Bulletin of the National Research Centre

RESEARCH Open Access

Detection of genetic variations in *orobanche crenata* using inter simple sequence repeat (ISSR) markers

M. M. F. Abdalla[1], Heba A. M. A. Saleh[2] and M. A. Khater[3*]

Abstract

Background: This recent investigation aimed to detect and characterize the genetic variabilities and genetic similarity in *Orobanche crenata* collected from naturally *Orobanche*-infested field at Gemmeiza Research Station, Agriculture Research Center (ARC), Egypt. Five different ISSR primers were used in this study.

Results: Obtained results showed that a huge genetic variation has been detected by these ISSR primers; moreover, there were clear relationship/genetic similarity between and within all collected *O. crenata* parasite plants. However, the results revealed that there were high relationships where similarity matrix ranged from 0.837 (the highest) between R4 and R17 followed by 0.831 for R4/R25 and in the end, the lowest one was R1/R29 similarity matrix 0.655.

Conclusions: It was noticed that the relationship and similarity did not reach 100% but the maximum was 83.7% (there were no identical plants); therefore, each *Orobanche* plant in this study was different genotype than neighboring plant within location.

Keywords: Faba bean, *Orobanche crenata*, ISSR markers, Genetic diversity, Genetic variability and similarity

Introduction

Faba bean (*Vicia faba* L.) is an important pulse crop grown worldwide as a source of protein both for human food and animal feed. However, its cultivation is strongly hampered in Mediterranean and Middle East farming systems by the parasitism of broomrape causing important yield losses (Rubiales et al. 2006; Joel et al. 2007; Parker 2009). Legumes are parasitized mainly by two different species of broomrapes, namely, crenata broomrape (*Orobanche crenata* Forsk) and foetida broomrape (*Orobanche foetida* Poir). *O. crenata* has threatened the legume cultivation in the Mediterranean Basin and Middle East crops since antiquity. On the contrary, *O. foetida* has been reported to damage faba bean in Tunisia only (Kharrat et al. 1992 and Diego Rubiales 2014).

Orobanche spp. seeds germinate in response to chemical signals exuded from host roots (Fernández-Aparicio et al., 2009, 2011). Subsequently, the seedling of the parasite develops into an appressorium, a specialized structure that penetrates the host root and then into a haustorium, which forms a connection between the host vascular tissue and the parasite. Then, the parasite develops into a tubercle, a bulbous structure from which a shoot arises to emerge from the soil to flower and set seeds (Joel et al. 2007).

Legume breeding for broomrape resistance is difficult considering the scarce and complex nature of resistance in legumes in general (Rubiales et al. 2006). This contrasts with the success experienced in other crops such as sunflower (*Helianthus annuus* L.), in which single genes governing resistance against *Orobanche cumana* Wallr. have been identified and exploited (Fernández-Martínez et al. 2008). This limitation has made selection

* Correspondence: mahmoudkhater2000@gmail.com
[3]Botany Department, National Research Centre, Giza, Egypt
Full list of author information is available at the end of the article

more difficult and slowed down the legume breeding programs.

Recent investigations supported by molecular taxonomy analyses have resulted in re-definition of *Orobanchaceae* family. According to this classification, *Orobanchaceae* consists of 89 genera, containing 2061 species. On the Balkans, the family *Orobanchaceae* is represented by 3 *Orobanche* which includes 25 species: three of which are present in Egypt (*crenata*, *aegyptiaca*, and *minor*), and 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 around 100 million farmers (Gevezova et al. 2012).

The genetic nature of broomrape resistance/ tolerance is not clear at 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 are known to farmers in Egypt under the commercial names "Giza"*. An acceptable level of resistance was found in Vf1071, an inbred line selected from cv. Giza 402 in Southern Spain. This line has been used in breeding programs to develop the well-adapted, high yielding cv. Baraca (Gnanasambandam 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).

Table 1 ISSR primer sequences used for *Orobanche crenata* fingerprint

	Primer code	Primer sequence (5' → 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

ISSR markers are suitable for investigating genetic diversity among *O. aegyptiaca* genetic groups and able to discriminate between individuals (Abedi et al. 2014). These markers have several benefits over other techniques: first, it is known to be able to discriminate between closely related genotypes (Fang and Roose 1997 and Hodkinson et al. 2002) and second, it can detect polymorphisms without any previous knowledge of the crop's DNA sequence. ISSR markers are also quick and easy to handle and more informative for the evaluation of genetic diversity (Korbin et al. 2002, Rakoczy-Trojanowska et al. 2004 ,and Abdalla et al. 2016).

So, this study aimed to distinguish and determine the differences and similarity among *O. crenata* plants (spikes) in infested field using ISSR molecular markers.

*Agric. Research Center (ARC) and "Cairo" (Cairo University).

Materials and methods

Plant material

Thirty plants from *O. crenata* Forsk were individually collected from naturally *Orobanche*-infested field at

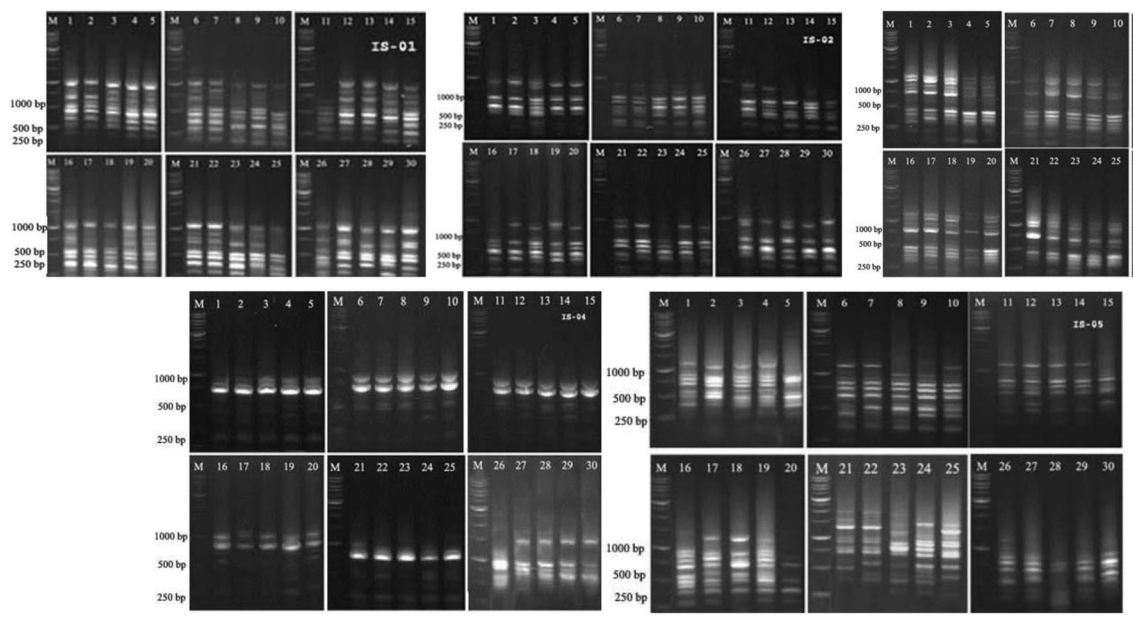

Fig. 1 ISSR profile of sample genotypes of *Orobanche crenata* generated by five ISSR primers. M, DNA standard marker

Gemmeiza Research Station, ARC, Egypt, during the growing season of 2017/2018 and prepared to molecular fingerprint using ISSR technique. However, in this study, five ISSR primers (Table 1) were used to study the similarity and the differences among *O. crenata* plants (spikes).

DNA extraction and ISSR amplification

Total DNA was extracted from 1 g of shoot tip of *O. crenata* spikes using Biospin plant genomic DNA extraction kit (Bio Basic Inc. Kit Leading Supplier and Manufactures of Life Science Products and services, Canada). One percent of agarose gel electrophoresis was used to check DNA quality.

Polymerase chain reaction (PCR) was carried out in a volume of 15 μl volume containing DNA (40 ng), Master Mix (Gene Direx one PCR TM), template DNA, and primer (five different ISSR primers were used) (Table 1).

The PCR mixture was subjected to 35 cycles in PCR with intial denaturation 94 °C for 30 s and annealing 52 °C for 45 s followed by a final extension step at 72 °C for 7 min and store 4 °C. Amplified products were stained with ethidium bromide and electrophoresed in a horizontal gel electrophoresis unit using 1.5% agarose gel (Maniatis et al. 1982).

Each experiment was repeated twice with each primer, and those primers which gave reproducible fingerprints were considered for data analysis.

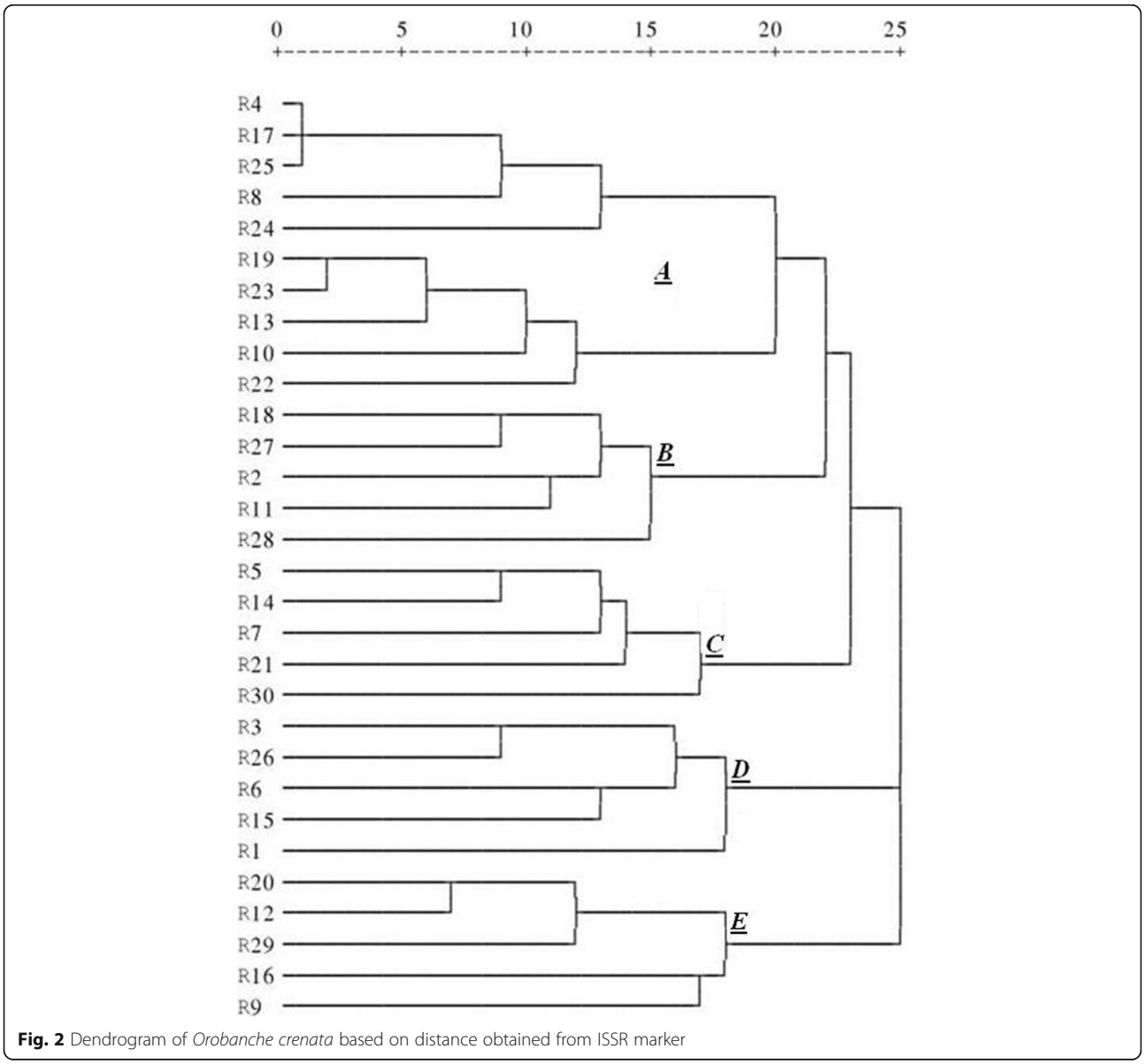

Fig. 2 Dendrogram of *Orobanche crenata* based on distance obtained from ISSR marker

Statistical analysis

A cluster analysis based on the similarity matrix was performed using the neighbor-joining method (Satiu and Nei 1987).

Genetic similarity

Genetic similarity matrix among all *Orobanche* samples 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). All measurements have been made using the NTSYS-pc version 2.1 (Rohlf 2010).

Results

The significant differences among selected *Orobanche* samples induced using the five ISSR primers were clearly shown and summarized in (Fig. 1). However, multiple bands varied in their molecular weight were detected in all tested plants using these different primers. Moreover, UPGMA dendrogram shows genetic relationship between all collected *Orobanche* plants using ISSR markers (Table 1). However, there were clear relationships or genetic similarity between and within all selected *Orobanche*; otherwise, a huge genetic variation has been detected by these ISSR primers.

Similarity and relationship between all tested *Orobanche* plants are shown in Fig. 1 and dendrogram (Fig. 2), and the comparison revealed that there were high relationship where similarity matrix ranged from 0.837 (the highest) between R4 and R17 followed by 0.831 for R4/R25 and at the end, the lowest one was R1/R29 similarity matrix 0.655 (Fig. 2).

The dendrogram (Fig. 2) based on neighbor joining method clearly showed that there are five major clusters (A, B, C, D, and E). The cluster (A) has 10 samples that distributed in two sub clusters; each one has 5 samples. Moreover, the highest similarity matrix (0.837) was recorded between R4 and R17 followed by R4/R25 in the first sub cluster and 0.824 between R19 and R23 in the second sub cluster. However, remaining clusters (B, C, and D), every cluster has the same numbers of samples (5), and similarity ratio differed between their individuals and between different clusters (Fig. 2). Therefore, each *Orobanche* plant in this study is considered as different genotype than neighboring plant within location.

Table 2 and Fig. 3 illustrated the genetic similarity or genetic relationship among all collected *Orobanche* plants in the same plot. However, it is noticed that there were no any identical plants; in other means, every plant has a unique genotype. However, there were many relationships between all samples and ratio of similarity scored as a percentage (0–100%) (Table 2). Moreover, these relationships or similarity ratio differed from one to another among samples, and these ratios reached more over than 80%, where as it recorded its maximum (83.7%) between plants R4 and R17, followed by (83.1%) between R4 and R25 (Table 2 and Fig. 3) which these plants consider the most related plants or the less distant plants in their genetic material. On the other hand, these ratios were gradually decreased among plants and reached the lowest (65.5%) between R1 and R29 which consider the more distant plants in this study.

Table 2 Similarity matrix (%) among collected *Orobanche crenata* samples using ISSR marker analysis

	1	2	Similarity %
1	**R4**	**R17**	83.7
2	**R4**	**R25**	83.1
3	**R19**	**R23**	82.4
4	**R13**	**R19**	79.5
5	**R20**	**R12**	79.1
6	**R8**	**R4**	77.8
7	**R5**	**R14**	77.4
8	**R18**	**R27**	77.4
9	**R3**	**R26**	77.4
10	**R10**	**R13**	77.1
11	**R2**	**R11**	76.2
12	**R10**	**R22**	75.5
13	**R29**	**R20**	75.3
14	**R5**	**R7**	74.9
15	**R2**	**R18**	74.7
16	**R24**	**R8**	74.6
17	**R6**	**R15**	74.5
18	**R5**	**R21**	73.6
19	**R28**	**R2**	72.9
20	**R6**	**R3**	72.6
21	**R16**	**R9**	72.0
22	**R30**	**R5**	71.7
23	**R29**	**R16**	71.3
24	**R1**	**R6**	71.0
25	**R24**	**R10**	69.5
26	**R28**	**R24**	68.4
27	**R28**	**R30**	67.4
28	**R1**	**R28**	66.2
29	**R1**	**R29**	65.5

Discussions

In this, study there were many plants closely related in its genotype to more than one plant with variable similarity ratios, for example, plant R4 was closely related to both R17, R25, and R8 with similarity ratio of 83.7, 83.1, and 77.8%, respectively. Furthermore, plant R1 was more similar to

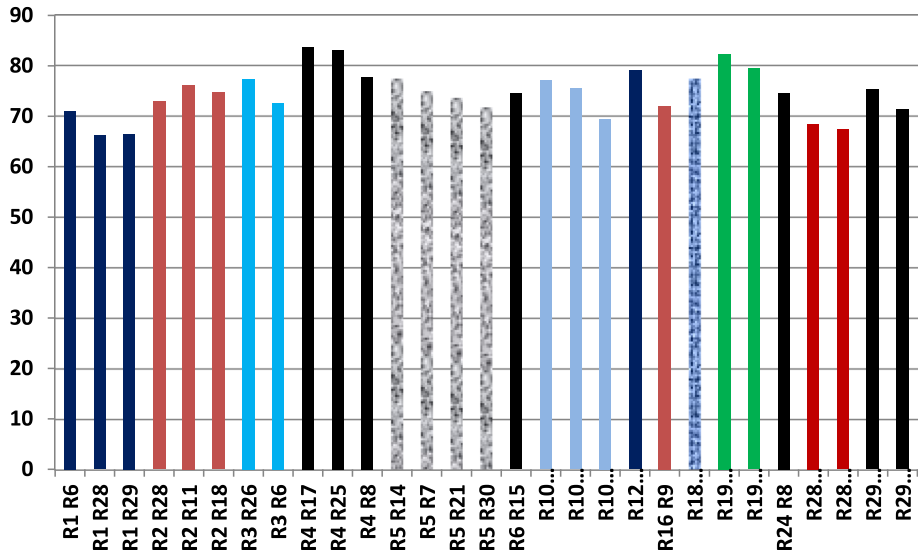

Fig. 3 Histogram of similarity (%) between/within collected *Orobanche crenata* samples

both R6, R28, and R29 with similarity ratio of 71.0, 66.2, and 65.5%, respectively; otherwise, these plants are considered the more distant plants or the less similar plants (R1R29 followed by R1R28) (Table 2 and Fig. 3).

Abedi et al. (2014) 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 30 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. Moreover, Abdalla et al. (2016) determined the genetic diversity of *Orobanche* in two locations (Giza and Sids). Molecular variance indicated greater variation within locations (97%) than between locations (3%); ISSR markers produced fragments covered 218 to 980 bp of the total *Orobanche* genome. The authors found cluster analysis which divided 96 *Orobanche* samples into five sub clusters and concluded that there was wide genetic variation among *O. 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*.

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 appears 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 detected 106 horizontally transferred genes (HTG genes), only in the genomes of the two obligate parasites (22 and 84 for *Oro. minor* and *Ae. indica*, respectively), whereas none in the three facultative parasites. Moreover, they found that almost all HGT genes retained introns at the same locations as their homologs in potential host species, indicating a crucial role of DNA-mediated gene transfer, rather than mRNA mediated retro transfer. Furthermore, some of the HGT genes might have transferred simultaneously because they located very closely in the host reference genome, indicating that the length of transferred DNA could exceed 100 kb. They confirmed that almost all introns are spliced in the genome of the parasite species, and that about half HGT genes do not have any missense mutations or frame shift-causing indels, suggesting that some HGT genes may be still functional.

Conclusion

From previous results, it noticed that the relationship and similarity did not reach 100% but the maximum was

83.7%, and this indicated that every *Orobanche* plant has its unique genotype which was partially similar to other plants in the same plot, and there are no any identical plants.

Authors' contributions

Abdalla M. M. F. and Heba A.M.A.Saleh performed the field experiments. M .A. Khater performed the analysis with the molecular markers. All authors read and approved the final manuscript.

Funding

This study was funded by the authors (Abdalla M. M. F. Heba A. M. A. Saleh and M .A. Khater).

Availability of data and materials

The authors declare that the experimental data and material are available.

Ethics approval and consent to participate

The authors declare that the work is ethically approved, and cssonsent to participate was obtained.

Consent for publication

The authors declare that the work has been consented for publication.

Competing interests

The authors declare that they have no competing interests.

Author details

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

Received: 26 March 2020 Accepted: 29 July 2020
Published online: 24 August 2020

References

Abdalla MMF, Shafik MM, Attia SM, Ghannam HA (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

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

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

Fernández-Aparicio M, Flores F, Rubiales D (2009) Recognition of root exudates by seeds of broomrape (Orobanche and Phelipanche) species. Annals Botany 103:423–431

Fernández-Aparicio M, Yoneyama K, Rubiales D (2011) The role of strigolactones in host specificity of Orobanche and Phelipanche seed germination. Seed Science Research 21:55–61

Fernández-Martínez JM, Domínguez J, PérezVich B, Velasco L (2008) Update on breeding for resistance to sunflower broomrape. Helia 31:73–84

George WB (1960) Etiology and Epiphytology of Victoria blight of Oats. Iowa State University

Gevezova M, Dekalska T, Stoyanov K, Heisteva T, Kostov K, Batchvarova R, Denev I (2012) Recent advance in broomrapes research. Journal of Bioscience & Biotechnolngy. 1(2):91–105

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

Hodkinson, TR, MW. Chase and SA. Renvoize (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

Joel DM, Y. Hershenhorn, H. Eizenberg, R. Aly, G. Ejeta, PJ. Rich, JK. Ransom, J. Sauerborn and D. Rubiales, (2007). Biology and management of weedy root parasites. In J Janick, ed, Horticultural Reviews, Vol 33. John Wiley & Sons, Hoboken, NJ, pp 267–349.

Kado T, Innan H (2018) Horizontal gene transfer in five parasite plant species in Orobanchaceae. Genome Biol. Evol. 10(12):3196–3210

Kharrat M, Halila MH, Linke KH, Haddar T (1992) First report of Orobanche foetida Poiret on faba bean in Tunisia. FABIS Newsletter 30:46–47

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

Maniatis, T., E.F.Frictsch and J.Sambrook, (1982). Molecular cloning.A laboratory manual. Cold Spring Habor Laboratory Publisher, New York.

Parker C (2009) Observations on the current status of Orobanche and Striga problems worldwide. Pest Management Science 65:453–459

Rakoczy-Trojanowska M and H. Bolibok, (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.

Rubiales D, A. Pérez-de-Luque, M. Fernández-Aparicio, JC. Sillero, B. Román, M. Kharrat, S. Khalil, DM. Joel and C. Riches, (2006). Screening techniques and sources of resistance against parasitic weeds in grain legumes. Euphytica 147 187–199.

Rubiales D (2014) Legume breeding for broomrape resistance. Czech J. Genet. Plant Breed. 50(2):144–150

Satiu N, Nei M (1987) The neighbor joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 4(4):406–425

Tomar, R.S., M.V. Parakhia, V.M. Rathod, , J.R. Thakkar and B.A. Golakiya, (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.

Publisher's Note