# Genetic diversity among some broomrape *(Orobanche)* species from Egypt using SRAP and ISSR markers

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## ABSTRACT

*Broomrape, a noxious parasitic weed from several families, causes severe damage to economically important vegetables and field crops in Egypt. It can reduce crop yield as much as 90 to 100%. Identification of broomrape species based on morphological characters faces major difficulties. Therefore, the precise discrimination of such parasitic weed is considered the first step toward effective and specific weed management. Thus, in this study molecular markers were used as powerful and rapid identification techniques for the most major Orobanche species in Egypt. Sixteen sequence related amplified polymorphism (SRAP) primers and nine inter simple sequence repeat (ISSR) primers were employed to assess the genetic diversity among three most important Orobanche species. The SRAP and ISSR analyses showed that, 132 out of 170 and 80 out of 106 markers, respectively, were detected as polymorphic markers (77.6 % and 75.4 %) among the tested Orobanche species. The Orobanche species were characterized by 32 genotype-specific markers, 20 for SRAP and 12 for ISSR that would be considered as useful markers for Orobanche species. UPGMA cluster analysis separated the three broomrape species into two main clusters, the first one comprised of two species O. ramosa which infect different plants and O. aegyptiaca. While, the second cluster included only O. crenata. This clustering indicates that the tested species have common genomic segments. Our results indicated that SRAP–based molecular could be used for characterization of the studied broomrape species as considered more reliable and robust than other molecular markers used. Moreover, investigating the variability in broomrape species is crucial when attempting to develop weed control means.*

***Keywords:*** *Broomrapes, Orobanche spp., Genetic diversity, ISSR markers, SRAP markers.*

## INTRODUCTION

Broomrape is chlorophyll-lacking root parasite of many dicotyledonous species, causing severe damage to economically important vegetables and crops from several families. Egyptian broomrape is one of the most common species, where it parasitizes a wide range of crops belonging to families such as *Solanacea, Fabaceae* and *Umbelliferae* (Joel *et al.,* 2007). ver 73 million hectares of farmland under cultivation in the Southern , Middle East and Eastern Europe, and regions of North Africa are infested with *Orobanche* (Amsellem *et al*., 2001 and Abang *et al.,* 2007). In some cases, crop yield reduction reaches up to 100% (Rolland *et a.,l* 2016 and Abdalla., *et al.* 2016). This crop

production loss is estimated as hundreds of millions of dollars that affecting globally the livelihoods of 100 million farmers (Abang *et al*., 2007 and Gevezova *et al*., 2012). Broomrape varies in host plant range; some parasitizing an enormous range of crops, while others are more specific. *Orobanche ramosa* L. has the widest host range, parasitizing many solanaceous crops such as tomato, tobacco and potato, members of *Brassicaceae, Leguminaceae*, and several other families. *O. aegyptiaca Pers*. has a host range similar to that of *O. ramosa*, and is also parasitic on carrot, legumes such as common vetch, and crucifers including oilseed rape. *O. crenata* is the most widespread and troublesome broomrape in Egypt, parasitizing mainly on legume crops. Management of this parasitic weed presents serious problems in agriculture, because it is closely associated with the host root and is hidden underground for most of its life. Accordingly, an economically means of controlling such weed is difficult and rather not effective. The distinguishing of *Orobanche* species among other flowering plants based on visual features faces great difficulties even when accurate observation of mature plants is performed and is often subject of debate (Zeid *et al*., 1997). Musselman (1994) explained that there is inherent morphological variability between Orobanche populations. Even the host may influence the morphology of the parasite (Musselman and Parker, 1982). This would lead to hindering the assessment of economic importance of broomrape as noxious agricultural weed species. Thus, the accurate identification of such parasitic weed comprises the first step in developing meaningful weed control strategies. For better control of this noxious weed, identification of the *Orobanche* species is particularly needed because of the differences in the host preferences of the various species (Portony *et al*., 1997 and Atanasova *et al.,* 2014). The number of available characters used in distinguishing species is limited posing taxonomic problems. Recently, molecular markers have been a beneficial tool for characterizing and differentiating many *Orobanche* species more precisely and specifically (Rolland *et al.*, 2016). Molecular markers ISSR and random-amplified polymorphic DNA (RAPD) offer many advantages over phenotype-based approach as they are stable and detectable in all tissues (Eizenberg *et al.,* 2012). The use of genetic markers to recognize broomrape species infestation in the field can contribute to determining its spatial distribution, which can be an important first step toward effective and specific weed management. More recently, sequence-related amplified polymorphism (SRAP) first raised by Li and Quiros (2001).This marker is dominant, easy, simple, inexpensive and effective for producing fragments with high reproducibility and versatility. Furthermore, SRAP markers are used to multiply coding regions of DNA with primers aiming open reading frames. SRAP markers have proven to be strong and highly variable, as AFLP, are attained through a significant and less technically demanding process (Robarts and Wolfe, 2014). These techniques had been applied extensively in genetic diversity analysis and comparative genetics of different species (Fan *et al*., 2010; Guo *et al*., 2012; Wang *et al*., 2012 and Aneja *et al*., 2013). To the best of our knowledge, there are a few studies examining the genetic diversity and relationship of broomrape using SRAP marker. Therefore, the present study aimed to investigating the molecular differentiation of the most noxious and spread *Orobanche* species in Egypt using SRAP and ISSR markers in order to relate morphological and molecular data for *Orobanche* species under the study. Genetic relationships among *Orobanche* species and the phylogenetic

relationships within this genus were also determined.

## MATERIALS AND METHODS

### Plant material

Eight mature *Orobanche* species were collected from natural populations in agricultural fields in Giza governorate, Egypt. The collected broomrape species under the study and their host plants are listed in Table (1).

### Morphological studies

The morphological characterization of *Orobanche s*pecies under the study was visually identified by the Department of Flora Research and Plant Taxonomy, Agricultural Research Center, Egypt. Morphological studies were based on nine different discriminating visual characters (Boulous 2002; Mohamed and Musselman 2008; and Sharawy and Karakish 2015) as shown in Table (3).

### Molecular studies

### DNA extraction

Total genomic DNA was isolated from frozen shoot and inflorescence which was grounded in liquid nitrogen using I-genomic plant DNA extraction mini kit (Intron biotechnology, Boston, USA; (CAT NO.17371) according to the manufacturer's guidelines.

### SRAP markers

Polymers chain reaction amplification was performed in a 20 µL reaction volume containing 1 µl DNA (50 ng/µL), 10 µL master mix (Gene DireX, CAT NO.MB208-0100), 1 µL from forward and reverse primers (2 µM/µL of primers) and 7 µL of nuclease free water. Sixteen SRAP primer (Bio Basic Canada Inc.) combinations (Table 2) were screened by PCR. Amplification of PCR was achieved according to Li and Quiros (2001) methods. The conditions were programmed with denaturation step at 94 ℃ for 4 min and followed by 5 cycles at 94 °C for 1 min, then annealing at 35 °C for 1 min, and extension at 72 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 1 min, and annealing step at 50 °C for 1 min., then extension step at 72 °C for 1 min Finally, the analysis was completed with one cycle of a final extension at 70 °C for 5 minutes.

### ISSR markers

Nine ISSR primers (Table 2) obtained from Bio Basic Canada Inc. were used. PCR analysis was carried out in 20 µl reaction mixture containing 1 µl genomic DNA (50 ng/µL), 10 µL master mix, 1 µl of 10 µM of ISSR Primers, then complete the total volume with $ddH_20$ to 20 µl. The amplification conditions were carried out in a thermocycler UNO II, Biometra. The PCR program started with denaturation step at 94°C for 4 min, followed by, 37 cycles at 94°C/ 1 min for denaturation, and annealing at 52 °C/1 min, then elongation step at 72°C/2 min. Finally a terminal extension cycle at 72°C/ 5 min (Zietkiewicz *et al*., 1994).

The products of PCR of both markers were resolved by 1.5 % agarose gel in 1x TAE buffer, DNA bands visualized with ethidium bromide staining (0.5 µg/mL), and photographed under UV light using gel documentation system (Bio-Rad® Gel Doc-2000). One hundred bp DNA ladder (Promega, USA) was used as molecular weight size marker.

### Data analysis

The PCR products were scored on the basis of presence (1) or absence (0) of the bands.The data obtained from SRAP and ISSR analyses were collected together to measure the genetic similarity coefficient between two samples according to the Dice coefficient (Sneath and Sokal, 1973). The tree diagram

was produced by clustering the similarity data with the UPGMA method using systat ver. 7 (SPSS Inc. 1997 SPSS Inc.3/97 standard version) software.

***Table (1): The Orobanche code, species and host plant.***

| No | *Orobanche* species (visual identification) | Host plant |
|---|---|---|
| 1 | *O. crenata* | Chickpeas (Ch) |
| 2 | *O. crenata* | Lentil (Le) |
| 3 | *O. crenata* | Safflower (Sa) |
| 4 | *O. crenata* | Faba bean (Be) |
| 5 | *O. ramosa* | Eggplant (Eg) |
| 6 | *O. ramosa* | Turnip (Tu) |
| 7 | *O. ramosa* | Cauliflower (Ca) |
| 8 | *O. aegyptiaca* | Tomato (To) |

***Table (2): The nucleotide sequences of ISSR and SRAP primers.***

| ISSR code | Sequence of Primer (5'-3') | SRAP primer | |
|---|---|---|---|
| | | Forward primer (5'-3') | Reverse primer (5'-3') |
| UBC808 | (AG)8C | EM1:GACTGCGTACGAATTAAT | ME1:TGAGTCCAAACCGGATA |
| UBC809 | (AG)9G | EM2:GACTGCGTACGAATTTGC | ME2:TGAGTCCAAACCGGAGC |
| UBC810 | (GA)8T | EM3:GACTGCGTACGAATTGAC | ME3:TGAGTCCAAACCGGAAT |
| UBC817 | (CA)8A | EM4:GACTGCGTACGAATTTGA | ME4:TGAGTCCAAACCGGACC |
| UBC823 | (TC)8C | | ME5:TGAGTCCAAACCGGAAG |
| UBC824 | (TC)8G | | |
| UBC826 | (AC)8C | | |
| UBC862 | (AGC)6 (GAC)2AGA | | |
| UBC873 | CAG ACA A | | |

## RESULTS AND DISCUSSION

### Identification of broomrape species based on morphological traits

Morphological distinguishing based on stem and flower (corolla, calyx and anther), as the most identification characters keys of *Orobanche*, revealed that the eight studied species are belonging to three distinguished species identified as *O. crenata*, *O. aegyptiaca* and *O. ramosa*. *O. ramosa* and *O. aegyptiaca* are morphologically similar to one another, have similar host ranges. However, *O. aegyptiaca* can be identified by its relatively more branched stem, larger corolla, relatively short calyx and blue flowers. While,*O. ramosa* has smaller corolla, short calyx, laxer inflorescent, and white to pale blue flower. Whereas, *crenata* has been discriminated by its unbranched stem, long calyx, denticulate margins of the corolla lobes and dense inflorescence (Table 3). Although the number of available characters used in distinguishing species are limited, the pattern of interspecies variation observed in this study was in agreement with previous taxonomical characterization based on morphological differences among broomrape species under the study (Mohamed and Musselman, 2008; Plaza *et al*., 2004 and Sharawy and Karakish 2015 ).

***Table (3): Morphological characters of different Orobanche species.***

| Species | Stem | | | Flower | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Diameter (cm) | branching | Calyx Length (mm) | Corolla length | Corolla margin | Corolla color | Anther | Capsule (mm) |
| ***O. crenata*** | Different | 3 | Un-branched | 15-18 | 3-5 | Crenate | White to dark violet or yellow | Hairy-glabrous | 9-11 |
| ***O. aegyptiaca*** | 15-20 | 1 | Branched | 10-13 | 2.5-4 | Rounded | Violet-blue | Hairy | 6-9 |
| ***O. ramosa*** | 10-40 | Less than 1 | Branched | 5-8 | 1.2-1.8 | Rounded | Violet-blue to bluish or whitish | Glabrous | 5-6 |

**SRAP analysis**

There is little information in Egypt about the genetic diversity among *Orobanche* populations based on the molecular markers. In this study genetic diversity among and within *Orobanche* populations was determined and compared with other studies. Due to the advances in DNA, molecular markers are now increasingly available for use in cultivar identification, molecular identification, and marker assisted selection in plants. Among various molecular markers, SRAP is a relatively new PCR marker extensively used recently for germplasm characterization, cultivar identification, molecular mapping and gene cloning (Esposito *et al*., 2007 and Mutlu *et al*., 2008). SRAP is a useful technique for the estimation of the genetic variability because it has shown a high degree of reproducibility and discriminatory power, as well as a high polymorphism rate in many genetic studies (Alghamdi *et al*., 2012). In our study sixteen SRAP primers were used to study the polymorphism levels among three (previously eight species which were morphologically identified) broomrape species collected from natural populations in agricultural fields in Giza governorate. SRAP primer combinations showed different levels of polymorphism among the broomrape species as illustrated in Fig (1). SRAP analysis resulted in a total of 170 markers detected among the eight broomrape species (Table 4). Only 132 of them were polymorphic markers (77.6 %). The highest number of bands (13 bands) was generated by using both EM1-ME1 and EM3-ME4 primers, while the lowest one was 8 bands that was generated with three different markers (EM2-ME2, EM2-ME5 and EM4-ME1). The EM3- ME3 primer recorded the highest polymorphism percentages (100 %) while the lowest percentage (58.3%) was recorded by both EM3-ME2 and EM2-ME4 primers. Ennami *et al.*, (2017) assessed the genetic variation among and within Moroccan *O. crenata* populations, growing in faba bean fields, using SRAP. They could identified 101 markers as 98 bands were polymorphic (97.02%), indicating considerable genetic variation of these *O. crenata* populations.

***Table (4): PCR amplicons obtained from SRAP markers in Broomrape species.***

| Primer name | Total bands | Polymorphic band | Polymorphism % | Band size range |
|---|---|---|---|---|
| EM1-ME1 | 13 | 11 | 84.6 | 41-1000 |
| EM1-ME2 | 9 | 6 | 66.6 | 59-1185 |
| EM1-ME3 | 9 | 7 | 77.7 | 66-417 |
| EM1-ME4 | 12 | 9 | 75 | 157-713 |
| EM1-ME5 | 12 | 11 | 91.6 | 89-914 |
| EM2-ME1 | 10 | 9 | 90 | 70-893 |
| EM2-ME2 | 8 | 5 | 62.5 | 85-928 |
| EM2-ME3 | 10 | 8 | 80 | 121-581 |
| EM2-ME4 | 12 | 7 | 58.3 | 54-853 |
| EM2-ME5 | 8 | 7 | 87.5 | 87-710 |
| EM3-ME1 | 10 | 8 | 80 | 68-444 |
| EM3-ME2 | 12 | 7 | 58.3 | 44-1174 |
| EM3-ME3 | 12 | 12 | 100 | 90-1220 |
| EM3-ME4 | 13 | 8 | 61.5 | 131-768 |
| EM3-ME5 | 12 | 11 | 91.6 | 120-1339 |
| EM4-ME1 | 8 | 6 | 75 | 84-1000 |
| Total | 170 | 132 | 77.6 | |

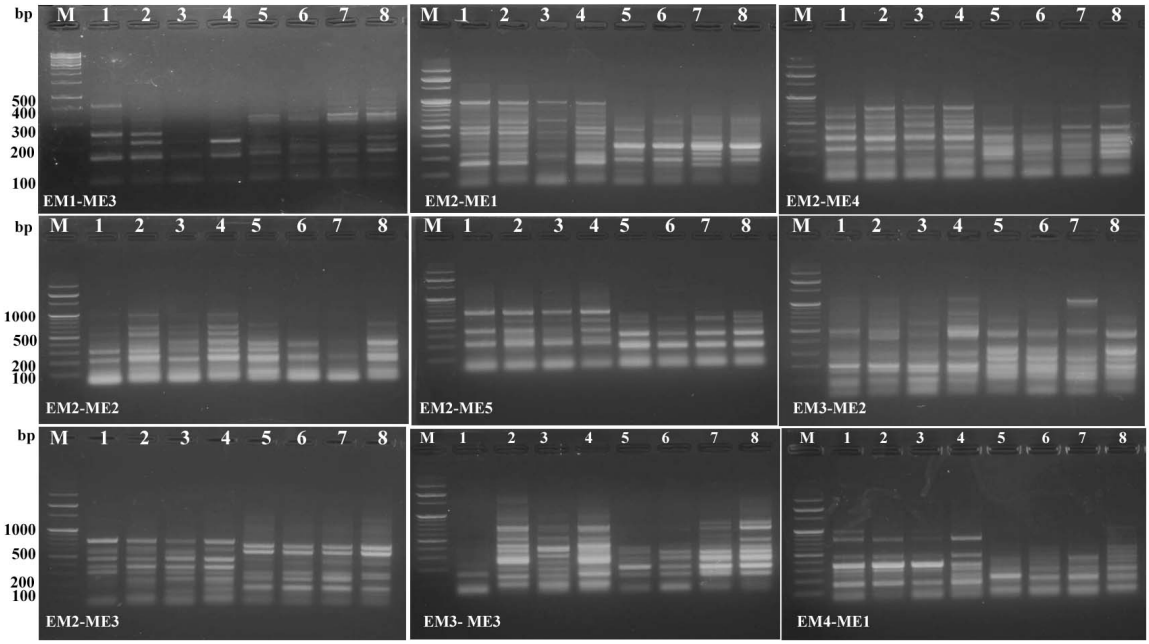


***Fig. (1): SRAP profile demonstrating polymorphism among the broomrape species. M refers to DNA marker of 100 bp ladder. Lanes 1-8 represent all broomrape host plant samples (Ch: Chickpeas, Le: lentil, Sa: Safflower, Be: Bean, Eg: Eggplant, Tu: Turnip, Ca: Cauliflower and To: Tomato), respectively.***

**ISSR analysis**

ISSR markers were the powerful tool in genetic fingerprinting and variation analysis (Aghaei *et al*., 2012). ISSR is the best method for fingerprinting and a useful alternative to single-locus because large numbers of DNA amplicons are amplified in reaction, representing multiple loci from across the genome (Román *et al*., 2002; Hristova *et al*., 2011 and Aghaei *et al*., 2012).Nine primers were used in the detection of polymorphism among three broomrape species. These primers generated reproducible and easily scorable ISSR profiles with a total of 106 markers (Table 5 and Fig.2). Only 80 out of them were polymorphic markers (75.4 %). The highest number of bands (20 bands) was generated using the primer UBC873, while the lowest one was (8 bands) generated by both UBC817 and UBC826 primers. The highest polymorphism percentage was recorded by using UBC817 and UBC824 primers (100 %) while the lowest percentage (50 %) was detected by using UBC826 and UBC873. The use of ISSR technique in studying broomrape diversity is relatively recent approach, which enable researchers in this field to identify several *Orobanche* species (Benharrat *et a.,* 2002), and to distinguish populations of *O. crenata* and *P. ramosa* (Benharrat *et al.,* 2002; Roman *et al*., 2002 and Buschmann *et al*., 2005). Abedi *et al*. (2014) studied the genetic polymorphism among 44 *O. aegyptiaca* individuals using inter-simple sequence repeat (ISSR) markers. According to their data, 261 discernible bands were amplified using 20 ISSR primers in which 245 (94%) were polymorphic, indicating considerable genetic variation among the examined individuals. Their results demonstrated the potential usefulness of ISSR markers for determination of genetic variation in *O. aegyptiaca.* Sharawy and Karakish (2015) used five ISSR primers to differentiate between the *Orobanche* species belonging to section *Orobanche* from species belonging to section *Trionychon*.

**Genotype identification**

The presence of unique SRAP markers among the various broomrape species indicated the utility of the approach for fingerprinting purposes. Twenty markers out of the 132 polymorphic SRAP markers were found to be genotype-specific (15.15 %). The three broomrape species were characterized by twenty (16 positive and 4 negative) unique SRAP markers (Table 6). Therefore, these SRAP markers would be used as associated markers for the broomrape species. Moreover, six ISSR primers produced twelve unique markers (7 positive and 5 negative) ranging from one for UBC826 and UBC808 to four for UBC824 as shown in Table (7). The results showed that all techniques used in this study, *i.e* SRAP and ISSR showed different genotype-specific molecular markers which can be used to discriminate between studied broomrape species and considered suitable tools for sufferable fingerprinting diagnostic markers for all broomrape species under the study.

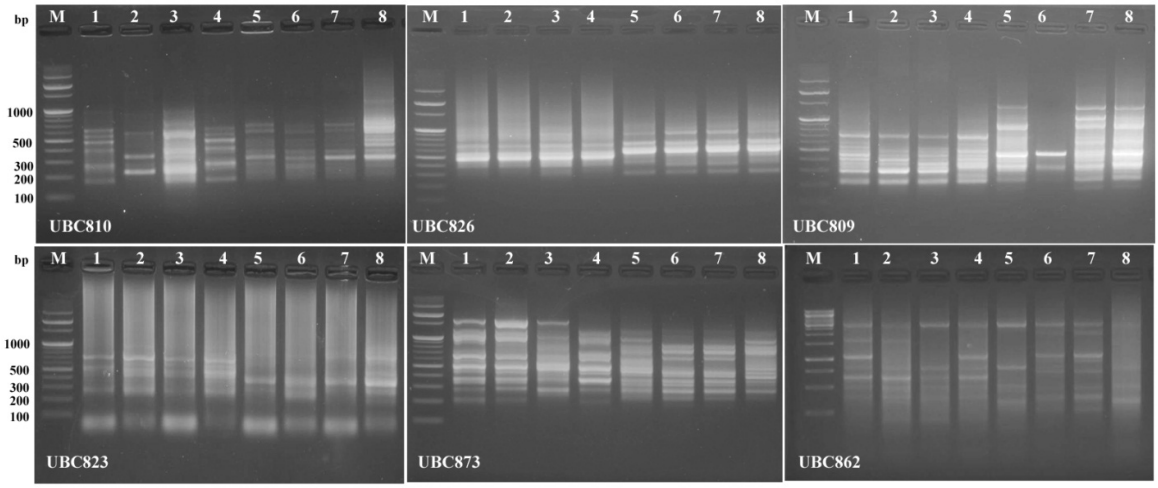


***Fig. (2): ISSR profile demonstrating polymorphism among the eight broomrape species. M refers to DNA marker of 100 bp ladder. Lanes 1-8 represent all broomrape host plant samples (Ch: Chickpeas, Le: lentil, Sa: Safflower, Be: Bean, Eg: Eggplant, Tu: Turnip, Ca: Cauliflower and To: Tomato), respectively.***

***Table (5): PCR amplicons obtained from ISSR markers in Broomrape species.***

| Primer name | Total bands | Polymorphic band | Polymorphism % |
|---|---|---|---|
| UBC809 | 13 | 10 | 76.9 |
| UBC810 | 10 | 7 | 70 |
| UBC817 | 8 | 8 | 100 |
| UBC808 | 16 | 13 | 81.2 |
| UBC823 | 10 | 9 | 90 |
| UBC824 | 10 | 10 | 100 |
| UBC826 | 8 | 4 | 50 |
| UBC862 | 11 | 9 | 81.8 |
| UBC873 | 20 | 10 | 50 |
| Total | 106 | 80 | 75.4 |

***Table (6): Broomrape species specific unique SRAP markers.***

| Species | SRAP unique markers | | Total markers |
|---|---|---|---|
| | Positive | Negative | |
| ***O. crenata* (Ch)** | EM3-ME5 (1100, 1600, 1800)<br>EM1-ME5 (610, 720, 890, 1000)<br>EM1-ME3 (215) | ---------- | 8 |
| ***O. crenata* (Le)** | EM3-ME3(1960,1990)<br>EM1-ME4 (1100) | EM2-ME1 (250) | 4 |
| ***O. crenata* (Sa)** | EM1-ME1 (195) | ---------- | 1 |
| ***O. ramose* (Eg)** | ---------- | EM2-ME3 (540) | 1 |
| ***O. ramose* (Tu)** | EM3-ME4 (760) | ---------- | 1 |
| ***O. ramose* (Ca)** | EM1-ME2 (150, 780) | EM3-ME5 (500, 700) | 4 |
| ***O. aegyptiaca* (To)** | EM4-ME1 (600) | ---------- | 1 |
| **Total** | 16 | 4 | 20 |

***Table (7): Broomrape species specific (unique) ISSR markers.***

| Species | ISSR unique markers | | Total markers |
|---|---|---|---|
| | Positive | Negative | |
| ***O. crenata* (Le)** | UBC824 (1300) | ---------- | 1 |
| ***O. crenata* (Be)** | UBC824 (270, 400, 780) | UBC826 (310)<br>UBC873 (700, 800) | 6 |
| ***O. ramosa* (Eg)** | ---------- | UBC809 (290) | 1 |
| ***O. ramosa* (Tu)** | ---------- | UBC808 (95) | 1 |
| ***O. aegyptiaca* (To)** | UBC823 (150, 930, 950) | ---------- | 3 |
| **Total** | 7 | 5 | 12 |

**Genetic similarity among broomrape species**

Determining the genetic dissimilarity between individuals is an important point for clustering and analyzing variation (Kosman and Leonard, 2005). The genetic similarity ranged from 0.510 between *O. crenata* (Le) and *O. aegyptiaca* (To) to 0.952 between *O. crenata* (Sa) and *O. crenata* (Be) using SRAP data (Table 8). The scoring data resulting from ISSR were analyzed as presented in Table (9). The estimated genetic similarity among the broomrape species based on ISSR ranged from 0.516 to 0.863. The highest genetic similarity (0.863) was between *O. ramosa* (Eg) and *O. aegyptiaca* (To), while the lowest genetic similarity (0.516) was between *O. crenata* (Sa) and *O.ramosa* (Ca). To obtain more balanced values for genetic similarity among species and an equilibrated dendrogram representation of the relationships among these species, data of SRAP and ISSR profiles were combined and summarized in Table (10). Combining data showed that the highest similarity was 0.867 between *O. crenata* (Sa) and *O. crenata* (Be), while the lowest similarity was 0.525 between *O. crenata* (Le) and *O.ramosa* (Ca).

***Table (8): Broomrape species similarity matrix based on SRAP analysis.***

| Species | O. crenata (Ch) | O. crenata (Le) | O. crenata (Sa) | O. crenata (Be) | O. ramosa (Eg) | O. ramosa (Tu) | O. ramosa (Ca) | O. aegyptiaca (To) |
|---|---|---|---|---|---|---|---|---|
| **O. crenata (Ch)** | 1 | | | | | | | |
| **O. crenata (Le)** | 0.876 | 1 | | | | | | |
| **O. crenata (Sa)** | 0.854 | 0.849 | 1 | | | | | |
| **O. crenata (Be)** | 0.864 | 0.849 | **0.952** | 1 | | | | |
| **O. ramosa (Eg)** | 0.669 | 0.634 | 0.588 | 0.588 | 1 | | | |
| **O. ramosa (Tu)** | 0.626 | 0.616 | 0.620 | 0.586 | 0.807 | 1 | | |
| **O. ramosa (Ca)** | 0.642 | 0.606 | 0.570 | 0.570 | 0.917 | 0.815 | 1 | |
| **O. aegyptiaca (To)** | 0.527 | **0.510** | 0.531 | 0.531 | 0.757 | 0.721 | 0.736 | 1 |

***Table (9): Broomrape species similarity matrix based on ISSR analysis.***

| Species | O. crenata (Ch) | O. crenata (Le) | O. crenata (Sa) | O. crenata (Be) | O. ramosa (Eg) | O. ramosa (Tu) | O. ramosa (Ca) | O. aegyptiaca (To) |
|---|---|---|---|---|---|---|---|---|
| **O. crenata (Ch)** | 1 | | | | | | | |
| **O. crenata (Le)** | 0.802 | 1 | | | | | | |
| **O. crenata (Sa)** | 0.791 | 0.828 | 1 | | | | | |
| **O. crenata (Be)** | 0.838 | 0.800 | 0.818 | 1 | | | | |
| **O. ramosa (Eg)** | 0.661 | 0.578 | 0.630 | 0.661 | 1 | | | |
| **O. ramosa (Tu)** | 0.666 | 0.551 | 0.682 | 0.698 | 0.821 | 1 | | |
| **O. ramosa (Ca)** | 0.558 | 0.525 | 0.516 | 0.564 | 0.733 | 0.806 | 1 | |
| **O. aegyptiaca (To)** | 0.666 | 0.569 | 0.666 | 0.682 | **0.863** | 0.854 | 0.819 | 1 |

***Table (10): Broomrape species similarity matrix based on the combined data between SRAP and ISSR analysis.***

| Species | O. crenata (Ch) | O. crenata (Le) | O. crenata (Sa) | O. crenata (Be) | O. ramosa (Eg) | O. ramosa (Tu) | O. ramosa (Ca) | O. aegyptiaca (To) |
|---|---|---|---|---|---|---|---|---|
| **O. crenata (Ch)** | 1 | | | | | | | |
| **O. crenata (Le)** | 0.842 | 1 | | | | | | |
| **O. crenata (Sa)** | 0.815 | 0.852 | 1 | | | | | |
| **O. crenata (Be)** | 0.846 | 0.835 | **0.867** | 1 | | | | |
| **O. ramosa (Eg)** | 0.666 | 0.575 | 0.610 | 0.629 | 1 | | | |
| **O. ramosa (Tu)** | 0.622 | 0.533 | 0.642 | 0.641 | 0.818 | 1 | | |
| **O. ramosa (Ca)** | 0.584 | **0.525** | 0.528 | 0.557 | 0.805 | 0.816 | 1 | |
| **O. aegyptiaca (To)** | 0.616 | 0.548 | 0.635 | 0.634 | 0.83 | 0.852 | 0.808 | 1 |

**Cluster analysis**

To determine the genetic relationships among the broomrape species, the scoring data resulting from each marker type assay was used to compute the similarity matrices and then used in cluster analysis to generate dendrogram using UPGMA analysis. The relationships among species have been represented as a dendrogram. In SRAP analysis, the dendrogram divided the broomrape species into two main clusters (Fig. 3), the first one included two species *O. ramosa* and *O. aegyptiaca* that branched into two sub-clusters; first sub-cluster included *O. ramosa* species that infect different plants (*O. ramosa* (Eg), *O. ramosa* (Ca)) with same linkage distance and *O. ramosa* (Tu), while the *O. aegyptiaca* (To) represent the second sub-cluster. In contrast, the second cluster included only *O. crenata* specie that branched into two sub-clusters; first sub-cluster included *O. crenata* (Sa) and *O. crenata* (Be) while, the second sub-cluster included *O. crenata* (Ch) and *O. crenata* (Le). Based on ISSR data, the cluster analysis divided the broomrape species into two main clusters (Fig. 4). The first one included two species *O. ramosa* and *O. aegyptiaca* that branched into two sub-clusters; first sub-cluster included *O. ramosa* (Eg) and *O. aegyptiaca* (To) with same linkage distance and *O. ramosa* (Tu) alone, while the *O. ramosa* (Ca) represent the second sub-cluster. The second cluster included *O. crenata* and branched into two sub-clusters; first sub-cluster included *O. crenata* (Ch) and *O. crenata* (Be) while the second sub-cluster included *O. crenata* (Le) and *O. crenata* (Sa).

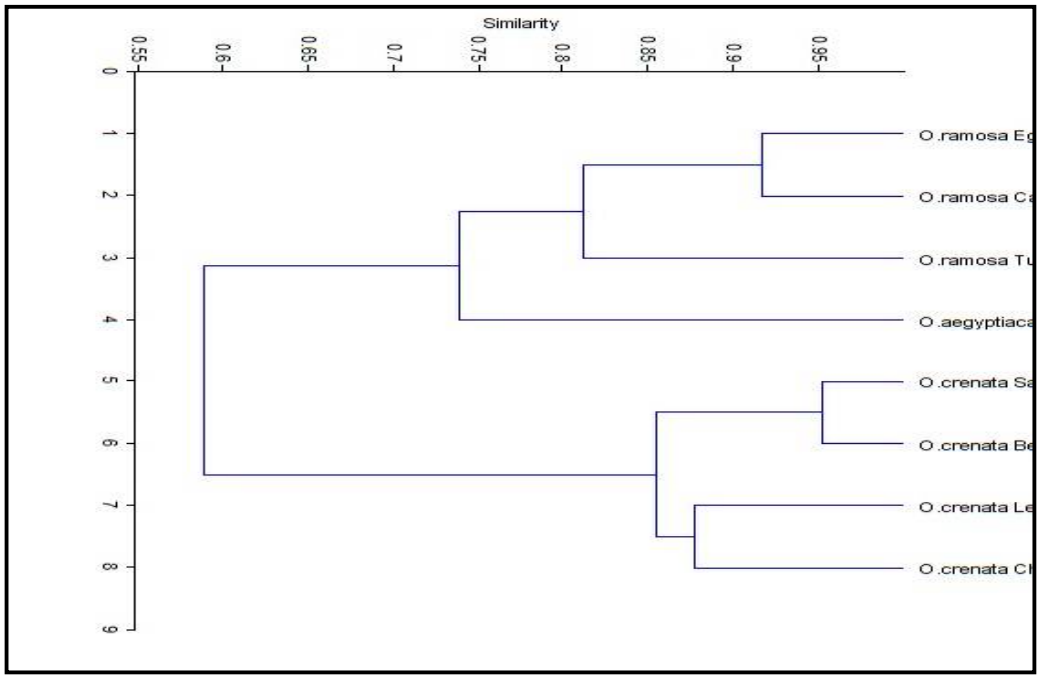


***Fig. (3): Cluster analysis of the broomrape species as revealed by SRAP data.***

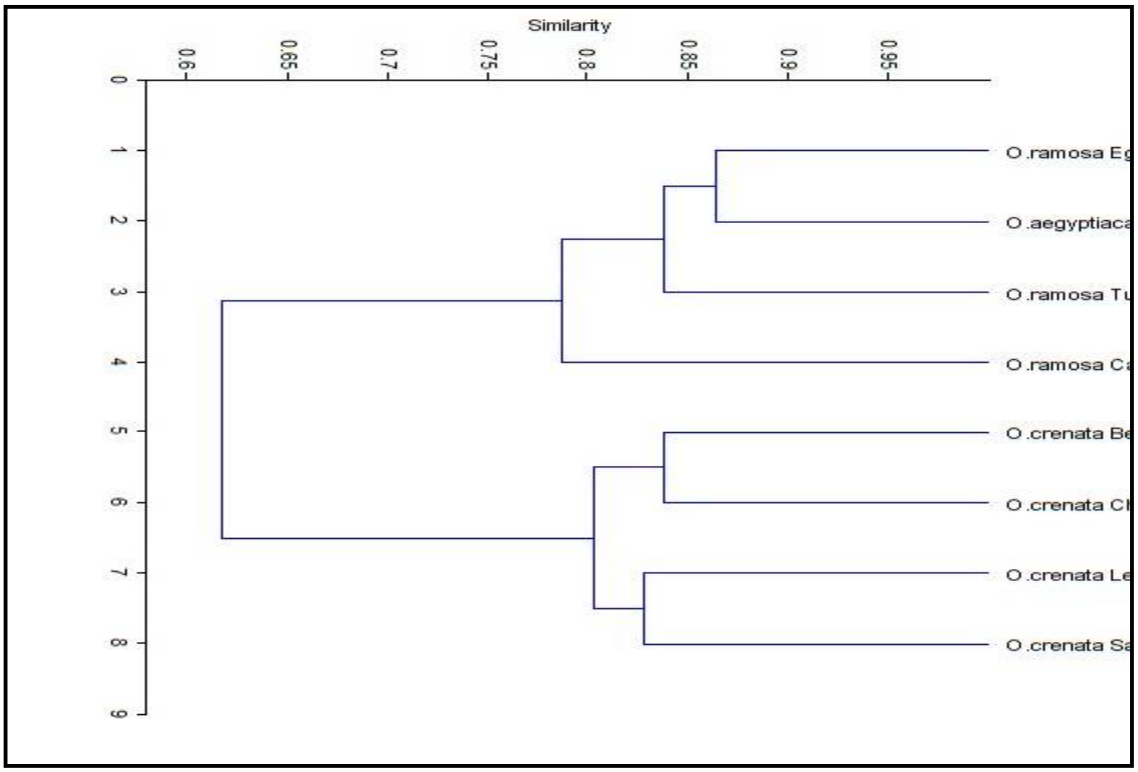


***Fig. (4): Cluster analysis of the broomrape species as revealed by ISSR data. A dendrogram was constructed based on the combined data from the two types of markers, SRAP and ISSR, as shown in Fig. (5).The dendrogram for combined data showed that the dendrogram divided the broomrape species into two main clusters. The first one included two species O .ramosa which infest different plants (Eg, Ca, and Tu) and O. aegyptiaca (To).The second cluster included O. crenata only that infest (Sa, Be, Ch and Le). In addition, the dendrogram of combined data was very similar to SRAP dendrogram than ISSR dendrogram. This clustering indicates that these species have common genomic segments. Furthermore, SRAP markers could be discriminate closely related species such as O. crenata and O. ramosa. Indeed, the results of this combined data were agreed with the visual identification of infested host plants with Orobanche in Table (1).***

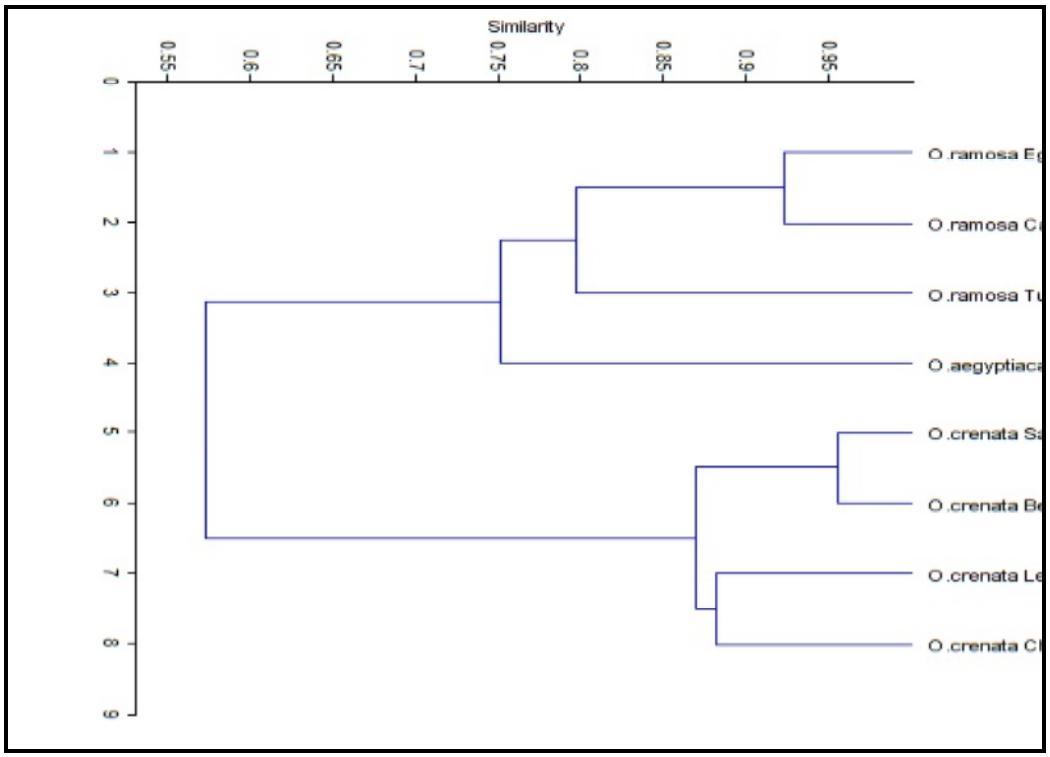


***Fig. (5): Cluster analyses of the broomrape species as revealed by the combined data across SRAP and ISSR analyses.***

SRAP markers were more trustworthy, as they have the highest average discriminating power among the different markers such as SSR, AFLP, ISSR and RAPD (Budak *et al*., 2004). Moreover, SRAP markers have the asset to amplify coding regions of the genome with primers targeting open reading frame, and explain the regions with inherent biological significance (Robarts and Wolfe, 2014).

## CONCLUSION

For better control of such parasitic and devastating weed, characterization of the broomrape species is particularly needed because of the differences in the host preferences of the various host plant species. In addition new and more virulent populations are currently appeared which are very difficult to discriminate using morphological characteristic alone. Consequently, the need for analyzing their genetic variability using molecular discrimination should be considered. Our results suggest that using SRAP and ISSR analyses represent efficient and rapid tools for determining the genetic diversity and relationships among broomrape species. Furthermore, the generated unique markers could be useful markers to differentiate between the closely related broomrape species used. The pattern of interspecific variability and genetic distances observed in this study using SRAP and ISSR markers were in agreement with previous taxonomical characterization based on morphological differences among the studied *Orobanche* species. Our findings showed that

SRAP–based molecular could be used for characterization of the studied broomrape species as considered more reliable and robust than other molecular markers used.

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## الملخص العربي

### التنوع الوراثي بين بعض أنواع الهالوك *(Orobanche)* المصرية بإستخدام المعلمات SRAP وISSR

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يعتبر الهالوك من الحشائش الطفيلية الضارة الذى يتبع العديد من العائلات التي تسببأضرارًا شديدة للخضروات والمحاصيل الحقلية المهمة في مصر. والذى يمكن أن يقلل من غلة المحاصيل بمقدار ٩٠ إلى ١٠٠ ٪ . أن تحديد أنواع الهالوك على أساس الصفات المورفولوجية يواجه صعوبات كبيرة. لذلك ، يعتبر التمييز الدقيق لهذه الأعشاب الطفيلية الخطوة الأولى نحو مكافحة والحد من أضرار هذا العشب الضار. ولذلك ، تم في هذه الدراسة إستخدام الواسمات الجزيئية كتقنيات فعالة وسريعة للتعرف على أكثر أنواع الهالوك *Orobanche* الرئيسية في مصر. وقد تم فى هذه الدراسة إستخدام ستة عشر بادئ للـ SRAP و تسعة بادئات خاصة بالـ ISSR لتقييم التنوع الوراثي بين ثلاثة أنواع من أهم عائلات ا لـ *Orobanche* وقد أظهرت نتائج المعلمات الجزيئية SRAP و ISSR ، أنه تم ظهور ١٣٢ أليل مختلف من ١٧٠ أليل خاص بالـ SRAP بنسبة تباين وراثى (٧٧.٦%) و ٨٠ أليل من ١٠٦ أليل خاص بالـ ISSR بنسبة تباين وراثى (٧٥.٤ %) ، بين أنواع Orobanche المختبرة. تميزت أنواع *Orobanche* بـ ٣٢ واسم خاصة بالنمط الوراثي منها ٢٠ لـ SRAP و ١٢ لـ ISSR والتي يمكن اعتبارها واسمات مفيدة لأنواع *Orobanche* وقد قسم تحليل مجموعة UPGMA أنواع الهالوك الثلاثة إلى مجموعتين رئيسيتين، الأولى تحتوى على نوعين *O. ramosa* و *O. aegyptiaca* بينما المجموعة الثانية شملت فقط *O. crenata*. يشير هذا التقسيم إلى أن الأنواع المختبرة لها أصول وراثية مشتركة. وبالتالى فقد اوضحت النتائج المتحصل عليها فى هذه الدراسة أنه يمكن استخدام المعلمات الجزيئية المعتمدة على SRAP لتوصيف أنواع الهالوك المستخدمة فى هذه الدراسة باعتبارها أكثر موثوقية وقوة من المعلمات الجزيئية الأخرى المستخدمة. علاوة على ذلك ، يعتبر دراسة التباين لأنواع الهالوكأمرًا بالغ الأهمية عند محاولة تطوير وسائل مكافحة الحشائش.