Theor Appl Genet (1996) 93:367–372

N. Katzir · V. Portnoy · G. Tzuri · M. Castejón-Muñoz
D. M. Joel

Use of random amplified polymorphic DNA (RAPD) markers in the study of the parasitic weed *Orobanche*

Received: 17 December 1995 / Accepted: 26 January 1996

Abstract Despite the tremendous economic impact of broomrapes (*Orobanche* spp.) on agriculture in many countries little is known of the pattern of genetic variation within this group of parasitic weeds. The present paper describes the use of RAPD markers for the study of five *Orobanche* species in agricultural fields in Israel. Pronounced genetic differentiation was found between the species, and RAPD markers were raised for the identification of each of them. Southern-hybridization patterns of RAPD products of the various species were used to confirm the interpretation. The same markers were valid both for broomrapes collected in agricultural fields and for those collected in natural habitats. The validity of the markers found for *O. cumana* and *O. crenata* was confirmed on plants of the same species that were collected in Spain. Parsimony analysis of 86 RAPD characters produced a tree that clearly distinguishes between the five studied *Orobanche* species, separates the two *Orobanche* species belonging to sect. Trionychon from those belonging to sect. Osproleon, and supports the separation of *O. cumana* from *O. cernua* and of *O. aegyptiaca* from *O. ramosa*.

Key words RAPD markers · DNA fingerprinting · *Orobanche* · Broomrape · Parasitic weed

Introduction

Broomrapes (*Orobanche* spp.), angiospermous root-parasites, are amongst the most devastating parasitic weeds, causing extensive damage to vegetables, legumes, sunflower and fodder crops in the Middle-East, southern Europe, Northern Africa and parts of Asia, and currently spreading gradually to new areas in other continents including America and Australia (Parker and Riches 1993). The areas infested are vast and ever growing. The impact of parasitic weeds on the world economy is tremendous, particularly in developing countries where farmers have no alternatives to the few crops they grow for their living (Musselman 1986; Parker and Riches 1993; Joel et al. 1995).

All *Orobanche* species except one that cause acute agricultural problems in the world (Musselman 1986; Parker and Riches 1993) are important weeds in Israel, seriously attacking agricultural crops. These include *O. aegyptiaca* Pers. and *O. ramosa* L. (=*O. mutelii* F.W. Schulz), both belonging to sect. Trionychon Wallr., and *O. cernua* Loefl., *O. cumana* and *O. crenata* Forsk., belonging to sect. Osproleon Wallr. (Feinbrun-Dothan 1978; Joel 1987). Most weedy species of *Orobanche* also grow in native habitats in Israel, together with six additional native species that do not penetrate agricultural fields (Feinbrun-Dothan 1978, 1986). Israel is therefore suitable for conducting genetic, evolutionary and taxonomic studies on this genus of parasitic weeds.

Orobanche taxonomy is a problem for several reasons (Musselman 1994). First, there is an inherent morphological variability within populations of the plant, which is reflected in several aspects of their biology: weediness, chromosome aberrations (e.g. Moreno et al. 1979) and reproductive strategies. Second, their holoparasitism results in a reduced number of characters for taxonomy: they are non-photosynthetic, they have no leaves, and they produce only short abnormal roots. Third, the host plant may influence the morphology of the parasite.

A controversy exists as to the validity of some species. *O. mutelii* F.W. Schultz and *O. ramosa* L. (Chater and Webb 1972; Feinbrun-Dothan 1978) are regarded as a single taxon by Musselman (1986). Further, the distinction between *O. ramosa* L. and *O. aegyptiaca* Pers. in the

Communicated by G. Wenzel

N. Katzir · V. Portnoy · G. Tzuri · D.M. Joel (✉)
Newe-Ya'ar Research Center, Agricultural Research Organization, Haifa 31900, Israel

M. Castejón-Muñoz
Institute of Sustainable Agriculture, Apartado 4084, 14080 Córdoba, Spain

field is sometimes difficult due to the inconsistent characters used in keys. For example, plants of *O. aegyptiaca* of the same maternal origin differ in size and morphology when parasitic on different hosts, some developing into small plants carrying small flowers that very much resemble those of *O. ramosa* (Musselman 1986; Joel, unpublished results).

O. cernua occurs in agricultural fields in two different forms. One attacks the sunflower in eastern Europe and in Mediterranean countries, the other attacks vegetables, mainly in Asian countries (Parker and Riches 1993). Whereas these forms are regarded by some researchers as variants or sub-species of *O. cernua* Loefl. (e.g. Chater and Webb 1972; Feinbrun-Dothan 1978), others (including us) regard them as two separate species: *O. cumana* Wallr. and *O. cernua* Loefl., respectively (Joel 1988), based on both morphological and behavioral characters.

Species identification is thus problematic, though very important for agriculture due to differences in host preference. However, recent discussions concerning *Orobanche* taxonomy and genetics have been published only in proceedings (Moreno et al. 1979; Musselman 1986, 1994; Verkleij et al. 1986, 1991a,b; Castejón-Muñoz et al. 1991; Verkleij and Pieterse 1994).

At least for one species (*O. cumana*) it is suspected that the agricultural populations in Israel have been introduced into the country only recently (Joel 1988). Whereas the agricultural populations of *Orobanche* are of higher economic importance, floras (e.g. Feinbrun-Dothan 1978) are mainly based on plant collections in native, non-agricultural, habitats.

The pattern and distribution of genetic variation within this important genus is virtually unknown. Some important information on species polymorphism has been gained from isozyme analysis of *O. crenata* and *O. cumana*, based on agricultural field populations from Syria, Egypt and Spain (Castejón-Muñoz et al. 1991; Verkleij et al. 1991a,b; Verkleij and Pieterse 1994). There are, however, two main disadvantages of isozyme markers: (1) they may be affected by environmental conditions and different stages of development, and (2) the number of relevant loci is limited and discrimination of different genotypes is not always possible. It appears that mislabelling of clones may be common with this method (Keil and Griffin 1994). In their study of broomrape populations Verkleij et al. (1991b) suggested that differences in environmental conditions might explain the relative isozyme uniformity between Syrian *Orobanche* populations, compared to the greater variability they found between Spanish populations: in the Syrian study the tested plants were grown under greenhouse conditions, whereas in Spain the plants were directly collected in the field.

DNA-based markers have largely overcome these disadvantages and have been applied successfully to discriminate between individual genotypes in a wide range of plant and animal species. The random amplified polymorphic DNA (RAPD) technique (Williams et al. 1990), based on the use of short primers of arbitrary nucleotide sequence in the polymerase chain reaction (PCR), has been shown to be useful for a wide range of applications (Williams et al. 1993), including the DNA fingerprinting of plants (Castiglione et al. 1993; Francisco-Ortega et al. 1993). These studies demonstrate that it is possible to obtain RAPD profiles that are reproducible and unique to different genotypes (Keil and Griffin 1994) and can be used for the estimation of genetic relationships within and between species (Landry et al. 1994; Thorman et al. 1994; Brummer et al. 1995). In contrast to the isozyme markers, the RAPD profiles are not dependent on environmental and developmental factors.

In the present paper we have used RAPD markers to study, for the first time, *Orobanche* species in agricultural fields. The objectives of the study were:
(1) to compare closely related species,
(2) to examine the genetic relation between native and weedy population of *Orobanche* species,
(3) to find RAPD markers for the identification of those *Orobanche* species that are serious weeds in agricultural fields.

Materials and methods

Plant material

Orobanche plants used in this study (Table 1) were either collected from the field as fresh material or harvested in the greenhouse from plants raised from seeds that were collected in the field. In most cases freshly harvested flower buds or young tubercles were stored at −80 °C until used.

DNA preparation

Orobanche genomic DNA was prepared from young flower buds or young (3–5 mm) tubercles using the microprep protocol described by Fulton et al. (1995).

RAPD analysis

RAPD analysis was performed according to Williams et al. (1990). Ten-mer arbitrary primers were obtained from the Nucleic Acid-Protein Service Unit of the University of British Columbia (Vancouver, Canada; primers designated UBC followed by their serial number) or Operon technologies Inc. (Alameda, Calif; primers designated OP followed by their serial number). *Taq* DNA polymerase was obtained mainly from AB (Advanced Biotechnologies Ltd., Surrey, UK) and used at 0.5 units in each sample. The designation of amplification products included both the primer name and the estimated size of the product in bp.

Southern hybridization

Southern hybridization of RAPD gels was performed as described by Sambrook et al. (1989). The probes were prepared by excising the amplification products (OPG6-400, OPG6-660, UBC215-470, UBC215-1400 and UBC250-750) from the relevant lane in 1.4% agarose gel, and purifying them using the Genclean II kit (BIO 101 Inc.). ^{32}P-radiolabelling was performed with the *rad*iprime random primer labelling kit (Amersham life science).

Table 1 *Orobanche* plants used in this study. N = number of sampled populations. Geographical names for Israel after Feinbrun-Dothan (1986)

Species	Origin		N	Host plant
O. aegyptiaca	Israel	Coastal Galilee	3	Tomato, melon
		Acco Plain	2	Tomato
		Esdraelon Plain	2	Tomato, Sunflower
		Lower Jordan Valley	1	Tomato
O. ramosa	Israel	Golan Heights	4	Potato, tomato, poppy
		Coastal Galilee	1	*Oxalis pes-caprae* L.
		Lower Galilee	1	*Stellaria media* Vill.
		Esdraelon Plain	1	*Tropaeolum majus* L.
O. cernua	Israel	Lower Galilee	1	Tomato
		Golan Heights	1	Tomato
		Western Negev	1	Potato
		Coastal Galilee	1	*Othanthus maritimus* Hoffmans. et Link
O. cumana	Israel	Lower Galilee	1	Sunflower
		Esdraelon Plain	1	Sunflower
		Esdraelon Plain	1	Tomato
	Spain	El Coronil (Sevilla)	1	Sunflower
		Cuenca	1	Sunflower
		Huescar (Granada)	1	Sunflower
O. crenata	Israel	Upper Jordan Valley	1	Pea
		Beit Shean Valley	2	Carrot
	Spain	Córdoba	2	Lentil, chickpea
		Coria del rio (Sevilla)	1	Chickpea

Data analysis

The PAUP 3.1.1 program (Swofford 1993) was previously used for parsimony analysis of data obtained from RAPDs of various organisms (Landry et al. 1994; Brummer et al. 1995; Voigt et al. 1995). Using this program we analysed the RAPD data accumulated for the different species of *Orobanche*, based on 86 clear polymorphic bands obtained with ten primers. In all cases we used a heuristic search with MULPARS and TBR branch swapping. Characters were unweighted, and polarity was not specified a priori. Polymorphic RAPD bands were treated as binary (present/absent) characters.

Results

Thirty one ten-mer arbitrary primers were used to amplify DNA of samples from five agriculturally important *Orobanche* species: *O. aegyptiaca*, *O. ramosa*, *O. cernua*, *O. cumana* and *O. crenata*. Two of the eighty six polymorphic amplification products (UBC300-600 and UBC212-300) gave unique clear bands that can be used for the identification of the different sections (sect. Osproleon and sect. Trionychon, respectively) of the genus *Orobanche*. Five other amplification products (UBC250-750, OPG6-400, OPG6-660, UBC215-470 and UBC215-1400) gave unique clear bands useful for the identification of the different species. The diagnostic information obtained from these seven products is summarized in Table 2.

An example of a RAPD pattern obtained with one primer (UBC215) used to amplify three individual plants (grown on different hosts or in different places, see Table 1) of each of the five species is presented in Fig. 1. This primer provided one band (UBC215-470) unique to *O. aegyptiaca* and another band (UBC215-1400) unique to *O. crenata*. In order to improve the visualization and to avoid ambiguity, RAPD patterns obtained with each of the informative primers were blotted and hybridized to the relevant probe (UBC215-1400 or UBC215-470, prepared as described in Materials and methods). As

Table 2 Polymorphic amplification products that give unique clear bands, useful for the identification of the two sections of the genus *Orobanche* and of the agriculturally important species. The table is based on RAPD analysis, and confirmed by using hybridization in all samples tested with UBC250, UBC215 and OPG6

Species	Primer	UBC300	UBC212	UBC250	OPG6	OPG6	UBC215	UBC215
	Bp	600	300	750	400	660	470	1400
Sect. Trionychon Wallr.								
O. aegyptiaca Pers.		−	+	−	+	−	+	−
O. ramosa L.		−	+	+	−	−	−	−
Sect. Osproleon Wallr.								
O. cernua Loefl.		+	−	−	−	−	−	−
O. cumana Wallr		+	−	−	−	+	−	−
O. cernata Forsk		+	−	−	−	−	−	+

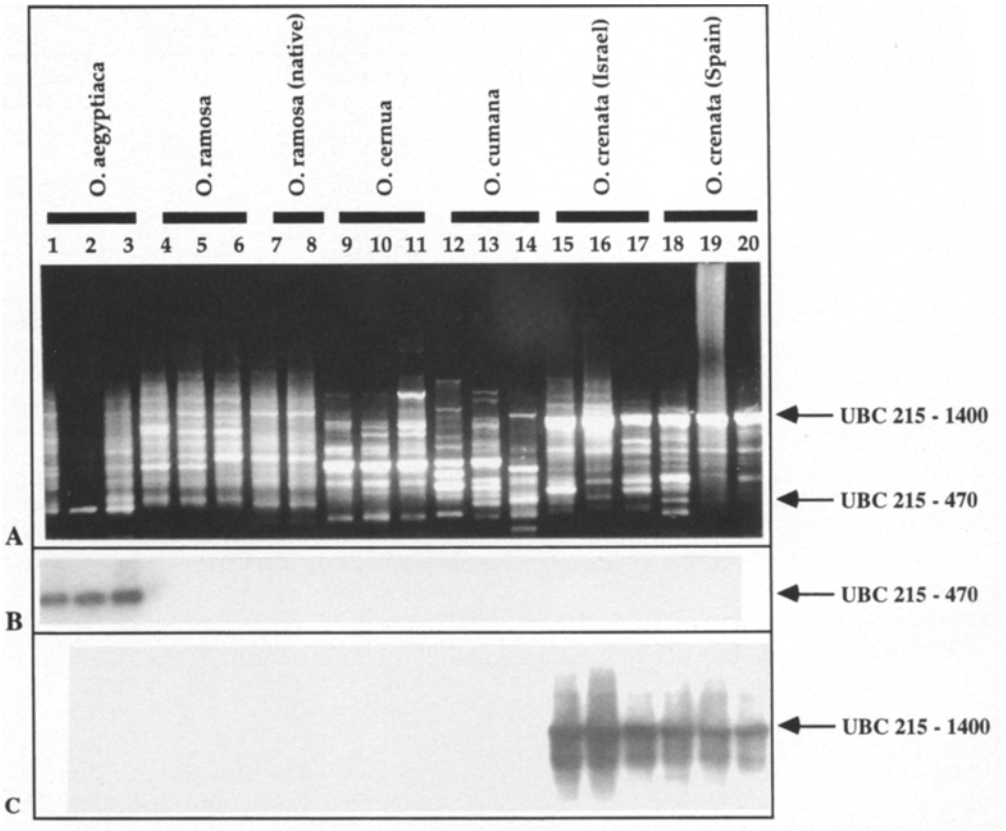


Fig. 1 RAPD pattern, obtained with UBC215, of individual plants of each of the five species of *Orobanche*. The band UBC215-470 is unique to *O. aegyptiaca* and UBC215-1400 is unique to *O. crenata*. Patterns obtained with each of the informative primers were blotted and respectively hybridized to the relevant probe. Samples of *O. crenata* from Spain show the same markers typical of the Israeli samples. *Lanes 7–8* and *9*: broomrapes from natural habitats

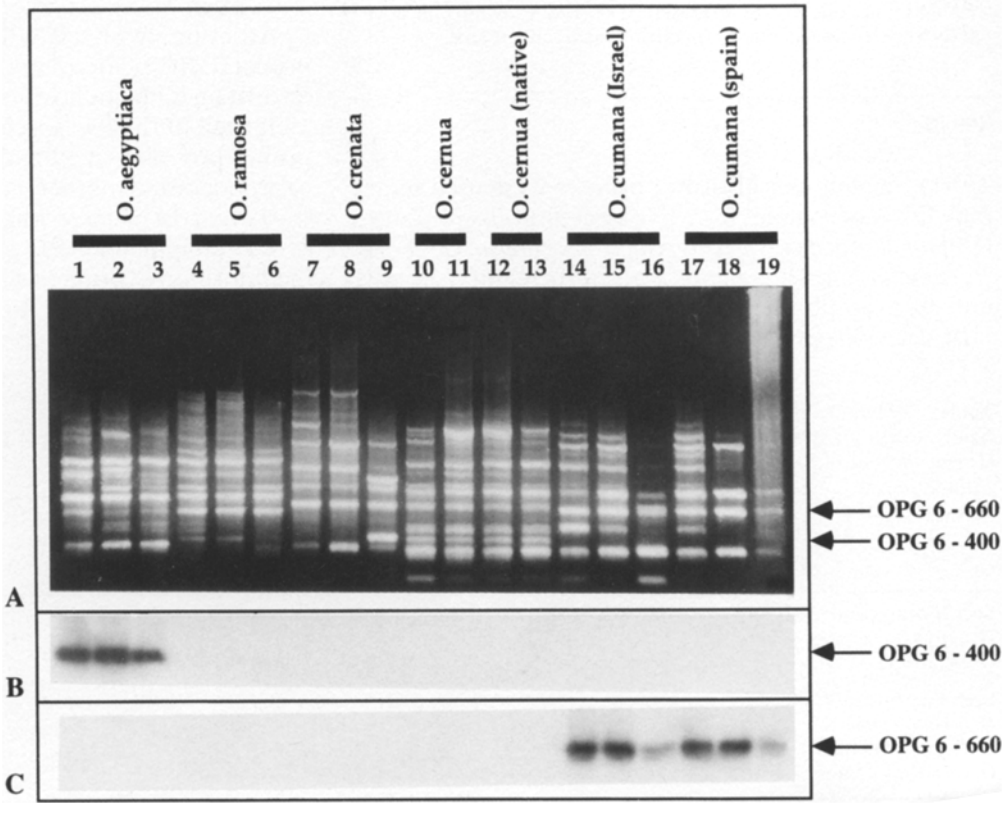


Fig. 2 RAPD pattern, obtained with OPG6, of individual plants of each of the five species of *Orobanche*. The band OPG6-400 is unique to *O. aegyptiaca* and OPG6-660 is unique to *O. cumana*. RAPD patterns obtained with each of the informative primers were blotted and respectively hybridized to the relevant probe. The samples of *O. cumana* from Spain also show the unique bands characteristic of the samples from Israel. *Lanes 6* and *12–13*: broomrapes from natural habitats

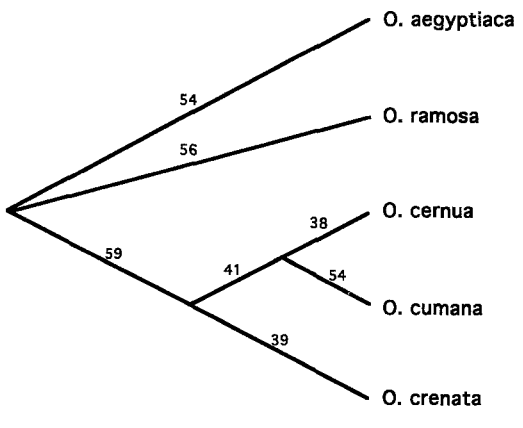
depicted in Fig. 1, the hybridization patterns were clearer and much easier to interpret than the RAPD patterns. Figure 2 summarizes the information provided by the OPG6 primer in a similar sample of plants. OPG6 gave one band (OPG6-400) unique to *O. aegyptiaca* and another band (OPG6-660) unique to *O. cumana.*

To ensure that three representatives of a species were adequate for this type of screening, one of the informative primers (OPG6) was tested on a sample of 24 individuals representing eight different populations of *O. aegyptiaca,* and on 24 more individuals representing four different populations of *O. ramosa,* some of which parasitized various host plants (Table 1). The unique band OPG6-400 was produced by all 24 *O. aegyptiaca* plants but not by any of the *O. ramosa* plants. In a similar manner, the marker UBC215-470 was amplified by all 24 *O. aegyptiaca* individuals but not by any of the *O. ramosa* individuals tested.

O. ramosa callected in agricultural fields in the Golan closely resembles the populations of this species in different native habitats in Coastal Galilee, Lower Galilee, and the Esdraelon Plain (Fig. 1). These populations could easily be distinguished from *O. aegyptiaca* by using the DNA probes that respectively hybridize to each of these species (Table 2). In a similar manner, RAPD patterns of *O. cernua* collected in agricultural fields in the Golan and the Negev closely resembled the RAPD patterns of samples of this species collected in a native habitat in Coastal Galilee (Fig. 2).

Two of the informative primers were also used to amplify DNA of samples obtained from Spain. Three samples of *O. crenata* are presented in Fig. 1, and three samples of *O. cumana* are presented in Fig. 2. In both cases the samples were collected in different locations in Spain, those of *O. crenata* parasitizing two different hosts (Table 1). Interestingly, the unique bands that were found to identify the different species in Israel were also produced by the Spanish plants of the same species.

Fig. 3 Tree based on parsimony (PAUP) analysis of the 86 RAPD characters of five *Orobanche* species. The tree is unrooted. Branch lengths are given above the lines (tree length = 341, CI = 0.619, RI = 0.386)



Parsimony analysis of the 86 RAPD characters produced a single MP (most parsimonious) tree (Fig. 3). This tree separates the two species belonging to sect. Trionychon from the species belonging to sect. Osproleon, discerns *O. aegyptiaca* and *O. ramosa,* distinguishes between *O. crenata* and *O. cernua,* and clearly shows up *O. cumana* as an autonomous entity near *O. cernua.*

Discussion

The results of this study demonstrate the potential of the RAPD analysis of the genus *Orobanche.* The difference between the agriculturally important taxa can also be shown using specific DNA probes for Southern hybridization of the RAPD products. Whereas parallel RAPD bands of a specific primer are only similar in their molecular size, parallel hybridization bands are similar not only in molecular size but also im molecular sequence. The diagnostic information obtained from RAPD products, which is summarized in Table 2, is a valuable tool for the study of *Orobanche* species in agricultural areas. Probes are now available for most relevant species. Using RAPD primers we were able to confirm the separation of species belonging to the section Trionychon from species belonging to the section Osproleon and to characterize problematic species.

O. ramosa and *O. aegyptiaca* are often difficult to separate in the field due to the inconsistent characters used in keys (Musselman 1986). *O. aegyptiaca,* when grown on some hosts develop into stunted plants resembling *O. ramosa* in their morphological characters (unpublished results). RAPD analysis easily overcomes this difficulty, clearly distinguishing between these two taxa. The difference between *O. aegyptiaca* and *O. ramosa* was shown using a variety of primers, and in a more accurate manner using specific DNA probes of each species for Southern hybridization of RAPD products.

In a similar manner, *O. cumana,* which occurs only in agricultural fields, could be easily distinguished from the various population of *O. cernua,* occurring both in agricultural fields and in native habitats in different parts of Israel.

In order to examine the validity of the new RAPD markers in the identification of *Orobanche* species we have tested them also on two *Orobanche* species in Spain, which represents the westernmost site of *Orobanche* distribution, on the other side of the Mediterranean sea. The markers for both *O. cumana* and *O. crenata* respectively hybridized with samples of these species collected in various locations in central and southern Spain. This is the first molecular indication confirming the wide distribution of particular *Orobanche* species around the Mediterranean sea.

It is very likely that *O. cumana,* a specific parasite of sunflower, has developed from a single population of *O. cernua* after sunflowers started to play an economic role in Russia in the nineteenth century (Putt 1978). The occurrence of *O. cumana* in Mediterranean countries

followed the introduction of the sunflower in this area during the twentieth century. The presence of *O. cumana* showing identical diagnostic markers in two distant countries on opposite sides of the Mediterranean, supports the hypothesis that all its populations are distinct from those of the widely distributed species *O. cernua*. The inter-relationship between these two species is not yet clear and further research is needed in order to finally decide on whether their separation as distinct species is justified in taxonomic terms. Nevertheless, from the agricultural point of view the distinction between them is highly relevant because they attack different crops, and accordingly should be identified for the benefit of the farmers.

Parsimony analysis of the data accumulated for the different species, based on 86 polymorphic RAPD bands, also provided a clear separation between the species (Fig. 4), similar to the known classical systematic division of this genus. *O. aegyptiaca* and *O. ramosa* were separated from the three other species, confirming their inclusion in a separate section (sect. Trionychon), while *O. cernua, O. cumana* and *O. crenata* were easily identified as belonging to another section (sect. Osproleon). *O. cernua* and *O. cumana* show a close relationship, though clearly separated from each other.

Our results are also relevant for the study of the native populations of *Orobanche*. Both *O. cernua* and *O. ramosa* which grow in natural habitats have the same diagnostic RAPD bands as their counterparts in agricultural fields.

In conclusion, our findings indicate that DNA analysis using RAPD markers can serve as a simple and reliable tool in the taxonomic analysis of the genus *Orobanche* as well as serving as a diagnostic tool.

Acknowledgments This research is supported by a grant No. 138-0282 from the Chief Scientist of the Israel Ministry of Agriculture and Development. Contribution from the Agricultural Research Organi-zation, Newe-Ya'ar Research Center, No. 138-1690-E, 1995 series.

References

Brummer EC, Bouton JH, Kochert G (1995) Analysis of annual *Medicago* species using RAPD markers. Genome 38:362–367

Castejón-Muñoz M, Suso MJ, Romero-Munoz F, Garcia-Torres L (1991) Isoenzymatic study of broomrape (*Orobanche cernua*) populations infecting sunflower. In: Ransom JK, Musselman LJ, Worsham AD, Parker C (eds) Proc 5th Int Symp Parasitic Weeds. CIMMYT, Nairobi, pp 313–319

Castiglione S, Wang G, Damiani G, Bandi C, Bisoffi S, Sala F (1993) RAPD fingerprints for identification and for taxonomic studies of elite poplar (*Populus* spp.) clones. Theor Appl Genet 87:54–59

Chater AO, Webb DA (1972) *Orobanche*. In: Tutin TG et al. (eds) Flora Europaea. Cambridge University Press, pp 286–293

Feinbrun-Dothan N (1978) Flora Palaestina. Vol. 3. Israel Acad Sci Humanities, Jerusalem

Feinbrun-Dothan N (1986) Flora Palaestina. Vol. 4. Israel Acad Sci Humanities, Jerusalem

Francisko-Ortega J, Newbury HJ, Ford-Lloyd BV (1993) Numerical analysis of RAPD data highlight the origin of cultivated tagasate (*Chamaecytisus proliferus* ssp. *Palmensis*) in the Canary Islands. Theor Appl Genet 87:264–270

Fulton TM, Chunwongse J, Tanksley SD (1995) Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol Biol Rep 13:207–209

Joel DM (1987) Identification of *Orobanche* seeds. In: Weber HC, Forstreuter W (eds) Parasitic flowering plants. Proc 4th ISPFP, Marburg, Germany, pp 437–444

Joel DM (1988) *Orobanche cumana* – a new adventive weed in Israel. Phytoparasitica 16:375

Joel DM, Steffens JC, Matthews DE (1995) Germination of weedy root parasites. In: Kigel J, Galili G (eds) Seed development and germination. Marcel Dekker, New York, pp 567–597

Keil M, Griffin AR (1994) Use of random amplified polymorphic DNA (RAPD) markers in the discrimination and verification of genotypes in *Eucalyptus*.. Theor Appl Genet 89:422–450

Landry BS, Li RQ, Cheung WY, Granger RL (1994) Phylogeny analysis of 25 apple rootstocks using RAPD markers and tactical gene tagging. Theor Appl Genet 89:847–852

Moreno MT, Cubero JI, Martin A (1979) Meiotic behaviour of *Orobanche crenata*. In: Musselman LJ, Worsham AD, Eplee RE (eds) Proc 2nd Symp Parasitic Weeds. Raleigh, North Carolina, pp 73–78

Musselman LJ (1986) Taxonomy of *Orobanche*. In: ter Borg SJ [ed] Biology and control of *Orobanche*. LH/VPO, Wageningen, The Netherlands, pp 2–10

Musselman LJ (1994) Taxonomy and spread of *Orobanche*. In: Pieterse AH, Verkleij JAC, ter Borg SJ (eds) Biology and management of *Orobanche*. Royal Tropical Inst, Amsterdam, pp 27–35

Parker C, Riches C (1993) Parasitic weeds of the world: biology and control. CAB International, Wallingford, UK

Putt ED (1978) Sunflower: history and present world status. In: Carter JF (ed) Sunflower science and technology. Am Soc Agron, Madison, 19:4–29

Sambrook J, Fritch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn., vols 1–3. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Swofford DL (1993) PAUP: phylogenetic analysis using parsimony, version 3.1. Illinois Natural History Survey, Champaign, Illinois

Thorman CE, Ferreira ME, Camargo LEA, Tivang JG, Osborn TC (1994) Comparison of RFLP and RAPD markers in estimating genetic relationships within and among cruciferous species. Theor Apple Genet 88:973–980

Verkleji JAC, Pieterse AH (1994) Genetic variability of *Orobanche* (broomrape) and *Striga* (witchweed) in relation to host specificity. In: Pieterse AH, Verkleij JAC, ter Borg SJ (eds) Biology and management of *Orobanche*. Royal Tropical Inst, Amsterdam, pp 67–79

Verkleij JAC, Janssen J, Pieterse AH (1986) A perliminary study of isozyme variation in *Orobanche crenata* and *Orobanche aegyptiaca* from Syria. In: ter Borg SJ (ed) Biology and control of *Orobanche*. LH/VPO, Wageningen, The Netherlands, pp 154–159

Verkleij JAC, Egbers WS, Pieterse AH (1991a) Allozyme variations in populations of *Orobanche crenata* from Syria. In: Wegmann C, Musselman LJ (eds) Progress in *Orobanche* research. Proc Int Wkshp on *Orobanche* Research. Obermarchtal, Germany, pp 304–315

Verkleij JAC, Lopez-Grandos F, Egbers WS, Garcia-Torres L, Pieterse AH (1991b) Genetic variability in populations of *Orobanche crenata* in Spain. In: Ransom JK, Musselman LJ, Worsham AD, Parker C (eds) Proc 5th Int Symp Parasitic Weeds. CIMMYT, Nairobi, pp 462–469

Voigt K, Schleier S, Brückner B (1995) Genetic variability in *Gibberella fujikuroi* and some related species of the genus *Fusarium* based on random amplification of polymorphic DNA (RAPD). Curr Genet 27:528–535

Williams JGK, Kubelic AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Williams JGK, Hanafey MK, Rafalski JA, Tingey SV (1993) Genetic analysis using random amplified polymorphic DNA markers. Methods Enzymol 218:704–740