Plant Archives Vol. 20, Supplement 1, 2020 pp. 3076-3083 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DETERMINATION OF THE SIMILARITY AMONG FABA BEAN (*VICIA FABA*) AND ITS *OROBANCHE* PARASITE USING ISSR MOLECULAR MARKERS

Abdalla M.M. F.[1], M.M. Shafik[1], M.I. Abd El-Mohsen[2], Heba A.M.A. Saleh[2], M.A. Khater[3] and M.M.A. Elashtokhy[4]

[1-]Agronomy Department, Faculty of Agriculture, Cairo University, Giza, Egypt
[2-] Field Crops Research Institute, ARC, Egypt
[3-]Botany Department National Research Centre, Giza, Egypt.
[4-]Genetics Department, Faculty of Agriculture, Zagazig University, Egypt.

Abstract

This study aimed to detect variations between four faba bean cultivars by using hybridization between minor and equina 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 *Orobanche crenata*. Five different ISSR primers were used, and general results showed that the total amplified fragments (TAF) induced in the three crosses using all primers were 270 bands (with an average 18 per primer) which distributed as 195 polymorphic bands (PB), 75 unique bands (UB), and finally there was not any monomorphic band (MB). The molecular similarity between faba bean host and *Orobanche* parasite may indicate some kind of complementary genes system controlling interaction of the host and the parasite.

***Keywords*:** 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 detected 73 fragments with an average of 14.6 fragment / primer were 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. Moreover the 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 Giza 843 (P_1), Sakha 4 (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. 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.

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

Name	Type*	Pedigree	Characteristics
Giza 843 (P_1)	*Equina*	Individual selection from Rebaya 40 **(FCRI)**	Tolerant to *Orobanche*
Sakha 4 (P_2)	*Equina*	81/35/2001 (Shkha 4) derived from Sakha 1 x Giza 3**	Susceptible 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.
** (See Amer *et al.* (2014).
FACU = Faculty of Agriculture, Cairo University (see Abdalla, 2015 for details).

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 parasitizing same host plants (a sample from the host and another from the attached parasite). 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.

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

	Primer Code	Primer Sequence ($5^{-}\rightarrow 3^{-}$)
1	ISSR-807	AGA GAG AGA GAG AGA GT
2	ISSR-810	GAG AGA GAG AGA GAG AT
3	ISSR-835	AGA GAG AGA GAG AGA GYC
4	ISSR-841	GAG AGA GAG AGA GAG AYC
5	ISSR-857	ACA CAC ACA CAC ACA CYG

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 percentage was calculated according to this equation:

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

Where:-

UB = Number of unique bands,

PB = Number of polymorphic bands

Note: Samples on gel Pic. 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*.

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. There wasn't any monomorphic fragment induced by these primers. 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 270 bands (with an average 18 per primer) which distributed as 195 polymorphic bands (PB), 75 unique bands (UB), and finally 0 monomorphic band (MB).

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	
Camilina × Giza 843	IS- 1	1288.15 – 191.95	22	-	4	18	100%
	IS- 2	971.54 – 185.12	22	-	5	17	100%
	IS- 3	1349.44 – 193.25	19	-	4	15	100%
	IS- 4	1841.13 – 303.21	16	-	3	13	100%
	IS- 5	1245.85 – 217.93	18	-	4	14	100%
Camilina × Sakha 4	IS- 1	1031.51 – 182.73	19	-	6	13	100%
	IS- 2	978.09 – 240.24	16	-	6	10	100%
	IS- 3	1224.74 – 180.21	20	-	8	12	100%
	IS- 4	1018.04 – 209.62	15	-	6	9	100%
	IS- 5	1223.74 – 185.04	19	-	6	13	100%
Cairo 33 × Sakha 4	IS- 1	1345.40 – 223.86	20	-	8	12	100%
	IS- 2	990.78 – 258.16	15	-	4	11	100%
	IS- 3	1069.91 – 205.82	15	-	4	11	100%
	IS- 4	1076.94 – 196.18	17	-	2	15	100%
	IS- 5	1948.37 – 268. 58	17	-	5	12	100%
	Total		270	-	75	195	-
	Average		18	-	5	13	100

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

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

1) **The cross Camilina × Giza 843:** The results revealed that there were 97 amplified bands with different molecular weights ranged from 185.12 – 1841.13 bp detected by the used primers (Table 3). Moreover, these amplified fragments were distributed between polymorphic bands (77 bands) and unique bands (20 bands) and there was not any monomorphic bands detected in this cross, so, polymorphism percentage was 100%.
2) **The cross Camilina × Sakha 4:** There were 89 amplified bands detected and distributed as polymorphic bands (57 bands), unique bands (32 bands) and there was not any monomorphic bands detected in this cross, so, polymorphism percentage was 100%. Moreover, the molecular weight of detected bands ranged 180.21 – 1224.74 bp (Table 3)
3) **The cross Cairo 33 × Sakha 4:** The polymorphism percentage recorded 100% in this cross, also, there were 84 amplified bands (23 unique bands and 61 polymorphic bands) detected using the five primers; and the molecular weights ranged 196.18 – 1948.37 bp (Table 3). Moreover, the highest number of amplified bands (61 bands) was achieved using primer IS-01, while, the lowest number (48 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, deletions, 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%.

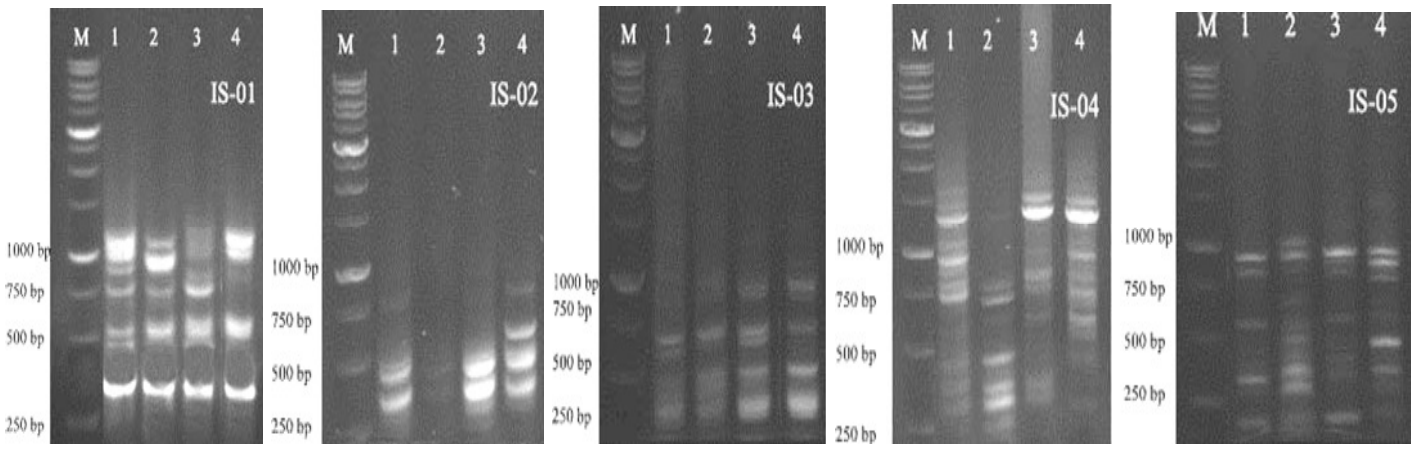

Fig. 1 : Banding pattern of tested faba bean parents generated by five ISSR primers M= DNA standard marker, 1= Giza 843, 2= Sakha 4, 3= Camiliena, and 4= Cairo 33.

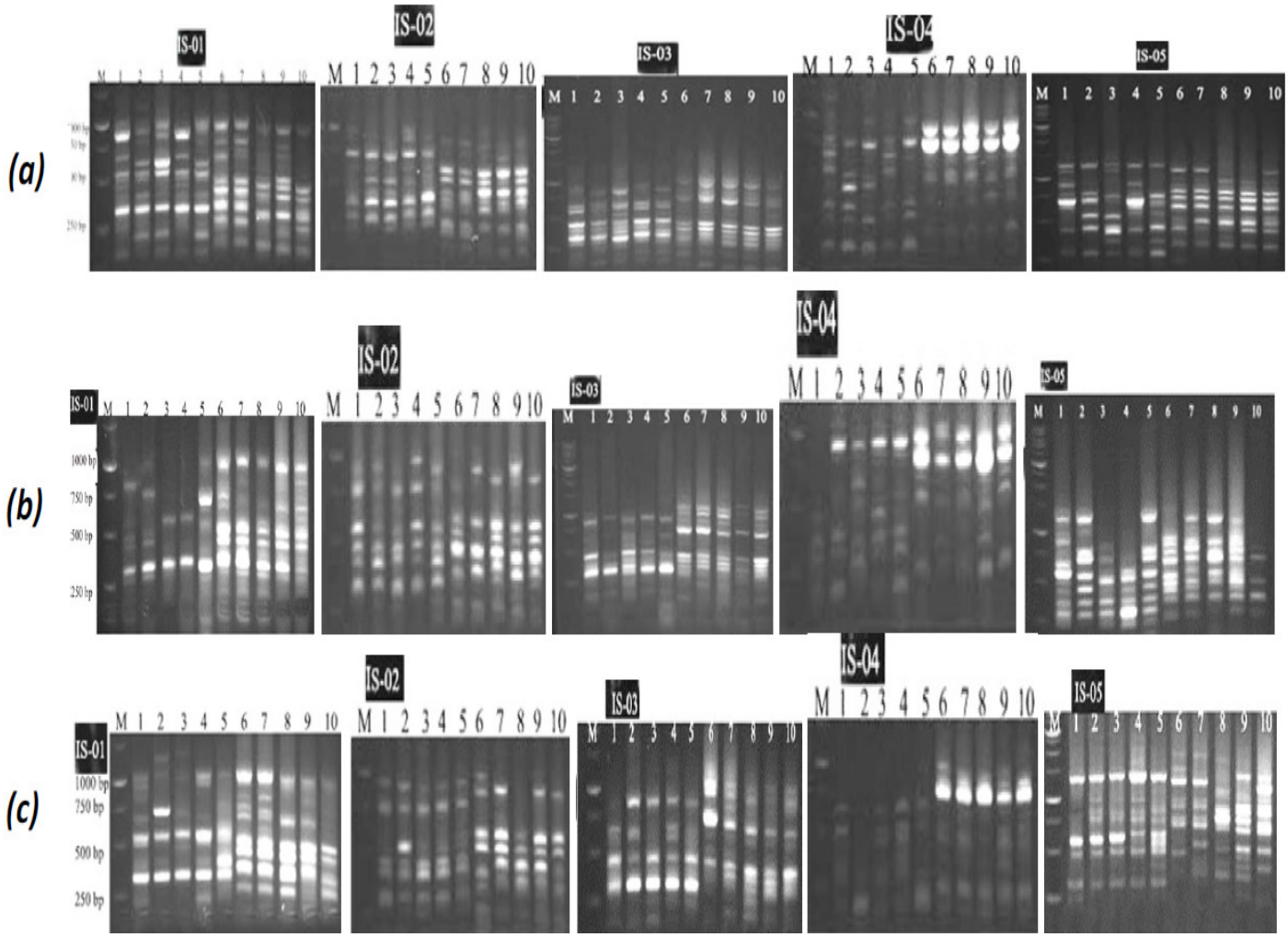

Fig. 2 : ISSR profile of sample genotypes of faba bean crosses and *Orobanche* generated by five ISSR primers. M= DNA standard marker, (1-5) = (F1- F5)= Faba bean samples and (6-10)= (OR1-OR5)= *Orobanche* samples. (*a*): Camilina × Giza 843, (*b*): Camilina × Sakha 4, and (*c*): Cairo 33 × Sakha 4.

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).

Camilina × Giza 843:

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. (3), 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 10 to 100% (Table 4). Moreover, there were close relationship between the two parents of this cross (Camilina, P_3 and Giza 843, P_1) and the percentage of this similarity reached 70.5%. This finding may support the volution of the major type from minor one through equina (Abdalla, 1979). Others else, there were relationship between Giza 843 and all segregant plants (similarity matrix of 65.3% for P1/F2 which was the highest). 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 percentage ranged from 56.8 – 47.4% for both P1/OR2, P1/OR5 and P1/OR3, respectively (Table 4 and Fig. 3). Also, similarity among Camilina (P3) and segregant plants was less than among P1 and segregant plants and ranged from 61.1 – 48.4% for P3/F4 and P3/F3, respectively, and ranged from 69.5 – 60.0% for P3/OR1 and P3/OR3, respectively in case of similarity between P3 and *Orobanche*. However, these previous results may draw the attention to a strong relationship between Camilina (P3) and *Orobanche* and this indicated the susceptibility of this genotype to *Orobanche*.

With regard to the similarity between and within plants of this cross, it was clearly shown that the most closely related plants were F2/F3 followed by F3/F5 (similarity matrix of 76.8 and 75.8%, respectively). The lowest relationships were recorded for F2/F5 (similarity matrix of 67.4%), followed by F3/F4 (similarity matrix of 68.4%) (Table 4).

However, the highest relationship between segregant plants and *Orobanche* was recorded between both F1 with OR1 (63.2%), and the lowest relationship was F3/OR2 (43.2%). On the other hand, between all *Orobanche* plants, there were high relationship where similarity matrix ranged from 81.1% (the highest) for OR4/OR5 followed by 80.0% for both OR2/OR3 and OR3/OR4, and at the end the lowest one was OR1with both OR4 and OR5 which similarity matrix scored 72.6% (Table 4 and Fig. 3).

Table 4: Similarity matrix (%) among the cross Camilina × Giza 843 (parents, five segregants faba bean plants (F1–F5) and *Orobanche crenata* (OR1–OR5) using ISSR molecular marker analysis.

	Giza 843	Camilina	F1	F2	F3	F4	F5	OR1	OR2	OR3	OR4
Camilina	70.5										
F1	64.2	57.9									
F2	65.3	50.5	69.5								
F3	56.8	48.4	71.6	76.8							
F4	61.1	61.1	69.5	72.6	68.4						
F5	62.1	55.8	70.5	67.4	75.8	71.6					
OR1	52.6	69.5	63.2	51.6	53.7	57.9	52.6				
OR2	56.8	65.3	48.4	49.5	43.2	49.5	44.2	76.8			
OR3	47.4	60.0	49.5	48.4	46.3	46.3	47.4	77.9	80.0		
OR4	54.7	61.1	48.4	45.3	47.4	51.6	50.5	72.6	76.8	80.0	
OR5	56.8	61.1	48.4	47.4	47.4	51.6	56.8	72.6	76.8	77.9	81.1

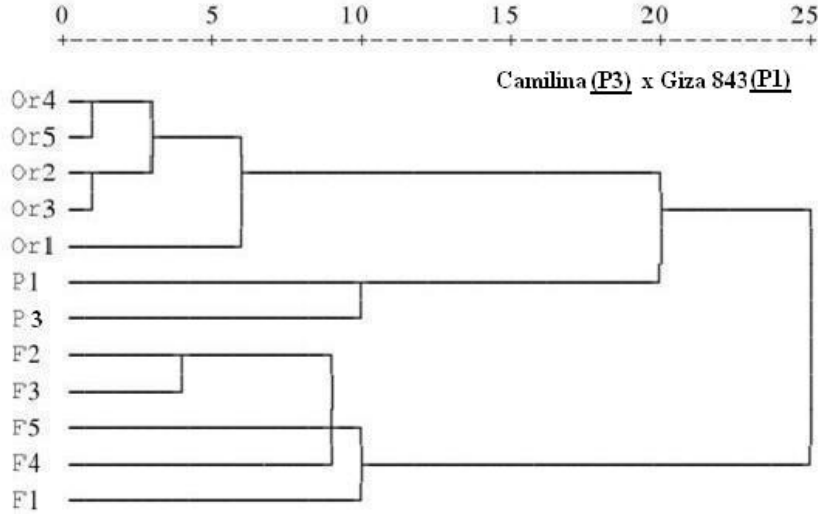

Fig. 3: Dendrogram of the genetic distances among and within Camilina × Giza 843 cross and *Orobanche* based on ISSR analysis.

The dendrogram (Fig. 3) is divided to two major clusters, however, the first cluster is sub divided into two subclusters one included (F1, F4 and F5) while the other subcluster involved (F2 and F3). Also, the second cluster was divided into two subclusters, the first cluster contained P1(Giza 843) and P3 (Camilina), and the other subcluster is sub divided into two sub-sub clusters, one of them contained OR1 and the second contained OR4, OR5, OR2 and OR3.

However, the highest degree of similarity and relationship was in the first cluster between OR4/OR5 (81.1%), followed OR2/OR3 (80.0%), OR2/OR4 (77.9%) and OR1/OR2 (75.0%). Moreover, all clusters were conjugated throw OR1/P1 (58.5%), and P1/F1 (52.3%).

Camilina × Sakha 4:

The dendrogram (Fig. 4) is divided to two major clusters, however, the first cluster divided into two subclusters (one included (OR1 and OR2) while the other subcluster involved (OR3, OR4 and OR5). Also, the second cluster was sub divided into four subclusters, the first one contained P3 (Camilina), the second contained P2 (Sakha 4), the third cluster divided into two sub-sub clusters (F1, F2 and F5), and the fourth subcluster also, divided into two sub-sub clusters (F3 and F4). (Fig. 4).

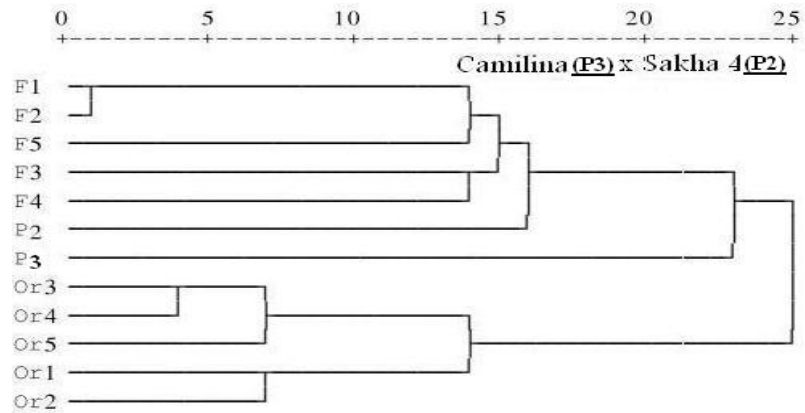

Fig. 4 : Dendrogram of the genetic distances among and within Camilina × Sakha 4 cross and *Orobanche* based on ISSR analysis.

Table 5: Similarity matrix (%) among the cross Camilina × Sakha 4 (parents, five segregants faba bean plants (F1–F5) and *Orobanche* (OR1–OR5) using ISSR molecular marker analysis.

	Sakha 4	Camilina	F1	F2	F3	F4	F5	OR1	OR2	OR3	OR4
Camilina	67.0										
F1	68.2	69.3									
F2	71.6	65.9	85.2								
F3	70.5	62.5	65.9	76.1							
F4	69.3	59.1	67.0	75.0	71.6						
F5	67.0	54.5	67.0	77.3	69.3	68.2					
OR1	56.8	58.0	63.6	62.5	61.4	53.4	58.0				
OR2	62.5	59.1	53.4	54.5	60.2	50.0	54.5	78.4			
OR3	69.3	61.4	55.7	54.5	62.5	56.8	52.3	67.0	68.2		
OR4	67.0	65.9	64.8	61.4	67.0	56.8	52.3	76.1	72.7	81.8	
OR5	72.7	69.3	63.6	62.5	70.5	58.0	55.7	70.5	76.1	76.1	80.7

Moreover, Table (5) illustrates the relationship and genetic similarity among and within faba bean plants (parents, crosses and *Orobanche* using ISSR markers, however, the relationship between parents (P2 and P3) recorded similarity of 67.0%, and the most closely related plant with the cultivar Sakha 4 was plant number 2 (F2) that recorded (71.6%) as similarity matrix followed by F3 (70.5%), while the lowest one was F5 (67.0%). With regard to relationship within segregant plants, there were a force relationship among these plants, and the highest one was F1/F2 and F2/F5 with similarity matrix (85.2% and 77.3%), respectively. On the other hand, F1/F3 recorded the lowest one (65.9%). With regard to the relationship among *Orobanche* plants, the most related plants were OR3/OR4 (81.8%) and the lowest one was OR1/OR3 (67.0%). (Table 5)

Moreover, there were relationship between all segregant plants and their *Orobanche* that grown on the same plants ranged from (70.5 – 50.0%), whereas, the most related plants were F3/OR5 followed by F3/OR4 that recorded (70.5 and 67.0%), respectively. On other side, F4/OR2 was the lowest one in its relationship (50.0%) (Table 5).

Cairo 33 × Sakha 4:

Table (6) and Fig. (5) 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: 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.

	Camilina	Cairo 33	F1	F2	F3	F4	F5	OR1	OR2	OR3	OR4
Cairo 33	66.7										
F1	70.2	56.0									
F2	70.2	58.3	78.6								
F3	69.0	57.1	77.4	86.9							
F4	65.5	51.2	71.4	81.0	86.9						
F5	70.2	56.0	78.6	78.6	82.1	78.6					
OR1	59.5	64.3	48.8	46.4	47.6	39.3	44.0				
OR2	59.5	66.7	56.0	53.6	52.4	46.4	53.6	78.6			
OR3	53.6	58.3	50.0	52.4	53.6	47.6	57.1	63.1	72.6		
OR4	58.3	60.7	50.0	52.4	53.6	45.2	57.1	67.9	77.4	85.7	
OR5	56.0	58.3	52.4	57.1	58.3	52.4	61.9	63.1	72.6	78.6	83.3

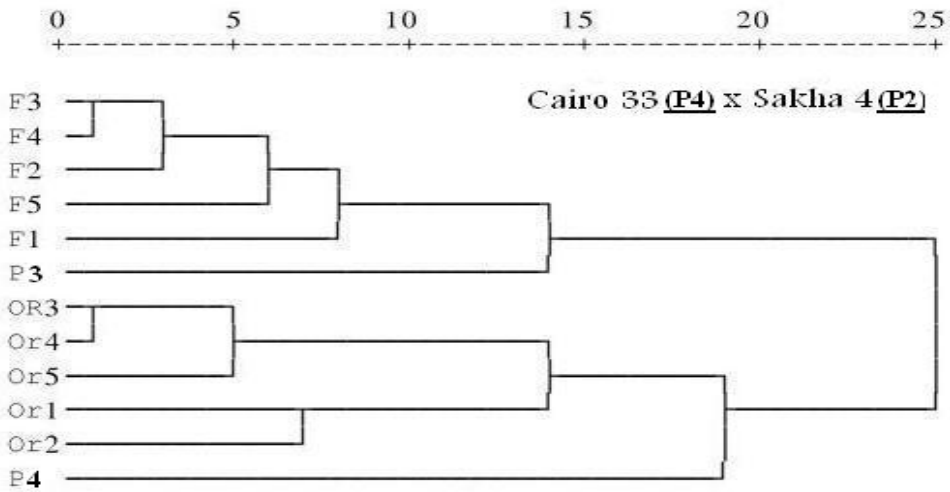

Fig. 5 : Dendrogram of the genetic distances among and within Cairo 33 × Sakha 4 cross and *Orobanche* based on ISSR analysis.

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.

There were a wide genetic distance in faba bean parents used in this study (Minor and Equina 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 not many studies estimated the genetic variations of *O. crenata* samples, but in the present work 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 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 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), only in the genomes of the two obligate parasites (22 and 84 for *O. minor* and *A. indica*, respectively), whereas none in the three facultative parasites. Moreover, they found that almost all HTG 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 HTG 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 HTG do not have any missense mutations or frame shift-causing indels, suggesting that some HTG may be still functional.

References

Abdalla, M.M.F. (1979). The origin and evolution in *Vicia faba* L. Proc. 1st Med. Conf. Genet. Cairo, 713–746.

Abdalla, M.M.F. (2015). Investigation on faba beans, *Vicia faba* L. 35. Cairo 33 a new variety with colourless hilums and tolerance to *Orobanche*. Egypt J. Plant Breed. 19(2): 233-245.

Abdalla, M.M.F.; Shafik, M.M.; Sabah, M.A. and Hend, A.G. (2016). Molecular Characterization of *Orobanche crenata* in Egypt Using ISSR Markers and Its Relation to Faba Bean Breeding. Biotechnology Journal International, 16(3): 1-11.

Abd-Elrahman Rehab, A.M. and Rasha, Y.S. Abd El-Khalek (2013). Morphological and Genetic Description of Some Faba Bean Cultivars. Egypt. J. Plant Breed., 17(3): 33– 44.

Abedi, S.; Darvishzadeh, R.; Bernousi, I.; Mandoulakani, B.A.; Maleki , H.H. and Shah, D. (2014). Genetic variability of *Orobanche aegyptiaca* infesting tobacco in Iran by bayesian analysis. Biologia. 69(12): 1652-1659.

Amer, M.I.; Ola, A.M.; El-Galaly, R.A.; Abou-Mostafa, M.A.; El-Borai, M.M.; Radi, S.; Attia, M.; El-Hady, M.M.; Saber, H.A.; Omar, M.A.; Khalil, S.A.; Samia A. Mahmoud, A.H. Hussein, M.A. El-Deeb, M.I. Abdel-Mohsen, A.R. Morsy, W.M. El-Rodeny, A.A.M. Ashrei, M.A. Ibrahim, Th.M. Abou-Sin, Gehan G. Abdel-Ghafar, Rehab A. Abel-Rahman, M.A. Ibrahim, A.G. Helal, A.M. El-Garhy, E.M. Rabie, Kh.M. Yamani, M.A. Bakheit, Nagat G.Abdalla, A.M. Gabra, G.A. Hafez, M.M. Soliman, M.A. El-Noby, E.A.

Sarhan* and K.M. Morsy., (2014). "SAKHA 4" A New Early Maturing Faba Bean Cultivar Tolerant To Foliar Diseases For Early Sowing In North Egypt. Egypt. J. Plant Breed. 18(2): 297 – 306.

Annathurai, G.; Jeff, P.; Ana, T.; Sukhjiwan, K.; Tony, L.; Haobing, Li , Xuxiao Zong , Tao, Y. and Michael, M. (2012). Impact of Molecular Technologies on Faba Bean (*Vicia faba* L.) Breeding Strategies. Agronomy, 2: 132-166.

Cubero, J.I. and Moreno, M.T. (1999). Studies on resistance to *Orobanche crenata* in *Vicia faba*. in Resistance to Broomrape—The State of the Art ; Cubero, J.I., Moreno, M.T., Rubiales, D., Sillero, J.C., Eds.; DGIFA: Junta de Andalucía, Sevilla, Spain, 9–15.

Ellingboe, A.H. (1976). Genetics of Host-Parasite Interactions. Physiological Plant Pathology, 761-778.

Elshafei, A.A.M.; Amer, M.A.; Elenany, A.M. and Helal, A.G.A. (2019). Evaluation of the genetic variability of faba bean (*Vicia faba* L.) genotypes using agronomic traits and molecular markers. Bulletin of the National Research Centre. 43:106. 1–10.

Fang, D. and Roose, M.L. (1997). Identification of closely related citrus cultivars with inter simple sequence repeat markers. Theor. Appl. Genet., 95: 408-417.

Flor, H.H. (1956). The Complementary Genic Systems in Flax and Flax Rust. Advances in Genetics. 8: 29-54.

Gadalla, N.O.; Eman, M.F.; Abd-Elsattar, A.; Ashry, N.A.; El-Enany, M.A. and Bahieldin, A. (2012). Evaluation of Gene Expression for *Orobanche* Tolerance in Faba bean (*Vicia faba* L.). JKAU: Sci., 24(1): 21- 38.

George, W.B. (1960). Etiology and Epiphytology of *Victoria blight* of Oats. Iowa State University.

Gillanders, Elizabeth M.A.B.; Julia, B.S. and Elizabeth, W. (2002). Responses to Abiotic Stress (Acadmic Press).

Gnanasambandam, A.; Paull, J.; Torres, A.; Leonforte, T.; Li, H.; Zong, X.; Yang, T. and Materne, M. (2012). Impact of Molecular Technologies on Faba Bean (*Vicia faba* L.) Breeding Strategies. Agronomy, 2: 132-166.

Hodkinson, T.R.; Chase, M.W. and Renvoize, S.A. (2002). Characterization of a genetic resource collection for Miscanthus (*Saccharinae, Andropogoneae,* Poaceae). Annals of Botany, 89: 627-636.

Jaccard, P. (1908). Nouvelles recherches sur la distribution florale. Bull.Soc. Vaud. Sci. Nat. 44: 223–270.

Kado, T. and Innan, H. (2018). Horizontal Gene Transfer in Five Parasite Plant Species in *Orobanchaceae*. Genome Biol. Evol., 10(12): 3196–3210.

Korbin, M.; Kuras, A. and Zurawicz, E. (2002). Fruit plant germplasm characterization using molecular markers generated in RAPD and ISSR-PCR. Cellular and Molecular Biology Letters. 7(2B): 785-794.

Mejri, S.; Mabrouk, Y.; Voisin, M.; Delavault, Ph.; Simier, Ph.; Saidi, M. and Belhadj, O. (2012). Variation in quantitative characters of faba bean after seed irradiation and associated molecular changes. African Journal of Biotechnology, 11(33): 8383-8390.

Muratuva, V. (1931). Common beans (*Vicia faba*). Bull. Appl. Bot. Genet. Plant Breed. Suppl., 50: 285.

Musselman, L.J. (1986). Taxonomy of *Orobanche*. In S.J. ter Borg (ed). Proc. Workshop on Biology and Control of *Orobanche*. Wageningen, the Netherlands. 2-10.

Rakoczy-Trojanowska M. and Bolibok , H. (2004). Characteristics and comparison of three classes of microsatellite-based markers and their application in plants. Cell. Mol. Biol. Lett., 9: 221-238.

Rohlf, J. (2010). NTSYSpc. Numerical Taxonomy and Multivariate Analysis System, Version 2.21 h. Exeter Software, Setauket, NY, USA.

Sillero, J.C.; Rubiales, D. and Cubero, J.I. (1996). Risks of *Orobanche* screenings based only on final number of emerged shoots per plant. In Advances in Parasitic Plant Research; Moreno, M.T., Cubero, J.I., Berner, D., Joel, D., Musselman, L.J., Parker, C., Eds.; Junta de Andalucìa: Córdoba, Spain, 652–657.

Tomar, R.S.; Parakhia, M.V.; Rathod, V.M.; Thakkar, J.R. and Golakiya, B.A. (2014). A comparative analysis of ISSR and RAPD markers for studying genetic diversity in *Trigonella foenum*-graecum genotypes. Res J Biotech. 9(10): 89- 95.

Wegmann, K. and Musselman, L.J. (1991). Eds.; Progress in *Orobanche* Research Eberhard-Karls-Universität: Tübingen, Germany, 257–277.