on 1.5% agarose in 1× TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system. Each experiment was repeated twice with each primer and those primers which gave reproducible fingerprints were considered for data analysis.

Statistical Analysis

Amplified fragments were scored manually for the presence (1) or absence (0) of homologous bands to develop a binary matrix of different ISSR phenotypes.

Polymorphism % was calculated according this equation:

Polymorphism percentage (PB%) = (UB + PB) / Total bands

Where:-

UB = Number of unique bands,

PB = Number of polymorphic bands

Note: Samples on gel fig. and tables were distributed as from 1 to 5 were for faba bean and from 6 to 10 were for *Orobanche*, moreover, each faba bean plant has a specific *Orobanche*.

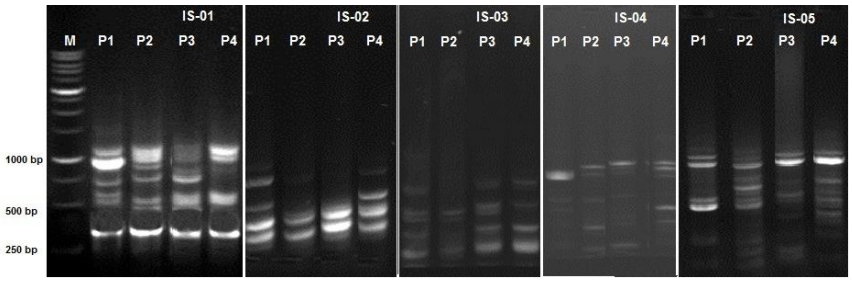

Fig. 1: Banding pattern of tested faba bean parents generated by five ISSR primers M= DNA standard marker, P_1= Nubaria 1, P_2= Giza 843, P_3= Camiliena and P_4= Cairo 33.

For example: 1(F1) match 6(OR1), 2(F2) match 7(OR2),…… and 5(F5) match 10(OR5).

Results and Discussion

Genetic polymorphism

The significant differences among faba bean plants, crosses and *Orobanche* induced using the five ISSR primers were clearly shown and summarized in table 3 and (Fig. 1 and 2). However, multiple bands varied in their molecular weight were detected in all tested plants using these different primers. The polymorphic percentage reached 100% in all tested plants with all five primers except the cross Cairo 33 × Giza 843 with primer IS-04 where polymorphism recorded 93.33%, whereas, there was only one monomorphic fragment induced by this primer. Moreover, all primers varied in inducing fragments (bands), whereas, every primer induced variable numbers of amplified bands which varied in total number, type (Monomorphic, Polymorphic and Unique) and finally in the range of molecular size of these amplified bands which varied also between primers. In general, the total amplified fragments (TAF) that induced in the three crosses using all primers were 305 bands (with an average 20.07 per primer) which distributed as 214 polymorphic bands (PB), 90 unique bands (UB) and finally 1 monomorphic band (MB).

As such as, these previous bands were distributed between crosses and primers as follow:

1. Cairo 33 × Nubaria 1: The results revealed that there were 104 amplified bands with different molecular weights ranged from154.75-2500 bp were detected by the used primers table 3. Moreover, these amplified fragments were distributed between polymorphic bands (78 bands) and unique bands (26 bands) and there was not any monomorphic bands detected in this cross, so, polymorphism percentage was 100%.

2. Cairo 33 × Giza 843: There were 115 amplified bands were detected and distributed as polymorphic bands (70 bands), unique bands (44 bands) and only 1 monomorphic band and for this reason the polymorphism percentage became 93.33%. Moreover, the molecular weight of detected bands ranged 153.15-1578.12 bp. (Table 3).

3. Cairo 33 × Camilina: The polymorphism percentage recorded 100% in this cross, also, there were 82 amplified

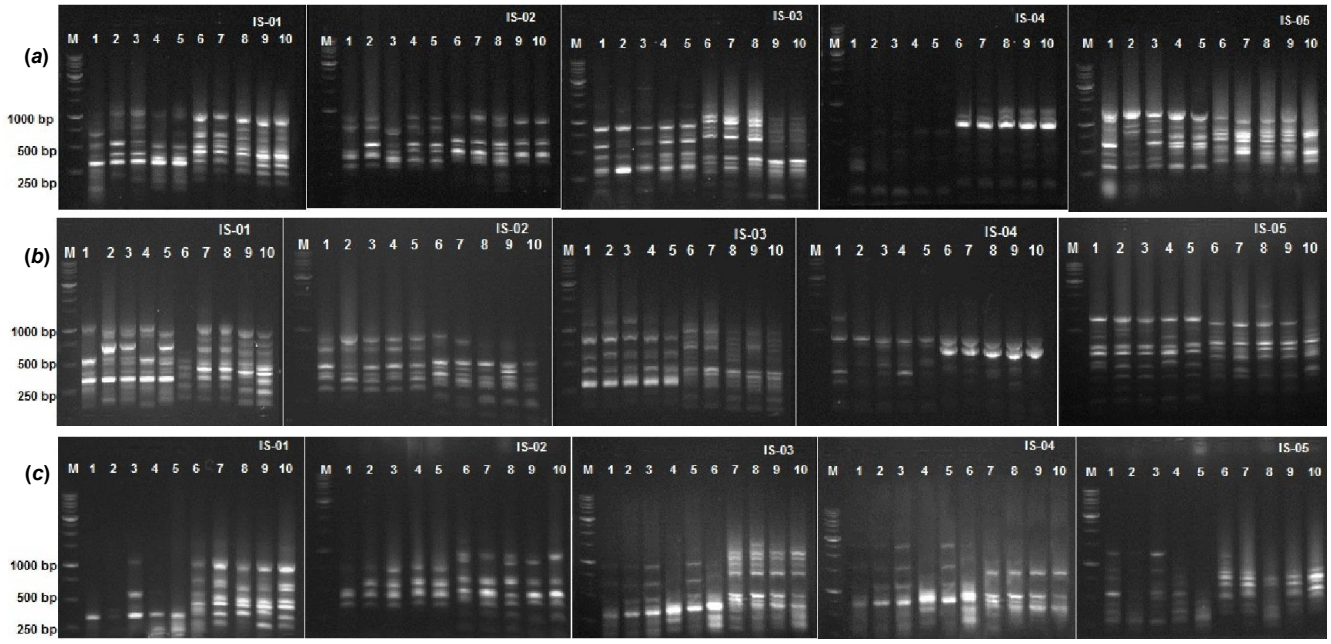

Fig. 2: ISSR profile of sample genotypes of faba bean crosses and *Orobanche* generated by five ISSR primers. M= DNA standard marker, (1-5) = (F1- F5)= Faba bean samples and (6-10)= (OR1-OR5)= *Orobanche* samples. (*a*): Cairo 33 × Nubaria 1, (*b*): Cairo 33 × Giza 843 and (*c*): Cairo 33 × Camilina.

Table 1: Types, pedigree and characteristics of faba bean parental genotypes used in the present study.

Name	Type	Pedigree	Characteristics
Nubaria 1 (P_1)	*Major**	Individual plant selection from Spanish variety	Tolerant to *orobanche*.
Giza 843 (P_2)	*Equina*	Individual selection from Rebaya 40 (FCRI)	Tolerant to *Orobanche* .
Camilina (P_3)	*Minor*	Introduction from Ethiopea.	Susceptible to *Orobanche*.
Cairo 33 (P_4)	*Equina*	Individual selection from breeding program (FACU)	Susceptible to *Orobanche*.

* (See classification of Muratova 1931) FCRI = Field Crops Research Institute. FACU = Faculty of Agriculture, Cairo University (see Abdalla, 2015 for details).

Table 2: ISSR primer sequences used for DNA fingerprinting of faba bean plants and *Orobanche*.

	Primer Code	Primer Sequence (5> ⟶ 3>)
1	ISSR-807	(AG)8 T
2	ISSR-810	(GA)8 T
3	ISSR-835	(AG)8 GYC
4	ISSR-841	(GA)8 YC
5	ISSR-857	(AC)8 YG

bands (20 unique bands and 62 polymorphic bands) were detected using the five primers, and the molecular weights ranged 185.04-2066.92 bp (Table 3). Moreover, the highest number of amplified bands (75 bands) was achieved using primer IS-01, while, the lowest number (49 bands) produced by IS-04.

Mejri *et al*., (2012) concluded that, appearance or disappearance of different DNA bands with variation of their intensity as well, might be connected with structural rearrangements in DNA caused by different types of DNA damages (breaks, transpositions, deletion, etc). Abd-Elrahman and Abd El-Khalek, (2013) used five RAPD primers to differentiate between seven Egyptian faba bean cultivars and obtained 40 bands 29 of them were polymorphic (72.5% polymorphism).

Abdalla *et al*., (2016) studied the genetic relationships among and within collected groups of *O. crenata* using ISSR markers. The results showed that there were 73 amplified fragments with an average of 14.6 fragment / primer induced by using five ISSR primers. Moreover, molecular weight of these fragments ranged from 218 to 980 bp with polymorphism percentage ranged from 86-94%.

Genetic Similarity

Genetic similarity matrix among all studied faba bean genotypes, crosses and *Orobanche* was obtained from amplified fragments shown by five ISSR markers using Jaccard coefficients (Jaccard, 1908). Moreover, cluster analysis based on similarity matrix was performed using UPGMA (unweighted pair group method with arithmetic mean) method of NTSYSpc ver. 2.21o (Exeter Software, Setauket, NY, USA) (Rohlf, 2010).

1. Cairo 33 × Nubaria 1: The UPGMA dendrogram showing genetic relationship between faba bean plants (Parents and crosses) and *Orobanche* using ISSR markers is presented in table 4 and fig. 3A, respectively. However, generally, there were clear relationships or genetic similarity between and within all tested plants of this cross and the degrees of this similarity varied and\ ranged from 0.0 to 1.0 (Table 4). Moreover, there were close relationship between the two parents of this cross (Cairo 33 (P4) and Nubaria 1 (P1)) and this similarity reached 0.709. Others else, there were relationship between Nubaria 1 and all segregant plants (similarity matrix of 0.709 for P1/F3 which was the highest and 0.563 which was the lowest), respectively. On the other hand, there were a large relationship between P1 and *O. crenata* that grown in the specific area of this cross similarity matrix ranged from 0.689-0.631 for both P1/OR3 and P1/OR5, respectively (Table 4 and Fig. 3A).

Table 3 : ISSR -markers for selected segregants of faba bean plants and *Orobanche* generated by five primers.

CROSS	Primers	Marker size (bp)	Amplified bands				PB %
			TAF	MB	UB	PB	
Cairo 33 × Nubaria 1	IS- 1	1285.37 - 167.25	25	-	10	15	100%
	IS- 2	929.34 - 180.69	19	-	2	17	100%
	IS- 3	2500.00 - 173.41	18	-	4	14	100%
	IS- 4	1342.76 - 154.75	19	-	3	16	100%
	IS- 5	1302.69 - 283.58	23	-	7	16	100%
Cairo 33 × Giza 843	IS- 1	1150.03 – 153.35	31	-	9	22	100%
	IS- 2	922.26 - 153.15	19	-	8	11	100%
	IS- 3	1246.22 – 226.41	30	-	18	12	100%
	IS- 4	1535.23 - 251.56	15	1	6	8	93.33%
	IS- 5	1578.12 – 157.49	20	-	3	17	100%
Cairo 33 × Camilina	IS- 1	1105.08 – 214.15	19	-	6	13	100%
	IS- 2	976.22 - 265.36	14	-	4	10	100%
	IS- 3	1452.10 – 206.22	19	-	7	12	100%
	IS- 4	2066.92 - 238.16	15	-	2	13	100%
	IS- 5	1205.79 – 185.04	15	-	1	14	100%
Total			301	1	90	214	-
Average			20.07	0.07	6	14.27	87.12

TAF = Total amplified fragments, MB = Number of monomorphic bands, PB = Number of polymorphic bands, PB% = Polymorphism percentage.

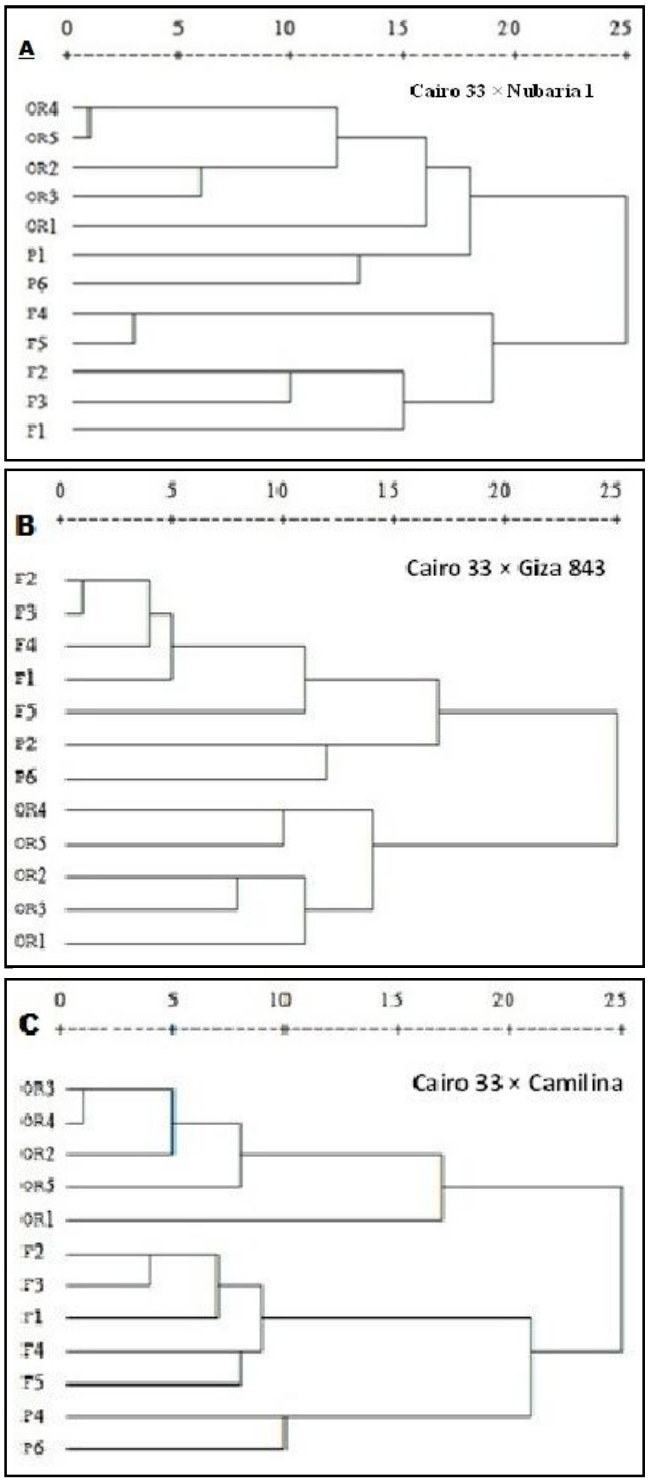

Fig. 3: Dendrogram of the genetic distances among and within three faba bean crosses and *Orobanche* based on ISSR analysis. (A): Cairo 33 × Nubaria 1, (B): Cairo 33 × Giza 843 and (C): Cairo 33 × Camilina.

Also, similarity among P4 and segregant plants was less than among P1 and segregant plants and ranged from 0.670-0.553 for P4/F2 and P4/F1, respectively and ranged from 0.689-0.592 for P4/OR2 and P4/OR3, respectively in case of similarity between P4 and *Orobanche*.

With regard to the similarity between and within plants of this cross, it was clearly shown that the most closely related plants were F4/F5 followed by F2/F3 (similarity matrix of 0.825 and 0.738, respectively. The lowest relationships were recorded for F3/F4 (similarity matrix of 0.592), followed by F1/F4 (similarity matrix of 0.602) (Table 4).

However, the highest relationship between segregant plants and *Orobanche* was recorded between both F4 and F5 with OR1 (0.602) and the lowest relationship was F3/OR4 (0.417). On the other hand, between all *Orobanche* plants, there were high relationship where similarity matrix ranged from 0.854 (the highest) for OR4/OR5 followed by 0.786 for OR2/OR3 and at the end the lowest one was OR1/OR5 which similarity matrix scored 0.602. (Table 4 and Fig. 3A).

The dendrogram (Fig. 3A) is divided to two major clusters, however, the first cluster is sub divided into two subclusters one included (F1, F2 and F3) while the other subcluster involved (F4 and F5). Also, the second cluster was divided into two subclusters, the first cluster contained P1 and P4 and the other subcluster is sub divided into two sub-sub cluster, one of them contained OR1 and the second contained OR4, OR5, OR2 and OR3.

2. Cairo 33 × Giza 843: The dendrogram (Fig. 3B) is divided to two major clusters, however, the first cluster divided into two subclusters (one included (OR1, OR2 and OR3) while the other subcluster involved (OR4 and OR5). Also, the second cluster was sub divided into two subclusters, the first one contained P2 and P4 and the other subcluster is divided into two sub-sub clusters, one of them contained F5 and the second contained F1, F2, F3 and F4. (Fig. 3B).

Moreover, table 5, illustrates the relationship and genetic similarity among and within faba bean plants (parents and crosses) and *Orobanche* using ISSR markers, however, the relationship between parents (P2 and P4) recorded similarity of 0.748 and the most closely related plant with the cultivar Giza 843 was plant number 2 (F2) that recorded 0.739 as similarity matrix followed by F3 (0.722), while the lowest one was F1 (0.670). With regard to relationship within segregant plant, there were a force relationship among these plants and the highest one was F1/F2 and F2/F3 with similarity matrix 0.861, while, F3/F5 recorded the lowest one (0.704). On the other hand, between *Orobanche* plants, the most related plants were OR2/OR3 and the lowest one was OR1/OR5. (Table 5).

Moreover, there were relationship between both segregant plants and between *Orobanche* that grown

Table 4 : Similarity matrix among the cross Cairo 33 × Nubaria 1 (parents, five segregants faba bean plants (F1-F5) and *Orobanche crenata* (OR1-OR5) using ISSR molecular marker analysis.

	Nubaria 1	Cairo 33	F1	F2	F3	F4	F5	OR1	OR2	OR3	OR4
Cairo 33	.709										
F1	.612	.553									
F2	.709	.670	.709								
F3	.563	.583	.660	.738							
F4	.583	.583	.602	.660	.592						
F5	.602	.602	.641	.680	.612	.825					
OR1	.641	.621	.583	.505	.456	.612	.612				
OR2	.650	.689	.573	.534	.524	.583	.563	.699			
OR3	.689	.592	.515	.476	.427	.544	.563	.699	.786		
OR4	.641	.641	.602	.485	.417	.553	.573	.650	.699	.738	
OR5	.631	.631	.553	.515	.447	.544	.563	.602	.709	.709	.854

on the same plants, whereas, the most related plants were F3/OR3 and F3/OR2 that recorded (similarity matrix for 0.652 and 0.643), respectively. On other side, F1/OR5 was the lowest one in its relationship (0.530). (Table 5).

3. Cairo 33 × Camilina: Table 6 and fig. 3C, show the genetic similarity between plants of cross Cairo 33 × Camilina. There was clear relationship between all plants, *Orobanche* and parents and also among plants/ *Orobanche* and between *Orobanche* plants. (Table 6 and Fig. 3C).

Abedi *et al.,* (2014) in Iran used ISSR markers for investigating genetic diversity among *O. aegyptiaca*. The fact that only five clusters emerged from the 96 samples of *Orobanche* (5%) indicates that the virulence/ aggressiveness of the parasite may not be that huge compared to its wide genetic diversity investigated by ISSR.

Table 7, shows the participated DNA fragments (polymorphic) that were detected between faba bean plants and *Orobanche* using ISSR primers. For each primer, the number and molecular weight of detected polymorphic bands varied from 1 to 9 bands and from 157.49 bp to 1235.59 bp, respectively. There was only on monomorphic band with molecular weights 805.55 bp detected in Cairo 33 × Giza 843 plants using primer IS-04. Moreover, it was also pronounced that Cairo 33 × Nubaria 1 plants and *Orobanche* plants had the same bands (26 bands) with the molecular weights varied from (167.25bp to 1235.59bp) and these participated fragments distributed between all tested faba bean plants and its parasitized *Orobanche* (Table 7).

However, there were 23 and 16 polymorphic bands with the molecular weights from (157.49bp to 1071.64bp) and from (268.01bp to 950.51bp) detected using all ISSR primers in plants of (Cairo 33 × Giza 843) and (Cairo 33 × Camilina) and their parasitized *Orobanche*, respectively and these fragments were distributed between all tested faba bean plants and its own *Orobanche* (Table 7).

Discussion

There were a wide genetic distance in faba bean parents used in this study (Minor, Equina, Major types), so that, the progeny or the segregants have a marked variation within others and this was proved by using ISSR molecular marker technique that detected a wide range of amplified fragments distributed between polymorphic and unique bands with polymorphism 100% in most cases. Elshafei *et al.,* (2019) reported that the UPGMA based dendrogram of the faba bean genotypes was generally based on their genetic background and place of origin.

On the other hand, till now there is no enough studies estimated the genetic variations of *O. crenata* samples, but in this study by using ISSR markers it have been proved that there were a wide similarity between faba bean plants and *O. crenata* + in the same growing plot and this similarity percentage differed between crosses under study. Moreover, the genetic variation in each sick plot which is expected because of several reasons such as seed dispersal by humans, animals, machinery, soil, water, wind and host seeds may have contributed to gene flow between plants of the *Orobanche* parasite. In addition, the genetic

Table 5 : Similarity matrix among the cross Cairo 33 × Giza 843 (parents, five segregants faba bean plants (F1–F5) and *Orobanche* (OR1–OR5) using ISSR molecular marker analysis.

	Giza 843	Cairo 33	F1	F2	F3	F4	F5	OR1	OR2	OR3	OR4
Cairo 33	.748										
F1	.670	.643									
F2	.739	.696	.861								
F3	.722	.696	.791	.861							
F4	.678	.704	.800	.817	.835						
F5	.704	.643	.774	.809	.704	.748					
OR1	.617	.643	.635	.617	.617	.626	.617				
OR2	.678	.635	.609	.609	.643	.635	.609	.748			
OR3	.670	.591	.565	.617	.652	.609	.583	.757	.783		
OR4	.643	.652	.557	.574	.609	.600	.574	.765	.722	.748	
OR5	.600	.591	.530	.548	.565	.557	.565	.687	.696	.722	.765

Table 6 : Similarity matrix among the cross Cairo 33 × Camilina (parents, five segregants faba bean plants (F1–F5) and *Orobanche* (OR1–OR5) using ISSR molecular marker analysis.

	Giza 843	Cairo 33	F1	F2	F3	F4	F5	OR1	OR2	OR3	OR4
Cairo 33	.750										
F1	.638	.613									
F2	.588	.638	.825								
F3	.588	.638	.750	.825							
F4	.588	.638	.775	.725	.750						
F5	.588	.638	.750	.775	.775	.775					
OR1	.525	.625	.663	.588	.613	.688	.563				
OR2	.563	.563	.550	.525	.500	.550	.475	.713			
OR3	.500	.550	.588	.563	.488	.538	.513	.625	.788		
OR4	.513	.588	.575	.550	.525	.600	.525	.663	.825	.863	
OR5	.575	.625	.588	.563	.563	.588	.513	.675	.738	.725	.838

variability between crosses and *O. crenata* per plot may be backed to the characteristics of parent's crosses (Table 1). Moreover, may be the most important reason is that *O. crenata* is reported to be predominantly an out-crossing parasitic weed (Musselman, 1986) and there were common genetic factors (susptability or resistance) between parents, their segregants and *O. crenata*. Therefore, in this study, from the evolutionary point of view the diversity of *O. crenata* matching the diversity of *V. faba* the host species which is an autogamous crop with cross pollination that may reach to 67% (Abdalla, 2015). The result will be host population in the field with very heterogenous nature that vary between complete homozygous and complete heterozygous and intermediate. In other words the dynamic nature of the host faba bean will result in populations in field where each plant will be different genotype (similar to the situation of *Orobanche*).

The molecular similarity between the host faba bean and the *Orobanche* parasite may indicate some kind of complementary genic system present between faba bean and *Orobanche* similar to that reported in flax and flax rust by Flor (1956) which was termed gene-for-gene hypothesis or complementary gene hypothesis.

The genetic data suggest that, for most host-parasite systems studied, the genes in host and parasite that confer specificity have their specific interaction for an incompatible relationship. The lack of specific interactions of these genes allows the development of compatible relationships between host and parasite. If this argument is correct, the specific interactions from the gene-for-gene relationships must be superimposed upon a basic compatibility between host and parasite. (Ellingboe, 1976).

The gene-for-gene relationships appear either to prevent the establishment of a compatible relationship or to destroy a compatible relationship once it is established. On the other hand, the data from studies of the blight of oats caused by *H. victoriae* strongly suggest that the specific interactions are needed for the parasite to successfully invade the host plant (George, 1960). The data suggest that the parasite must alter the host in order to develop. In some host-parasite combinations there appear to be no gene-for-gene relationships that can be demonstrated.

Kado and Innan (2018), sequenced genomes of five

Table 7: Molecular weight of participated DNA fragments between faba plants and *orobanche* using ISSR technique.

Cross	Primer	MW(bp)	Faba plant NO.	*Or.*
Cairo 33 × Nubaria 1	1	656.59, 457.49, 453.84, 330.06, 167.25	5, 4, 2-3, 1-2-3-4, 5	10, 9, 7-8, 6-7- 8-9, 10
	2	867.64, 465.87, 394.31, 342.42	2, 4, 5, 4, 5, 1, 4, 5, 4, 5	7, 9, 10, 9, 10, 6, 9, 10, 9, 10
	3	894.15, 825.48, 750.00, 658.74, 445.45, 333.71, 308.97, 222.72, 173.41	2, 1, 4-5, 3-4-5, 1-2-3, 4-5, 1, 1, 3	7, 6, 9-10, 8-9-10, 6-7-8, 9-10, 6, 6, 8
	4	428.99, 359.12, 345.78	1-3, 4, 1	6-8, 9, 6
	5	1235.59, 888.29, 830.15, 585.97, 489.61	1-4, 1, 2-3, 1-4, 1-2-4-5	6-9, 6, 7-8, 6-9, 6-7-9-10
Cairo 33 × Giza 843	1	1071.64, 769.38, 498.03, 454.89, 335.15, 287.67, 218.64, 198.73	3-4, 3, 1-2-3-4, 5, 2-3-5, 3, 1-2-3, 4	8-9, 8, 6-7-8-9, 10, 7-8-10, 8, 6-7-8, 9
	2	287.67, 218.64	2, 1	7, 6
	3	198.73, 613.46, 550.83, 411.96	1-2-3, 3, 1-3, 1-2	6-7-8, 8, 6-8, 6-7
	4	805.55, 609.38, 461.88, 365.267, 251.56	all, 3-4, 1-4, 4, 3-5	all, 8-9, 6-9, 9, 8-10
	5	896.20, 760.35, 673.14, 157.49	1, 5, 5, 1-2-3-4	6, 10, 10, 6-7-8-9
Cairo 33 × Camilina	1	323.28	1	6
	2	569.48	2-4-5	7-9-10
	3	950.51, 481.11, 428.62, 286.07	1-2-3-5, 5, 2-3, 1-2	6-7-8-10, 10, 7-8, 6-7
	4	464.38, 421.93, 366.47, 268.01	2-3-4-5, 1, 1-3-4, 1	7-8-9-10, 6, 6-8-9, 6
	5	726.97, 641.70, 525.84, 401.71, 328.68, 268.92	4, 3-4, 1-4-5, 1-4, 2-3, 1	9, 8-9, 6-9-10, 6-9, 7-8, 6

parasite species in family *Orobanchaceae* to explore the evolutionary role of horizontal gene transfer in plants. *Orobanche minor* and *Aeginetia indica* are obligate parasites with no photosynthetic activity, whereas the other three (*Pedicularis keiskei*, *Phtheirospermum japonicum* and *Melampyrum roseum*) are facultative parasites. Their results showed that by using reference genome sequences and/or transcriptomes of 14 species from *Fabaceae* and *Poaceae*, their major host families, it detected 106 horizontally transferred genes (HTG genes), only in the genomes of the two obligate parasites (22 and 84 for *Oro. minor* and *Ae. indica*, respectively), whereas none in the three facultative parasites. Moreover, they found that almost all HGT genes retained introns at the same locations as their homologs in potential host species, indicating a crucial role of DNA-mediated gene transfer, rather than mRNA mediated retro transfer. Furthermore, some of the HGT genes might have transferred simultaneously because they located very closely in the host reference genome, indicating that the length of transferred DNA could exceed 100 kb. They confirmed that almost all introns are spliced in the genome of the parasite species and that about half HGT genes do not have any missense mutations or frame shift-causing indels, suggesting that some HGT genes may be still functional.

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