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Analysis of Genetic Diversity of *Orobanche foetida* Population Parasitizing Crops Legume

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Abstract: Broomrape (*Orobanche foetida* Poir.) is an holoparasite that attacks crop legumes in Tunisia mainly faba bean which limits seriously crop cultivation and causes severe damage in several important regions of the North-West of the country. The use of molecular markers may give good appreciation of genetic diversity in parasite populations. For this purpose, 35 specimens of *O. foetida* representing five populations collected respectively on *Vicia faba*, *Cicer arietinum*, *Medicago scutellata*, *Vicia sativa* and *Vicia narbonensis* were studied. Analysis of the genetic variability was assessed through the use of 10 RAPD markers. Among the markers used, 7 provided good electrophoretic profiles therefore they were selected for the analysis of genetic diversity of the broomrape samples studied. A total of 75 alleles were detected at the seven RAPD regions with an average of 10.71 alleles per locus. The dendrogram obtained using the Dice coefficient allowed us to distinguish unambiguously all the specimens of *O. foetida* studied except 2 which belong to the same parasite population infesting common vetch (*Vicia sativa*). Significant genetic differentiation among *O. foetida* populations was detected; diversity was attributed to differences among individuals within populations and among populations. The need of availability of good markers which could give a higher degree of resolution for discriminating closely related germplasm, could be useful to better understand the structure of populations and to well identify parasitic groups.

Keywords: *Orobanche foetida*, Populations, Genetic diversity, RAPD markers, legumes, Tunisia.

I. Introduction

The broomrapes (*Orobanche* spp.) are parasitic plants devoid of chlorophyll. They parasitize the roots of some plant species and are completely dependent on their hosts causing extensive damage to crops. *Orobanche* species reproduce by seed. They belong to the angiosperms section, to the class of dicotyledons, order of Lamiales, family of Orobanchaceae and genus *Orobanche*. There are slightly more than 200 species of broomrape worldwide. *Orobanche* is often distinguished by its host. Several species of broomrape parasitize many crops with important agro-economic interest. Indeed, the damage and losses caused by this parasite can be very important and even reach 100% [1]. The most harmful species of broomrape are: *O. crenata*, *O. foetida*, *O. aegyptiaca*, *O. minor*, *O. ramosa* and *O. cernua* [2]. Broomrapes were found mainly in temperate regions. They extend south-west Asia to other Mediterranean climate regions such as Western Australia and California. However, the center of distribution is essentially the Mediterranean region. In Tunisia, the three species *O. foetida*, *O. crenata* and recently *O. cumana* were reported causing severe damages on many cultivated crops [1]; [2]. Both *O. foetida* and *O. crenata* are reported to be a serious problem for grain legume crops causing important and significant yield losses [1].

Comparative studies of broomrape biotypes in their natural habitats and in cultivated fields would be very useful to clarify the evolution of pathogenicity of this parasite [4]. Indeed, the study of the variability within and among broomrape populations could be helpful in preventing the development of new races and help researchers to better establish their breeding programs. Therefore, it is important to study the genetic diversity of this parasitic plant mainly the specie *O. foetida* which is the most harmful in Tunisia. Indeed, the existence of *O. foetida* infecting food legume fields in Tunisia mainly faba bean is of relevant importance because this species only parasitized wild species in other Mediterranean countries except in Morocco where it was reported on cultivated vetch [5]. The study of genetic variability of parasite populations is of great interest to understand the variability among and within them. This kind of studies should help us to find correlation between the most

harmful populations and their level of genetic diversity. Molecular markers such RAPDs, AFLPs and SSRs were successfully used in several works on genetic diversity of *Orobanche* spp. [6; 7; 8; 9; 5; 10; 11].

The present work represents a preliminary study on the genetic diversity of 35 specimens belonging to 5 broomrape populations of *O. foetida* parasitizing 5 legume species from Beja (Tunisia), through the use of 10 RAPDs markers.

II. Plant Material And DNA Extraction

The plant material used consists of 35 specimens of *O. foetida* collected from 5 different parasitic populations in the experimental station of the Regional Centre for Research on Field Crops of Beja (CRRGC) parasitizing 5 species of grain legumes which are faba bean (*Vicia faba*), chickpea (*Cicer arietinum*), the annual Alfalfa (*Medicago scutellata*) the vetch (*Vicia sativa*) and Narbonne vetch (*Vicia narbonensis*). DNA extraction was performed from the flower buds of broomrape populations studied according to the protocol of [11] slightly modified. DNA quality was examined by electrophoresis in 1% agarose and estimated by visual comparison of DNA bands on gel with known concentrations of phage lambda DNA.

2.1, DNA amplification

Ten RAPD primers (Table 1) were used for the molecular characterization of the five broomrape populations. The sequences of these primers are shown in Table 1. Amplification reaction were carried out in a final volume of 20 µl containing 30-50 ng of template DNA, 1 X reaction buffer (20 mM (NH₄) SO₄, 75 Mm Tris-HCl pH 8.8), 1.5 mM MgCl₂, 0.1 mM of dNTPs (50uM of each), 2 µM of primer, and 1 U of Taq DNA polymerase. The PCR amplification were carried out on a thermocycler (MuliGene OPTIMAX) using the following temperature cycles: 1 cycle of 5 min at 94°C, 40 cycles of 1 min at 94°C, 2 min at the annealing temperature 35°C and 2 min at 72°C. The last cycle was followed by a final incubation for 8 min at 72°C and the PCR products were stored at 4°C before analysis. The DNA amplification products were loaded on 8% non-denaturing polyacrilamide sequencing gels in 1X TBE buffer. Gels were run for 3h at 250 V, stained by ethidium bromide and visualized under UV; the fragment sizes were estimated with the 2Kb DNA step Ladder of Promega DNA sizing markers (Fig.1).

Table1: Sequences of the 10 RAPD primers used

Primers	Sequences
OPA07	5 - GAAACGGGTG -3
OPA08	5 - GTGACGTAGG-3
OPB03	5 - CATCCCCTG -3
OPB07	5 - GGTGACGCAG -3
OPB10	5 - CTGCTGGGAC -3
OPE17	5 - CTA CTGCCGT -3
OPF16	5 - GGAGTACTGG -3
OPJ01	5 - CCCCCATAA-3
OPJ13	5 - CCACACTACC -3
OPV09	5 - TGTACCCGTC -3

2.2, Data analysis

To evaluate the information obtained with the 10 RAPDs studied, we calculated the next parameters: The number of alleles per locus (N) was counted from the gel profile analysis. The binary matrix was obtained from the reading of the electrophoretic patterns corresponding to all the RAPDs analyzed. Amplified fragments for each locus were scored as present allele (1) or absent allele (0). The binary matrix was used to calculate the genetic distance between each pair of accession using the coefficient of Dice [12]. A dendrogram was constructed from the genetic distance matrix using the unweighted pair group method average (UPGMA) clustering. Data were analyzed using the NTSYS-pc, version 2.1 program [13].

III. Results And Discussion

3.1, Genetic diversity of RAPD markers

The evaluation of 35 specimens of *O. foetida* belonging to 5 different parasite populations and infesting 5 different host plants of legumes was made by studying 10 RAPDs regions of broomrape genome. From the 10 markers used, 7 (OPA-07, OPB-07, OPB-10, OPE-17, OPF-16, OPJ-01 and OPV-09) showed readable amplifications revealing polymorphism between *O. foetida* samples, while 3 (OPA-08, OPB-03 and CPO-13) were unable to give clear amplification and were discarded from this study. Seven RAPD markers selected for this study have shown a multiallelic profiles and produce from 3 (OPB-10) to 18 alleles (OPJ-01) with a total of 75 alleles and an average of 10.71 alleles per locus (Table 2). The work of [8] on the study of the genetic diversity of two populations of *O. foetida* comprising a total of 35 specimens using RAPDs markers detected between 5 (OPV09) and 30 alleles (OPJ13) with a total of 120 alleles, and an average of 10 alleles per locus,

which coincides with the results that we have found in the present work indicating a high level of polymorphism between *O. foetida* samples studied. The size of the amplified fragments with each RAPD markers varies between 220 bp and 820 bp. The genetic diversity detected using the variability parameters between 7 RAPDs markers allowed us to separate all individuals of broomrape studied except 2 which belong to the same population infesting common vetch (*V. sativa*).

The PD values of different markers inform us about the power of each marker to differentiate among the genotypes studied. The PD values in this work were ranged from 0.62 (OPB-10) to 0.97 (OPJ-01) with an average of 0.73 (Table 2). The most informative locus of this study was OPJ-01, with a PD value of 0.97 and a Ne of 10.93, while the less informative locus was OPB-10, with a PD and Ne values of 0.62 and 1.95, respectively (Table 2). The selection of the three most polymorphic loci which revealed the highest PD and Ne (OPA-07, OPE-17 and OPJ-01) allowed us to distinguish the same specimens discriminated using the 7 RAPD markers. These markers could be considered as good markers to be used in future work on genetic diversity of *O. foetida* and even other species.

Table 2: Parameter of variability calculated for the 7 RAPD markers in 35 specimens of *O. foetida*

Locus	N	Ne	PD
OPA-07	13	8.67	0.93
OPE-17	10	5.53	0.81
OPV-09	11	5.42	0.79
OPB-10	3	1.95	0.62
OPB-07	8	2.53	0.70
OPF-16	12	3.86	0.81
OPJ-01	18	10.93	0.97
Total	75	30.89	5.16
Mean	10.71	4.41	0.73

N : Number of alleles ; Ne : Effectif number of alleles ; PD: Power of discrimination

3.2, Genetic relationships among *O. foetida* specimens studied based on RAPD variation

To elucidate genetic relationships among the 35 specimens of *O. foetida* studied, a dendrogram was performed using UPGMA cluster analysis basing on the similarity matrix calculated using the Dice coefficient [12] over the 7 RAPD loci (Figure 1). Twenty five individual broomrapes were clustered in two main groups, while the rest of specimens were clustered outside of these groups. The specimens sharing a large number of alleles were clustered together within the same group.

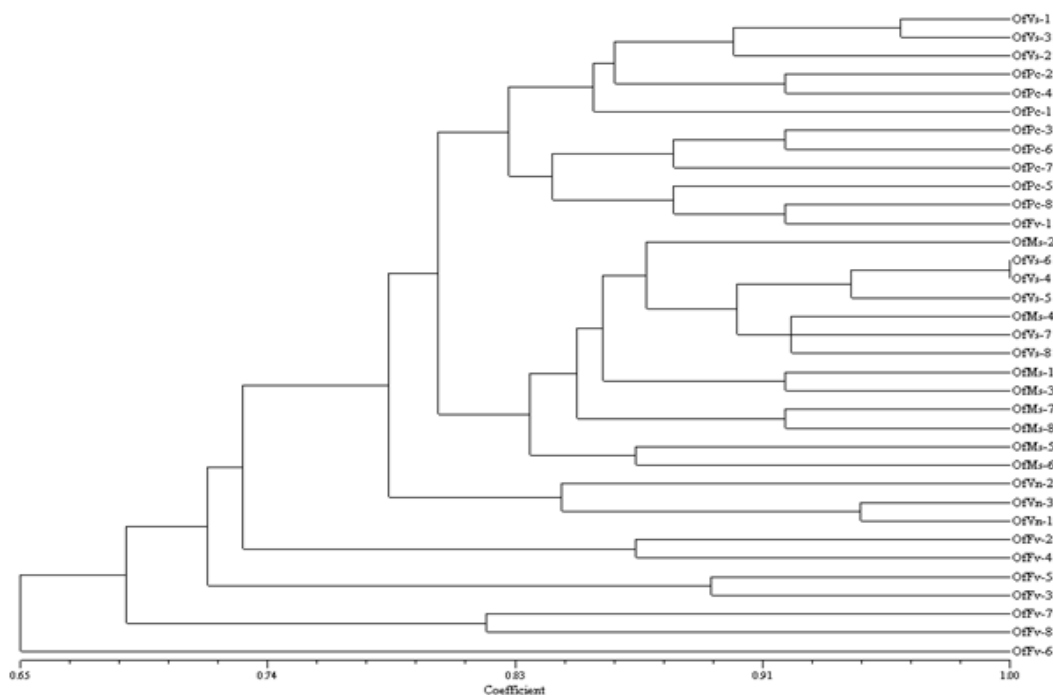


Fig. 1: UPGMA dendrogram of 35 specimens of *O. foetida* from five populations and parasitizing 5 different crop legumes based on their variation at 7 RAPD loci.

According to the dendrogram of Fig. 1, it is possible to identify a first group composed by 3 broomrape specimens belonging to the population parasitizing common vetch (*V. sativa*). Within this group we can find all

orobanche specimens growing on chickpea (*C. arietinum*). It is important to notice that an *O. foetida* specimen from the population parasitizing faba bean (offV-1) is positioned within the same group and appears closely related to the specimen OfPc-8 growing on chickpea with a genetic similarity of 0.92. This result can be explained by the proximity of the two plots of faba bean and chickpea, and probably offV-1 specimen belongs rather to the population parasitizing chickpea. A second group can be observed from the dendrogram and includes 13 broomrape specimens growing on two different crop legume populations, alfalfa (*M. scutellata*) and common vetch (*V.sativa*). In this group all specimens of *O. foetida* growing on alfalfa are situated with 5 specimens belonging to the population that parasite common vetch. This can give us information on the genetic proximity between both populations parasitizing two legume species.

On the dendrogram we can identify the clustering of 3 specimens of broomrape from the population infecting Narbonne vetch (*V. narbonensis*). This result is not surprising since they belong to the same parasitic population which explains a close relationship among them.

It is relevant to notice that the most genetic divergent population was the population collected on faba bean. Indeed, on the Fig. 1 we can observe the clustering of all specimens of this population at a high genetic distance from each other. This high diversity within this population which can results in a high parasitic power should be taken into account. Indeed, it offers for this population the capacity to attack other crops and become a real threat for agriculture.

The study of [8] showed that the UPGMA cluster analysis based on Dice distance matrix detected a clear differentiation among *O. foetida* samples collected on chickpea and those on faba bean, suggesting a host differentiation process. Moreover, the analysis of the genetic variability conducted on five *O. foetida* populations infecting wild and cultivated plants in Morocco using AFLP markers, suggested the existence of genotypic differentiation among populations [4].

Further studies should be conducted using codominant markers such SSR markers. Moreover, an evaluation of parasitic power of the five populations of *O. foetida* studied in this work was already underway; this should offer the possibility to detect correlation between genetic diversity and parasitic power of each population. Indeed, as mentioned [8], a parasite population with a high genetic diversity has the capacity to infect new host overcoming their resistance. Until now, only one work was conducted on the study of host-specialization detected on *O. foetida* samples growing on different cultivated host [8]. This leads as to deeply explore the host differentiation process by assessing different host responses to infection by different *O. foetida* populations. This will be possible by examining the germination rate and percentage of regenerated tubercles of broomrape seeds on different hosts.

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