Biotechnology Journal International
16(3): 1-11, 2016, Article no.BJI.27216
Previously known as British Biotechnology Journal
ISSN: 2231–2927, NLM ID: 101616695

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Molecular Characterization of *Orobanche crenata* in Egypt Using ISSR Markers and Its Relation to Faba Bean Breeding

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Authors' contributions

All authors conceived and designed the study, participated in drafting and correcting the manuscript critically and gave the final approval of the version to be published.

Article Information

DOI: 10.9734/BJI/2016/27216
Editor(s):
(1) Samuel Peña-Llopis, University of Texas Southwestern Medical Center, USA.
(2) Ghousia Begum, Indian Institute of Chemical Technology, Hyderabad, India.
(3) Kuo-Kau Lee, National Taiwan Ocean University, Taiwan.
Reviewers:
(1) Chen-Chin Chang, University of Kang Ning, Taiwan.
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(3) Martha Isabel Torres-Morán, Universidad De Guadalajara, México.
Complete Peer review History: http://www.sciencedomain.org/review-history/16777

Original Research Article

Received 24th May 2016
Accepted 18th October 2016
Published 5th November 2016

ABSTRACT

Orobanche crenata is an annual parasitic weed that causes heavy losses to its host crop faba bean (*Vicia faba*). Determining the genetic diversity in *Orobanche germplasm* is a preliminary crucial step in faba bean breeding.It helps in identifying liable criteria of host tolerance. The current study aimed to determine the genetic diversity for *Orobanche* collected from two divergent locations in Egypt (Giza and Sids). The inter simple sequence repeats (ISSR) markers were used to determine genetic relationships among and within the collected groups. The number of produced fragments covered 218 to 980 bp of the total of *Orobanche crenata* genome. The five used primers generated a total of 73 ISSR products with an average of 14.6 products / primer. The polymorphic information content (PIC) ranged from 0.86- 0.94.

Analyses of molecular variance indicated significant difference within each region. The difference between the two locations was not significant (3%) due to great variation within locations (97%). The cluster analysis devided the 96 *Orobanche* samples into five sub-clusters. The study signified

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the efficiency of ISSR molecular technique in estimating genetic relationships across the two divergent locations at the genomic level. It also was consistence with the predominantly allogamous behaviour of *O. crenata*. Because of *Orobanche* diversity, faba bean breeders should develop host varieties with heterogeneous background and don‟t release any pure line host in infested soils.

Keywords: Genetic variability; Orobanche crenata; ISSR; diversity; Faba bean.

1. INTRODUCTION

Faba bean, *Vicia faba* L. is an important pulse crop in Egypt. It is used mainly as human food to prepare several local popular dishes. The *Orobanche crenata* Forsk is an annual parasitic plant that causes heavy losses to its host faba bean. Recent investigations supported by molecular taxonomy analyses have resulted in re-definition of *Orobanchaceae* family. According to this classification *Orobanchace ae*consists of 89 genera, containing 2061 species. On the Balkans the family *Orobanchaceae* is represented by 3 genera *Orobanche* includes 25 species; three of which are present in Egypt (*crenata*, *aegyptiaca* and *minor*) they are widely spread in Bulgaria, Southern Europe, Russia, Middle East and Northern Africa. They cause losses in crop productivity estimated at hundreds of millions of dollars annually that affect the livelihoods of 100 million farmers [1]. However, Grenz and Sauerborn [2] hypothesized that *Orobanche* spp. may have a geographic distribution in all Mediterranean climate areas, part of monsoon, savanna and winter dry climate regions of Central America, Australia and South Asia. *Orobanche* seeds live in soil for many years, until germinated by exudates from proper host(s) [3,4]. Faba bean losses vary according to host genotype, level of parasitism, sowing date, soil moisture, temperature and other factors [5,6,7,8,9]. Host yield loss may reach 100% crop failure. In their review on breeding faba bean for tolerance to *Orobanche* in Egypt, Abdalla and Darwish [9] reported on *Orobanche* parasitism, host variability, variability of parasite, screening techniques for tolerance/resistance, genetics of host resistance / tolerance, nature of host resistance / tolerance and finally achievements and future prospects. Some authors were of the idea that *Orobanche* species does not vary in aggressiveness / virulence. However, other authors reported on the variability of this parasite [10,11,8,12]. Relation of faba bean and *Orobanche* was studied during three seasons 1979/80, 1980/1981 and 1981/82 [8] using 209 faba bean land races. They reported variation for both host and parasite. *Orobanche* varied in colour and was of single or multi-spiked nature. *Orobanche* parasitizing a host plant ranged from 13 to 114 g fresh weight and from 2 to 14 g dry weight. The authors recommended combining different tolerance genes from local and exotic faba bean genotypes differing in their genetical back grounds to breed faba bean for uniform resistance to *Orobanche*. Fischbeck et al. [10] and Radwan et al. [11] conducted pot experiments using 22 faba bean lines against 9 accessions of broomrape, 8 of which were collected from five Governorates in Egypt and the ninth was obtained from Syria. The authors reported clear differences in aggressiveness/ virulence among parasite accessions. This was reflected in different effects on vegetative and reproductive growth of the faba bean hosts. Host-parasite relationships were reported to be also dependent upon environmental conditions. Nawar et al. [12] studied the parasitic ability of three broomrape accessions collected from El-Behera Governorate (Egypt) on two faba bean varieties. Accessions varied in their effects at different growth stages of the two faba bean cultivars.

Breeding faba bean to broomrape tolerance and the interaction between the host and the parasite are by no means an easy task because:(a)faba bean is partially self-pollinated crop, cross pollination may reach 67% [13] in presence of pollinating agents. Growing faba bean lines under insect free cages may maintain the line identity but will result in inbreeding effects. However, growing faba bean lines in open field may result in cross breeding and loosing the line identity in a few generations [14]. (b) *Orobanche* is an allogamous plant with expected genetic diversity. (c) Interaction between host and parasite is liable to environmental effects. (d) Characters of the host plants are variable both vegetative and generative characters are affected by *Orobanche* parasitism and liable criteria of host tolerance might be No. of podded plants / line, No. pods / plant, seed yield / plant …etc. (e) Absence of differential set of hosts that may discriminate between virulent (aggressive) accessions of the parasite.

The previous difficulties complicating the host x parasite interaction, therefore, molecular markers may be used to investigate the diversity in *O. crenata.* Molecular markers are segments of chromosomes which don‟t necessarily encode any traits and are not affected by the environment, but which are inherited in a Mendelian fashion [15]. The Inter-simple sequence repeats (ISSR) are semi-arbitrary markers amplified by polymerase chain reaction (PCR) in the presence of one primer complementary to a target microsatellite. ISSR markers are suitable for investigating genetic diversity among O. *aegyptiaca* genetic groups and able to discriminate between individuals [16]. Each band corresponds to a DNA sequence delimited by two inverted microsatellites [17,18,19]. ISSR markers have several benefits over other techniques: first, it is known to be able to discriminate between closely related genotypes [20,21] 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 [22,23]. ISSRs have been used in genetic diversity studies in different crop plants [24,25,26]. Measuring diversity among *O. crenata* parasitic populations is not easy in terms of virulence because differential host sets are still not available in any of the cultivated hosts. Few studies have found low differentiation among *O. crenata* populations according to "race-specificity" [27,11]. Molecular marker analysis (RAPD) carried out among *O. crenata* populations from Spain and Israel from faba bean, chick pea and vetch, indicated within population diversity [28]. In Egypt Zeid et al. [29], have shown low inter population divergence. Hassanien et al. [30] used molecular marker analysis (RAPD) among three isolates of *O. crenata* and reported variation between them. Genetic variation also determinate by using SSR markers for *O. cumana* [31]. The main objective of this work was the comparison of *O. crenata* populations from two locations in Egypt using ISSR markers.

2. MATERIALS AND METHODS

2.1 Plant Materials

Ninety six *O. crenata* Forsk plants were sampled as individuals from naturally infected faba bean plants of two locations in Egypt: Giza (1 to 44) and Sids (45 to 96) during the growing season of 2013/2014. Five primers (Table 1) were used to differentiate *O. crenata* plants [28].

Table 1. Inter simple sequence repeat DNA primers used in the analysis of *O. crenata* populations

Primers	Sequence (5–3)
ISSR-807	AGA GAG AGA GAG AGA GT
ISSR-810	GAG AGA GAG AGA GAG AT
ISSR-835	AGA GAG AGA GAG AGA GYC
ISSR-841	GAG AGA GAG AGA GAG AYC
ISSR-857	ACA CAC ACA CAC ACA CYG

2.2 DNA Extraction and PCR Analysis

The genomic DNA was isolated from the shoot tip of *Orobanche* plants using CTAB protocol [32]. *Orobanche* shoot tips were ground to a fine powder in liquid nitrogen and extracted with hot CTAB. The purity of isolated DNA was quantified spectrometrically (Nano Drop, ND-1000, Nanodrop Technologies Wilmington, Delaware, USA) by measuring absorbance at 260 nm and visualized under UV light after electrophoresis on 1% agarose gel.

PCR amplifications were carried out within 20 µl reaction volumes. containing 1 µl plant genomic DNA, 4 µl 5x buffer, 2 µl MgCl2 (25 mM), 2 µl dNTPs (2 mM), 2 µl primer(10 pmol), and 0. 3 µl Taq (5U/ µl) DNA polymerase. PCR condition was an initial denaturation at 94℃ for 7 min followed by 35 cycles each of 94℃ for 30 s, 52℃ for 0.45s, 72℃ for 2 min. with a final extension at 72℃ for 5 min. Amplifications products were electrophoresed first on gels 1.5% agarose in TAE buffer and also electrophoresed in QIAxcel high resolution kit.

2.3 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. A cluster analysis based on the similarity matrix was performed using the neighbour joining method [33]. A cluster analysis based on the similarity matrix was performed using the UPGMA (unweighted pair group method with arithmetic mean) method of NTSYSpc ver. 2.21o (Exeter Software, Setauket, NY, USA) [34]. The analysis of molecular variance (AMOVA) was used to partition the total phenotypic variance into between and within individuals within regions and between regions. The polymorphic information content (PIC) value for each locus was calculated using the formula of Roldan-Ruiz et al. [35]. The STRUCTURE software [36] was used to identify the number of

genetic clusters within the data set, and to assign all the individuals to these clusters using the admixture model. First, we analysed the „reference" data set by modeling cluster assignments for K=2–12 clusters. We made 11 independent runs for each K to confirm consistency across runs. In all simulations, we performed a burn-in period of 100 iterations and 1000 Markov chain Monte Carlo iterations.

3. RESULTS

An initial screening of the ISSR primers for clear and repeatable band profiles showed that 5 primers (Table 1) of 17–18 bp, of which all of them were anchored at 3" end for increased specificity yielded amplified products. Number of products generated by these primers was found to range from 9 to 20 bands of different sizes ranging from 218 to 980 bp (Table 2). The primer ISSR 857 gave the maximum number of bands 20 and primer ISSR 807 produced the least with only 9 amplicons. PIC value shows high variation between primers where it ranged between 0.86-0.94 (Table 2).

Analysis of molecular variance (AMOVA) showed that the source of variation within regions used in this study was 97% of the comparing to only 3% among the regions (Table 3).

In each location, huge genetic variation proved to exist as detected by ISSR primers (Figs. 1 and 2). Fig. 3 comprised 44 samples collected from Giza. The dendrogram based on UPGMA clearly showed that there is two major clusters, at the 20% level of similarity the first cluster has two individuals (G7 and G9). The second cluster contained the rest of the individuals that splitted into four lines. Fig. 4 showed that Sids samples (52 samples) divided into two main clusters and had from six sub -clusters and sample (S24y) it was different about the rest of samples and came in separate sub cluster, also the samples (S23y and S26hy) came in separate sub-cluster and they were closest to each other with 22%.

The dendrogram based on neighbour joining method clearly showed that there are five major clusters (A, B, C, D and E). The cluster (A) has 29 samples where 55% were from Giza. Cluster (C) 14 samples most of them from Sids except two samples (G28, G33) from Giza. Cluster C has 15 samples all of them from Giza. Cluster D and E were mixed between Giza and Sids. Cluster D has 24 samples where 71% were collected from Sids (Fig. 5). The majority in cluster Structured analysis based on clustering groups produced four clear groups and did not gave the same results of neighbour joining method and divided the individuals into distanced groups. Groups (1, 2 and 4) have 16, 28 and 18 samples respectively, distributed equally between the two locations. In group (3) Sids samples were prevalent on Giza samples. The yellow color of stem and flowers was dominated on the violet color (Figs. 6 and 7).

Table 2. Summary of inter simple sequence repeat primers used in the analysis of *O. crenata*

Marker	No. of loci	PIC
ISSR-807	9	0.86
ISSR-810	13	0.91
ISSR-835	17	0.94
ISSR-841	20	0.94
ISSR-857	14	0.91

4. DISCUSSION

In this study we estimated the genetic diversity of 96 *O. crenata* individuals collected from Giza (46%) and Sids (54%). In each location (Giza and Sids) samples were collected from about one acre used as *Orobanche* sick plots in the two locations. However, still no enough studies estimated the genetic variations of *O. crenata* samples collected from different regions in Egypt where faba bean is considered as the major legume crop. This study identified a significant 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. Maybe the most importat reason is that *O. crenata* is reported to be predominantly an out-crossing parasitic weed [37]. Therefore, each *Orobanche* plant in this study considered as different genotype than neighbouring plant within location. 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% [13]. 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*).

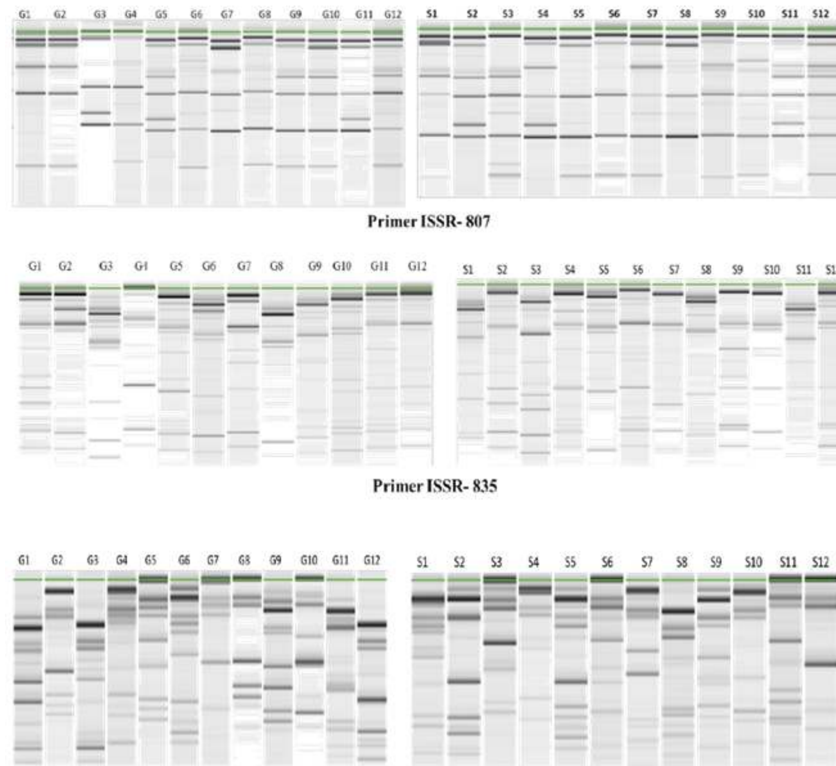

Fig. 1. ISSR profile of sample genotypes of *Orobanche crenata l* produced with primers (ISSR 807, ISSR 810 and ISSR 835 G (1-12) = samples collected from Giza, S (1-12) = samples collected from Sids

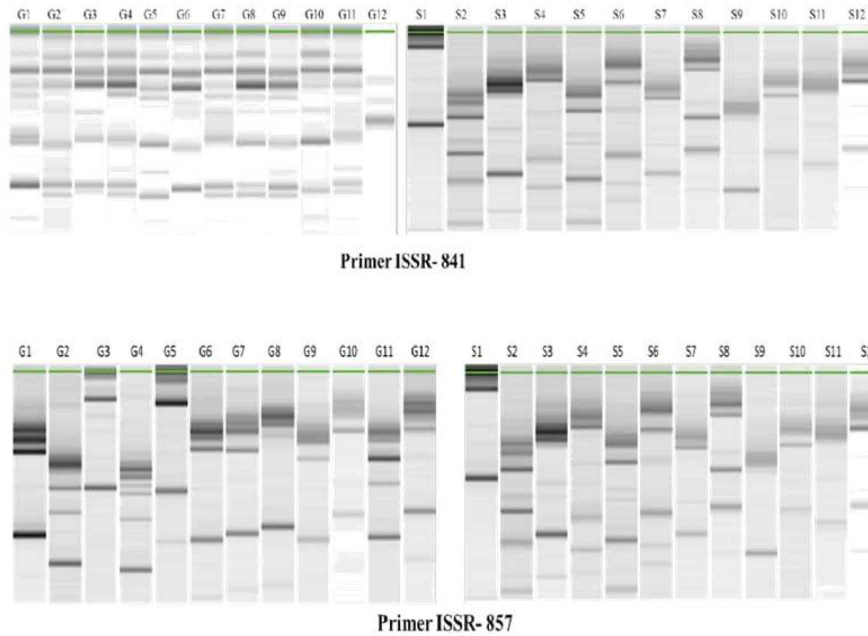

Fig. 2. ISSR profile of sample genotypes of *Orobanche crenata l* produced with primers ISSR 841 and ISSR857G (1-12) = samples collected from Giza, S (1-12) = samples collected from Sids

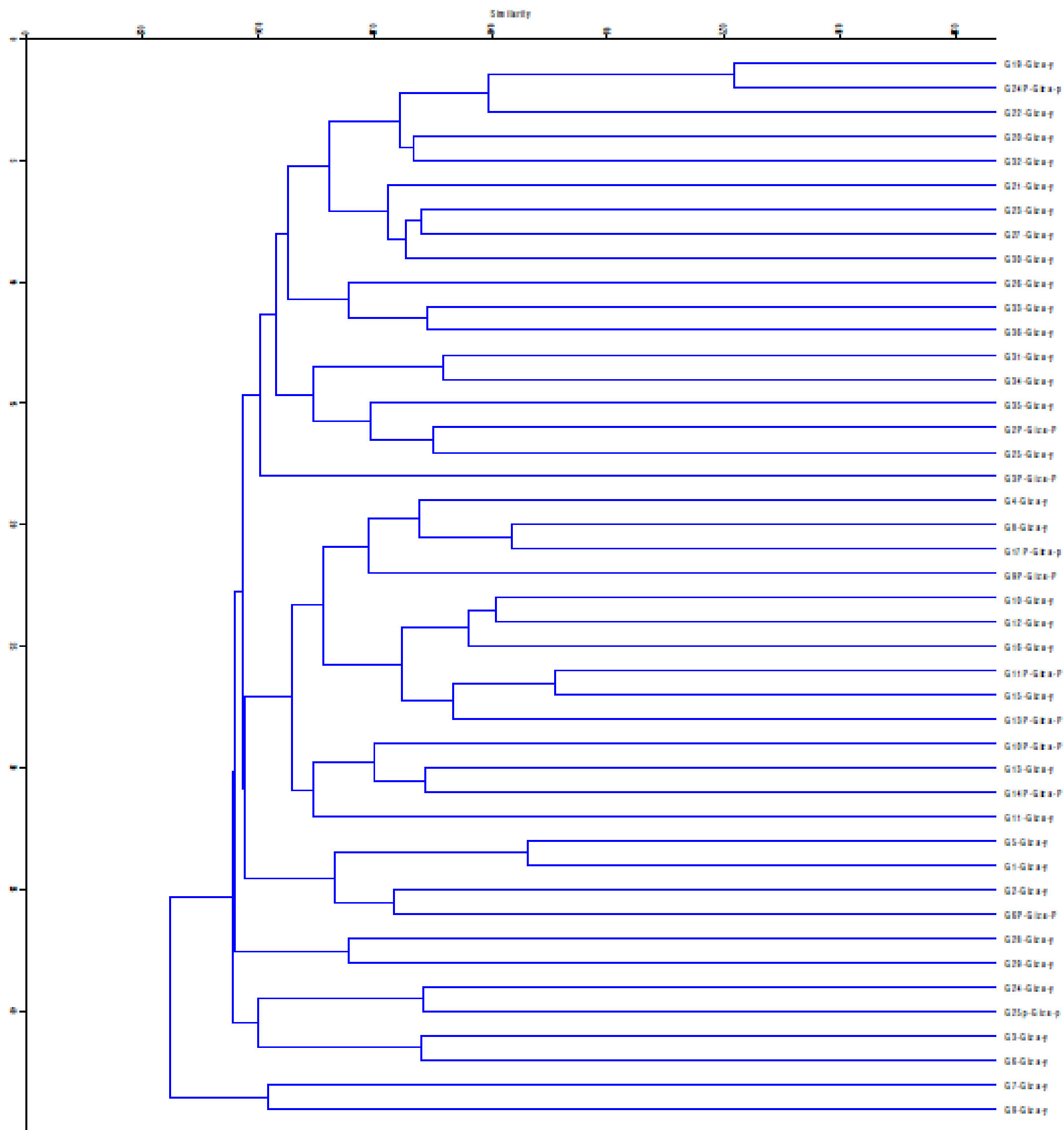

Fig. 3. Dendrogram of *Orobanche crenata* based on distance obtained from ISSR marker using UPGMA method in Giza, (y, p) refers to samples colors yellow and purple respectively

Table 3. Analysis of molecular variance for the partitioning of random amplified polymorphic DNA variation among regions and within regions

SOV	df	SS	MS	Est. Var.	%	Stat	P-value
Among regions	1	32.75	32.75	0.42	0.03		
Within regions	94	1186.19	12.62	12.62	0.97	PhiPT	0.03
Total	95	1218.94	45.36	13.04			

The use of molecular markers may be a suitable method for the identification of parasitic groups in parasite populations [38]. AMOVA analysis revealed that only 3% of *Orobanche* total variation was attributed to the variability between locations and 97% among individuals (within locations). This agreed with results of Romàn et al. [28] who reported high variation (71%)

within individuals compared to the (24%) between regions and (5%) between populations. The variability in our study (97%) higher than that reported by Roman et al. [28] (71%) however, we have to keep in mind that Roman et al. [28] studied only six populations from faba bean, chick pea and vetch. Moreover, in their studies they referred to locations (6) as populations and introduced in the AMOVA among regions (countries) source of variation.

Such variability reported in *O. crenata* by different authors (this study, Fischbeck et al. [10], Radwan et al. [11], Abdalla and Fischbeck [8], Nawar et al. [12], Zeid et al. [29], Romàn et al. [28] and Hassanien et al. [30]) should inforce the faba bean breeders to follow specific strategy for breeding for *Orobanche* tolerance in order to overcome the heterogeneous nature of the parasite.

The cluster analysis (Fig. 5) showed that our 96 samples from two locations, irrespective of their genetic diversity were finally divided into five different sub-clusters (A, B, C, D and E). However, the dendogram does not show clear separation of the samples based on the region. Abedi et al. [16] found similar results by using ISSR markers for investigating genetic diversity among *O. aegyptiaca* in Iran. The fact that only five clusters emerged from the 96 samples of *Orobanche* (5%) indicates that the virulence/ aggressiveness of the parasite may not that huge compared to its wide genetic diversity investigated by ISSR.

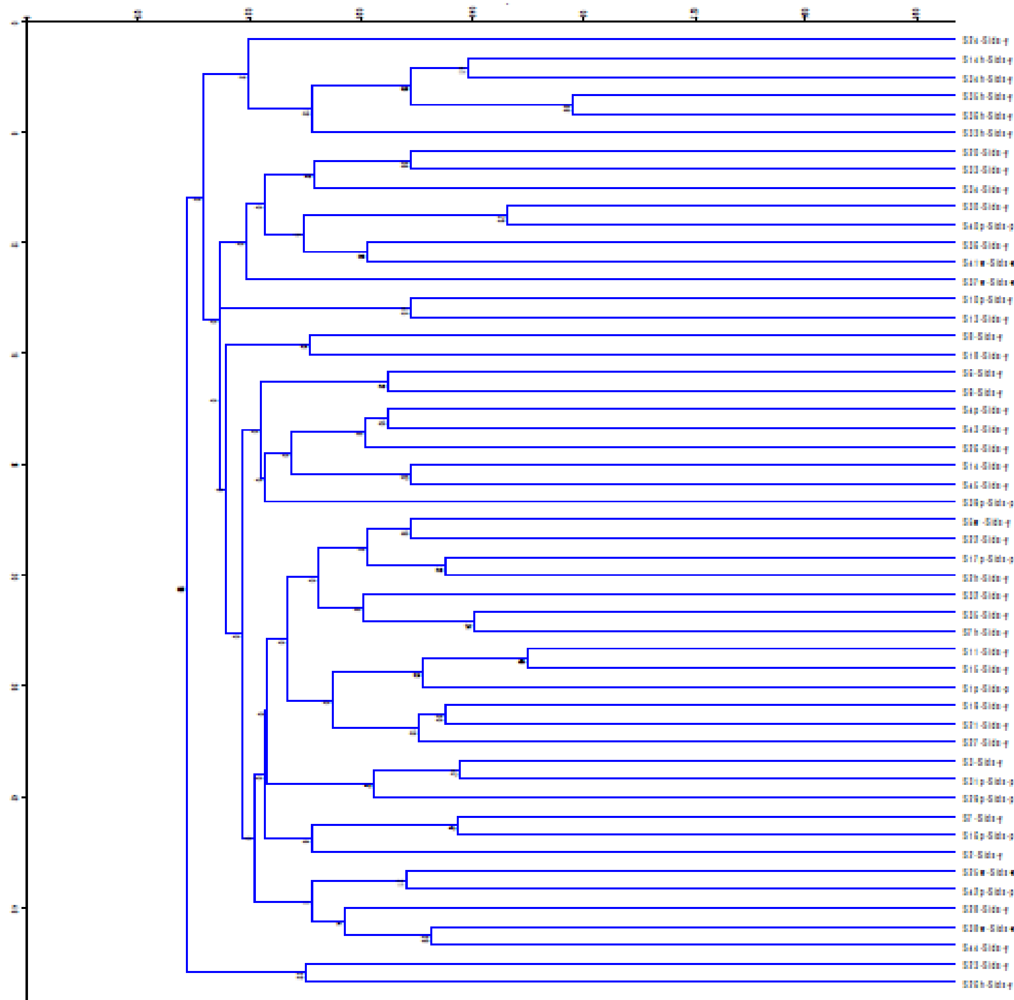

Fig. 4. Dendrogram of *Orobanche crenata* based on distance obtained from ISSR marker using UPGMA method in Sids, (y, p) refers to samples colors yellow and purple respectively

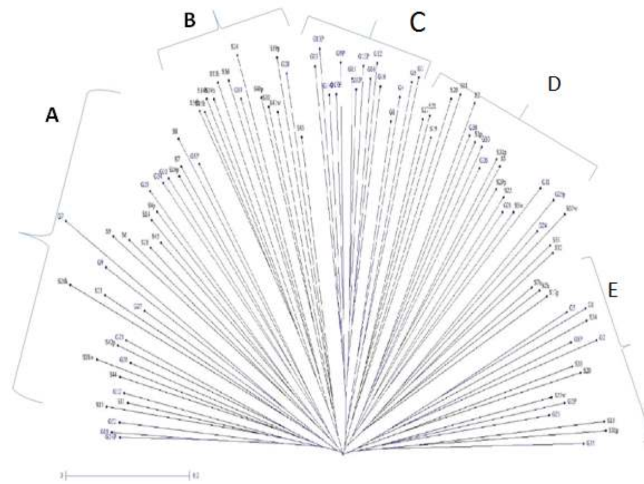

Fig. 5. Dendrogram of *Orobanche crenata* based on distance obtained from ISSR marker using. Neighbor joining method. (y, p) refers to samples colors yellow and purple respectively

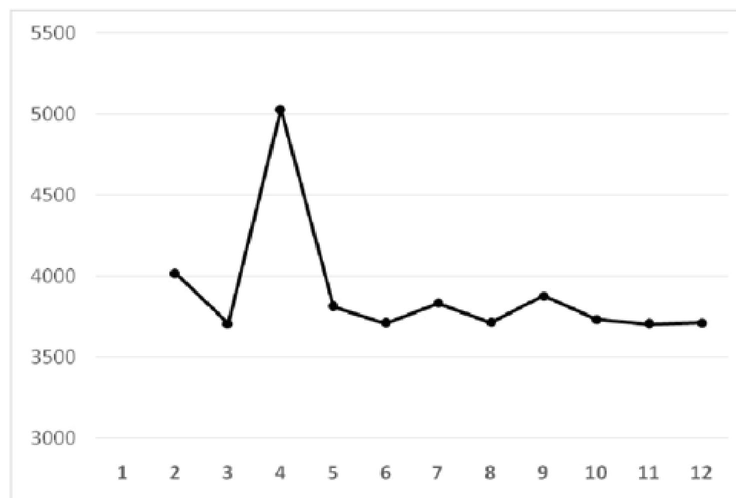

Fig. 6. Bilateral charts to determine the optimal number of K

4.1 Implications on Faba Bean Breeding Strategy

Breeding strategy of faba bean for *Orobanche* tolerance necessitates the following: (a) Ruling out the possibility of developing pure line host varieties to be grown in an *Orobanche* infested field because of the genetic variability of the parasite. (b) Developing faba bean varieties of heterogenous nature to tolerate *Orobanche* parsitizm. (c) The new developed varieties should be “location specific” as far as possible.

Geographically wide adapted varieties should not be recommended in the faba bean breeding strategy to tolerate *Orobanche.* (d) The recommended synthetic varieties should be composed of high yielding components with different genes controlling natures of resistance/ tolerance to *Orobanche.* In a related concern, Abdalla et al. [39] compared inbreds, open crosses and poly-crosses of faba bean. They found poly-crosses to be superior and best performing especially those poly-crosses with less numbers of parents. Similarly Ghaouti and Link [40] compared inbred, poly-crosses and check varieties for organic farming in five locations in Germany. They reported synthetic population to be the best performing stock.

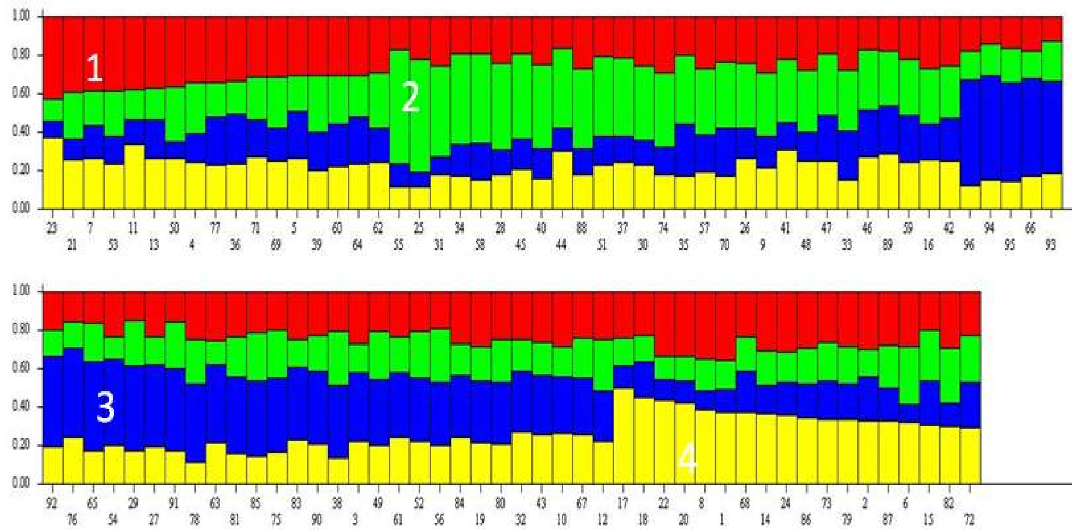

Fig.7. Genetic relatedness of 96 genotypes of *Orobanche crenata* analysed by structure program. Numbers on the y-axis indicate the membership coefficient and on the x-axis indicate the genotypes code. Genotypes with the same color belong to the same genetic groups

Some authors questioned presence of *O. crenata* races. It is clear from this study that *O. crenata* had great genetic variability. The existence of physiological races of *O. crenata* needs the development of a set of differential hosts that may be able to discriminate between races. Whenever, such hosts (or other means) are available, then races of *O. crenata* could then be named.

5. CONCLUSION

In conclusion, this research revealed the existence of wide genetic variation among *Orobanche crenata* plants from Egypt collected from faba bean naturally infested field. ISSR markers were suitable to study identifying genetic diversity among *O. crenata* individuals. The breeders have to consider this high genetic variation in *O. crenata* when they breed faba bean for tolerance / resistance to *Orobanche.*

ACKNOWLEDGEMENTS

The authors would like to thank International Center for Agriculture Research in Dry Area (ICARDA) for supporting study by using their biotechnology lab. We also pleasure to thank Dr. Alaa Hamawieh for guided data analysis and interpretation. Thank you for helping us to publish this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gevezova M, Dekalska T, Stoyanov K, Heisteva T, Kostov K, Batchvarova R, Denev I. Recent advance in broomrapes research. Journal of Bioscience & Biotechnolngy. 2012;1(2):91-105.
2. Grenz JH, Sauerborn J. Mechanisms limiting the geographical range of the parasitic weed *Orobanche crenata* Agric. Eco. Sys. and Environment. 2007;122:275-281.
3. Tewfic HA. Developmental stages of seedlings of *Orobanche crenata* Forsk in relation to its host *Vicic faba* (Linn). M.Sc. Thesis, Fac. Agric. Ain Shams Univ. Egypt; 1956.
4. Kasasion L. Miscellaneous observations on the biology of *Orobanche crenata* and *Orobanche aegyptiaca.* Symposium on parasitic weeds. Proceeding of the 1st EWRC. Parasitic Weeds: 68-75. Research Group. Wageningen, Netherlands; 1973.
5. Abdalla MMF. Characteristics of a local faba bean collectin and its reaction to *Orobanche.* In Hawtin G, Webb C, (Eds.).

Faba Bean Improvement. 207-212. Martinus Nijhoff (Netherlands) ICARDA / IFAD Nile Valley Project; 1982.

6. Nassib AM, Ibrahim AA, Kallil SA. Breeding for resistance to *Orobanche.* In Hawtin G, Webb C, (Eds.). Faba Bean Improvement. 199-206. Martinus Nijhoff (Netherlands) ICARDA/IFAD Nile Valley Project; 1982.
7. Zaitoun FMF. Studies on the resistance and susceptibility of broad bean (*Vicic faba* L.) to broomrape (*Orobanche crenata* Forsk). Ph.D. Thesis, Fac. Agric. Alexandria Univ. Egypt; 1990.
8. Abdalla MMF, Fischbeck G. Investigation on faba beans, *Vicia faba* L 2. Parasitizm of *Orobanche crenata* and selection for tolerance in host plants. Proc. 5th Conf. Agron. Zagazig. (Egypt), 13-15 Sept. 1992; 1:345-352.
9. Abdalla MMF, Darwish DS. Breeding faba bean for *Orobanhce* tolerance using the concept of breeding of uniform resistance. In Kroschel J, Abderabihi M, Betz H, (Eds.). Advances in parasitic weed control at on farm level. Join action to control *Orobanche* in the WANA region 205-213. (Magraf Verlag, Weikersheim, Germany. 1999;2.
10. Fischbeck G, Abdalla MMF, Metwally AA, Darwish DS. Variation of *Vicia faba* L. genotypes and populations of *Orobanche crenata* Forsk. Proc. 2ndAgron. Conf. Alex. Egypt. 1986;2:489-514.
11. Radwan MS, Abdalla MMF, Fischbeck G, Metwally AA, Darwish DS. Variation in reaction of faba bean lines to different accessions of *Orobanche crenata* Forsk. Plant Breed. 1988;101:208-216.
12. Nawar AA, Zaid MM, Zaitoun FMF, Madkour MA, Koraiem YS. Variation among three *Orobanche crenata* accessions in their virulence in relation to growth and yield characters of the faba bean cultivar Giza 3 and Giza 429. In. Kroschel J, Abderabihi M, Betz H, (Eds.). Advances in parasitic weed control at on farm level. Join Action to Control *Orobanhce* in the WANA Region 215- 225 (Magraf Verlag, Weikersheim, Germany. 1999;2.
13. Abdalla MMF. Investigation on faba beans, *Vicia faba* L. 35. Cairo 33 a new variety with colourless hilums and tolerance to *Orobanche*. Egypt J. Plant Breed. 2015; 19(2):233-245.
14. Von Kittlitz. Some observations on heterosis in succeeding generations of single crosses of *Vicia faba* in: Proceeding of Siminar in EEC Programme of. Coordination of Research on Improvement of the Production of Plant Proteins, Wagenningen, Netherlands. (Ed. Thompson R.) Martinus Nijhoff. World Crops. 1981;4:266-279.
15. Abdel-Mawgood AL. DNA Based Techniques for Studying Genetic Diversity, Genetic Diversity in Microorganisms; 2012. Available: www.intechopen.com
16. Abedi S, Darvishzadeh R, Bernousi I, Mandoulakani BA, Maleki HH, Shah D. Genetic variability of *Orobanche aegyptiaca* infesting tobacco in Iran by bayesian analysis. Biologia. 2014;69(12): 1652-1659.
17. Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 1994;20: 176-183.
18. Tsumura Y, Yoshimura K, Tomaru N, Ohba K. Molecular phylogeny of conifers using PCR - RFLP analysis of chloroplast genes. Theor. Appl. Genet. 1995;91:1222–1236.
19. Nagaoka T, Ogihara Y. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison toRFLP and RAPD markers. Theor. Appl. Genet. 1997;94: 597-602.
20. Fang D, Roose ML. Identification of closely related citrus cultivars with inter simple sequence repeat markers. Theor. Appl. Genet. 1997;95:408-417.
21. Hodkinson TR, Chase MW, Renvoize SA. Characterization of a genetic resource collection for Miscanthus (*Saccharinae, Andropogoneae, Poaceae*). Annals of Botany. 2002;89:627-636.
22. Korbin M, Kuras A, Zurawicz E. Fruit plant germplasm characterization using molecular markers generated in RAPD and ISSR-PCR. Cellular and Molecular Biology Letters. 2002;7(2B):785-794.
23. Rakoczy-Trojanowska M, Bolibok H. Characteristics and comparison of three classes of microsatellite-based markers and their application in plants. Cell. Mol. Biol. Lett. 2004;9:221-238.
24. Nagaraju J, Kathirvel M, Kumar R, Siddiq EA, Hasnain SE. Genetic analysis of traditional and evolved basmati and non-

Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Proc. Natl. Acad. Sci. 2002; 99(9):5836- 5841.
25. Reddy PM, Sarla N, Siddiq EA. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica. 2002;128-2:9-17.
26. Obeed RS, Harhash MM, Abdel-Mawgood AL. Fruit properties and genetic diversity of fiveber (*Ziziphusm auritiana* Lamk) cultivars. Pakistani J. Biological Sciences. 2008;11:888-893.
27. Cubero JI, Moreno MT. Agronomical control and sources of resistance in *Vicia faba* to *Orobanche crenata*. 41-80 in: Some current research on *Vicia faba* in Western Europe. Bond DA, Scarascia-ugnozza GT, Poulsen MH, Eds. Comm. Eur. Communities, Luxemburg; 1979.
28. Román B, Satovic Z, Rubiales D, Torres AM, Cubero JI, Katzir N, Joel DM. Variation among and within populations of the parasitic weed *Orobanche crenata* from Spain and Israel revealed by inter simple sequence repeat markers. Phytopathology. 2002;92:1262-1266.
29. Zeid M, Madkour M, Koraiem Y, Nawar A, Soliman M, Zaitoun F. Molecular studies on *Orobanche*. J. Phytopathol. 1997;145: 351-355.
30. Hassnien Aziza M, Attia Sabah M, Abd El-Rahman Rehab M, Ibrahim MAM. *Orobanche crenata* effects on some faba bean genotypes, the genetic variation among three isolates and chemical constituents. Egypt. J. Plant Bred. 2016; 20(1):105-118.
31. Pineda-Martos R, Pujadas-Salvà AJ, Fernández-Martínez JM, Stoyanov K, Velasco L, Pérez-Vich B. The genetic structure of wild *Orobanche ecumana* (*Orobanchaceae*) populations in Eastern Bulgaria reflects introgressions from weedy populations. Sci. World. J. 2014;1-15.
32. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 1980;8:4321-4325.
33. Satiu N, Nei M. The neighbor joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 1987;4(4):406-425.
34. Rohlf J. NTSYSpc. Numerical Taxonomy and Multivariate Analysis System, Version 2.21 h. Exeter Software, Setauket, NY, USA; 2010.
35. Roldan-Ruiz I, Dendauw J, Vanbockstaele E, Depicker A, De Loose M. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Mol. Breed. 2000;6:125–134.
36. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155:945-959.
37. Musselman LJ. Taxonomy of *Orobanche.* In S. J. ter Borg (ed). Proc. Workshop on Biology and Control of *Orobanche.* Wageningen, the Netherlands. 1986;2-10.
38. Gagne G, Roeckel-Drevet P, Grezes-Besset B, Shindrova P, Ivanov P, Grand-Ravel C, Vear F, Tourvieille de Labrouhe D, Charmet G, Nicolas P. Study of the variability and evolution of *Orobanche cumana* populations infesting sunflower in different European countries. Theor. Appl. Genet. 1998;96:1216-1222.
39. Abdalla MMF, Darwish DS, Abo- Hegazy SRE. Investigation on faba bean, *Vicia faba* L. Poly-crosses, open crosses and inbreds. The 2nd plant breed. Conf. October 2 (Assiut Univ. Egypt). 2001;375-389.
40. Ghauti L, Link W. Local *vs.* formal breeding and inbred line *vs.* synthetic cultivar for organic farming: Case of *Vicic faba* L. Field Crops Research. 2009; 110:167-172.

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