Variation Among and Within Populations of the Parasitic Weed *Orobanche crenata* from Spain and Israel Revealed by Inter Simple Sequence Repeat Markers

Belén Román, Zlatko Satovic, Diego Rubiales, Ana M. Torres, José Ignacio Cubero, Nurit Katzir, and Daniel M. Joel

First and fourth authors: Departamento de Mejora y Agronomía CIFA -Alameda del Obispo, Apdo 3092, 14080 Córdoba, Spain; second author: Faculty of Agriculture, Department of Seed Science and Technology, Svetosimunska 25, 10000 Zagreb, Croatia; third author: CSIC-Instituto de Agricultura Sostenible, Apdo. 4084, 14080 Córdoba, Spain; fifth author: Departamento de Genética, ETSIAM-UCO, Apdo 3048, 14080 Córdoba, Spain; sixth author: The Genetic Lab, Newe-Ya'ar Research Center, ARO, P.O. Box 1021, Ramat-Yishay 30095, Israel; and seventh author: Department of Weed Research, Newe-Ya'ar Research Center, ARO, P.O. Box 1021, Ramat-Yishay 30095, Israel.

Accepted for publication 23 July 2002.

ABSTRACT

Román, B., Satovic, Z., Rubiales, D., Torres, A. M., Cubero, J. I., Katzir, N., and Joel, D. M. 2002. Variation among and within populations of the parasitic weed *Orobanche crenata* from Spain and Israel revealed by inter simple sequence repeat markers. *Phytopathology* 92:1262-1266.

The patterns of genetic variation among *Orobanche crenata* populations from Spain and Israel were studied using radiolabeled inter simple sequence repeat amplification products that were separated in sequencing polyacrylamide gels. The analysis of molecular variance indicated that most of the genetic diversity was attributable to differences among indi-

viduals within a population although significant divergences were found between regions. The Jaccard's similarity matrix was analyzed by un-weighted pair-group method with arithmetic average and the resultant dendrogram clearly divided six populations by region, with the Spanish populations being more similar to each other than the Israeli populations. These results are consistent with the predominantly allogamous behavior of *O. crenata* and the extremely efficient dispersal of its seeds.

Additional keywords: homogeneity of molecular variance, *Orobanche* resistance, population structure.

Broomrapes (*Orobanche* spp.) are parasitic weeds that have a devastating effect on many important crops (2). *Orobanche* spp. are holoparasites, completely lacking chlorophyll, causing significant reductions in yield and quality. The seedlings attach to the root system of the host plant, establish a connection with the vascular system of the host via a specialized organ (haustorium), and deprive the host of water, mineral nutrients, and metabolites (18).

O. crenata Forsk. has its main area of distribution in the Mediterranean-Middle East region and is particularly harmful to cool season food legumes, such as faba bean and lentil, causing complete loss of yield with severe infestations (2). Other crops affected are chickpea, pea, carrot, and lettuce. The mode of reproduction of the parasite makes its control extremely difficult; it produces thousands of tiny seeds that remain viable in soil for many years and are dispersed by humans, machinery, animals, and wind.

Although the main interest in *Orobanche* has been its control, there are some other important areas of both theoretical and practical interest for this parasitic weed. Its adaptation, from a physiological point of view, as an obligate parasite, its origin and evolution out of green plants, and its population structure are also aspects to consider. When the major emphasis in parasitic weed control strategies is the breeding of host varieties for resistance, the genetic variability of the parasite is of major importance because resistance can be overcome as shown for some crops. Knowledge of genetic diversity in parasitic plant species is still very limited and needs to be extended (30). The first studies on *O. crenata* populations used isozymes to investigate the level of variability

among populations from Syria and Spain (28,29). Thereafter, genetic diversity studies have been carried out using random amplified polymorphic DNA (RAPD) markers on populations from Israel, Egypt, and Spain (17,22,31).

Measuring diversity among *O. crenata* parasitic populations is not easy in terms of virulence because a differential set is still not available in any of their cultivated hosts. Several studies have found low differentiation among *O. crenata* populations according to race-specificity (3,21). Molecular marker analysis carried out among *O. crenata* populations from Spain (22), Egypt (31), and Israel (17) have also shown low interpopulation divergences within the three countries. Because no clear differences have been detected among populations within countries, the main objective of this work has been the comparison of *O. crenata* populations from two distant zones of the Mediterranean area (Spain and Israel) to explore the genetic differentiation among them using inter simple sequence repeat (ISSR) markers. Because the ISSR technique amplifies large numbers of DNA fragments per reaction, representing multiple loci from across the genome, it is an ideal method for fingerprinting and a useful alternative to single-locus or hybridization-based methods (8,32). ISSR markers have been used to measure genetic diversity in a wide range of crops (7,12,23, 27), and are considered more reliable and robust than RAPD markers possibly due to the fact that their primers are longer and hence polymerase chain reaction (PCR) conditions are more stringent.

MATERIALS AND METHODS

Plant material. *O. crenata* populations were sampled in agricultural fields in two Mediterranean regions: Andalusia in southern Spain (western Mediterranean) and Israel (eastern Mediterranean). Spanish populations were sampled at Córdoba (C) (northern

Corresponding author: B. Román
E-mail address: broman.cifao@cap.junta-andalucia.es

Andalusia), Mengibar (M) (Province of Jaén in northeastern Andalusia), and Pinos Puente (PP) (Province of Granada in south-eastern Andalusia). The Israeli populations were sampled in Alonin (Galilee, in northern Israel) (N), Latroon (Judea, in central Israel) (C), and Masuot (Negev, southern Israel) (S). The Spanish samples were collected in faba bean (*Vicia faba* L.) fields. The samples from the north and the south of Israel were collected in chickpea (*Cicer arietinum* L.) fields and the samples from the center of Israel were collected in a vetch (*V. sativa* L.) field. Eight *Orobanche* plants were analyzed per population.

ISSR and RAPD analysis. DNA was isolated from floral terminal buds of single plants according to the mini-prep protocol described by Fulton et al. (5) in the case of the Israeli genotypes, and according to the method proposed by Lassner et al. (15), modified by Torres et al. (26), in the case of the Spanish genotypes. A total of 30 ISSR primers were surveyed on six individual DNA samples from different countries and populations. ISSR primers of oligonucleotide set number 9 were purchased from the University of British Columbia, Nucleic Acid Protein Service Unit, Vancouver, Canada (primer numbers 807, 810, 814, 816, 817, 819, 822, 825, 826, 827, 828, 835, 836, 840, 841, 842, 845, 846, 847, 851, 853, 855, 856, 857, 858, 859, 860, 868, 869, and 870). From this set, five primers that provided clear and reproducible amplification profiles on agarose gels were chosen for further use with all Spanish and Israeli samples (Table 1). An RAPD primer (OPG-06) purchased from Operon Technologies (Alameda, CA) was also used because it produced intense and consistent amplification products for broomrape species in a previous study (13). Nonradiolabeled PCR reaction mixture was used to separate amplified products on agarose gels, whereas the polyacrylamide gels separated radiolabeled amplified fragments. Amplification products, obtained with five ISSR primers that yielded polymorphic patterns on agarose gel, were tested on sequencing gels. OPG-06 RAPD amplification products were analyzed only on agarose gel. The resolution of the sequencing gel was greater than that of the agarose gel showing a higher number of polymorphic products per primer.

Nonradiolabeled PCR. PCR reaction mixtures for separation on agarose gel contained 30 ng of plant genomic DNA, 7.5 pmol of primer, 265.6 μ M each of the dNTPs, 10 $\times$ RedTaq buffer (Sigma Chemical, St. Louis) (100 mM Tris-HCl [pH 8.3], 500 mM KCl, 11 mM MgCl₂, and 0.1% gelatin), and 1.3 units of RedTaq

polymerase (Sigma Chemical) in a total volume of 25 μ l. The ISSR amplification program was as follows: initial denaturation for 7 min at 94°C, 35 cycles of 30 s at 94°C, 45 s at 45°C, and 2 min at 72°C in a thermocycler (PTC-100; MJ Research, Watertown, MA). The RAPD amplification program was as follows: initial denaturation for 3 min at 94°C, 44 cycles of 1 min at 94°C, 1 min at 36°C, and 2.5 min at 72°C. PCR products were separated by electrophoresis in 1.2 or 1.4% agarose gels (ISSR and RAPD, respectively) (Techcomp. Ltd., Hong Kong) for 3 to 4 h and stained with ethidium bromide.

Radiolabeled PCR. Amplification products of ISSR selected primers with radiolabeled nucleotide were separated in sequencing gels. PCR mixtures contained 30 ng of plant genomic DNA, 10 pmol of primer, 277 μ M dATP, dTTP, and dGTP, 3.3 μ M dCTP, 0.1 μ l of 3,000 Ci/mmol [α -33P] dCTP, 10 $\times$ RedTaq buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl, 11 mM MgCl₂, and 0.1% gelatin), and 2 units of RedTaq DNA polymerase in a total volume of 15 μ l. The PCR conditions were the same as for the nonradiolabeled ISSR but with 27 cycles. PCR products (3 μ l per lane) were separated on a DNA sequencing gel containing 6% polyacrylamide, 8 M urea, and 1 $\times$ Tris-borate-EDTA at 60 W constant power for 5.5 to 6.5 h. After drying, the gels were exposed to a Kodak MR film.

Statistical analysis. Amplified fragments were scored manually for the presence (1) or absence (0) of homologous bands to create a binary matrix of the different ISSR and RAPD phenotypes. The analysis of molecular variance (AMOVA) was used to partition the total phenotypic variance into within populations, among populations within regions, and between regions (4). The AMOVA was performed by treating an ISSR/RAPD profile as a haplotype (10) using WinAMOVA version 1.55 software (L. Excoffier, LGB, University of Geneva, Switzerland). Distance among individuals was measured as an Euclidean metric distance that was calculated between all possible pairwise combinations of molecular genetic markers (ISSR and RAPD bands) for individual plants. The variance components were tested statistically by non-parametric randomization tests using 1,000 permutations.

A nonparametric test for the homogeneity of molecular variance (HOMOVA), based on Bartlett's statistics (1), was performed to test heteroscedasticity among regions and among populations (25). Bartlett's statistics null distributions were obtained after 1,000 permutations.

Pairwise population comparisons examined with AMOVA resulted in values of ϕ_{st} that were equivalent to the proportion of the total variance that is partitioned between two populations. ϕ_{st} values between each pair of populations were interpreted as the average interpopulation distance between any two populations in order to obtain a distance matrix (9,10). A cluster analysis based on ϕ_{st} matrix was performed using unweighted pair-group method with arithmetic average (UPGMA) of the TFPGA 1.3 software package (M. Miller, Northern Arizona University).

Cophenetic correlation coefficient was calculated and Mantel's test (16) was performed to check the goodness of fit of a cluster analysis to the matrix on which it was based. The randomization procedure as implemented in the TFPGA software package included 1,000 permutations.

TABLE 1. Inter simple sequence repeat and random amplified polymorphic DNA primers (number of polymerase chain reaction products on polyacrylamide gels used in the analysis of *Orobanche crenata* populations)^a

Primers	Sequence (5'-3')	Polymorphic bands (polyacrylamide)
807	AGA GAG AGA GAG AGA GT	34
810	GAG AGA GAG AGA GAG AT	15
835	AGA GAG AGA GAG AGA GYC	29
841	GAG AGA GAG AGA GAG AYC	18
857	ACA CAC ACA CAC ACA CYG	15
OPG-06	GTGCCTAACC	—

^a Single letter abbreviations for mixed base positions: Y = (C,T).

TABLE 2. Analysis of molecular variance and homogeneity of molecular variance analysis for the partitioning of random amplified polymorphic DNA variation among regions (Israel versus Spain), among populations (within regions), and within populations as well as among and within each of two regions

Source of variation	df	Variance components	% Total variance	ϕ -statistics	P value	Bartlett's index	P value
Among regions (Israel versus Spain)	1	4.29	24.27	0.243	<0.001	0.227	<0.001
Among populations within regions	4	0.89	5.01	0.066	<0.001	0.181	0.198
Within populations	42	12.49	70.72	0.293	<0.001	...	...
Among populations (Israel)	2	1.76	11.76	0.118	<0.001	0.079	0.105
Within populations	21	13.20	88.24	...	...	...	...
Among populations (Spain)	2	0.01	0.09	0.001	0.546	0.037	0.449
Within populations	21	11.79	99.91	...	...	...	...

RESULTS

An average of 22.2 clear reproducible polymorphic products per ISSR primer was detected on polyacrylamide gels (Table 1). After excluding bands that were monomorphic for the whole data set, the six primers yielded a total of 115 polymorphic bands. The number of bands ranged from 5 (RAPD OPG-06) to 34 (ISSR 807). Out of the 115 bands, only one band showed fixed differences among the geographical regions and only seven of them (6.09%) were fixed in one region and variable in the other. Fifty-five bands (47.83%) were variable in one region but absent from the other, whereas 52 bands (45.22%) were variable in both regions.

Hierarchical analysis of phenotypic diversity using a two-way nested AMOVA was performed to analyze the partitioning of the variation between two regional groups (Israel versus Spain) as well as among populations within the groups and among individuals (Table 2). Highly significant ϕ values for all the sources of variation provided clear evidence for the existence of presumed levels of phenotypic structure. Nevertheless, most of the genetic diversity was attributable to differences among individuals within a population (70.72%). The remaining phenotypic diversity was unequally distributed between regions (24.27%) and between populations (5.01%). Moreover, corresponding two-way nested HOMOVA revealed that molecular variances were significantly heterogeneous between regions ($B_p = 0.227$) but not among populations within a region ($B_p = 0.181$). For the within-region analysis of Spanish populations, all the diversity was attributable to differences among individuals within populations (99.91%) because the among-population partitioning was not significant ($\phi_{st} = 0.001$; $P = 0.546$). Nonsignificant Bartlett's test provided further evidence of the lack of population structure ($B_p = 0.037$; $P = 0.449$). On the other hand, the within-region analyses of Israeli populations revealed that in spite of substantial internal variation (88.24%), there is still significant divergence among populations (11.76%; $\phi_{st} = 0.118$; $P < 0.001$). However, Bartlett's heteroscedasticity index, although higher than that among Spanish populations, was still nonsignificant ($P = 0.105$).

ϕ_{st} values between each pair of populations were significant in all cases except among Spanish populations (Table 3). The resultant dendrogram based on UPGMA clearly divided six populations by region (Fig. 1). Spanish populations were generally more similar to each other than the Israeli populations. Cluster analysis using UPGMA showed a good fit to the matrix on which it was based giving significant cophenetic correlation coefficient ($r = 0.9917$; $P = 0.012$).

DISCUSSION

This study has detected significant genetic differentiation among *O. crenata* populations from Spain and Israel and considerably high variation within populations in both countries. Effective seed dispersal by humans, machinery, animals, and wind as well as on host seeds may contribute to gene flow between populations of the parasitic weed. In the case of Spanish *O. crenata* populations, diversity was attributable to differences among individuals

within populations (99.91%) and the among-population partitioning was not significant ($\phi_{st} = 0.001$; $P = 0.546$). *O. crenata* plants sampled in three geographically distant locations in Andalusia (Spain) could be considered members of the same population. In fact, the significant negative value of ϕ_{st} observed between the Spanish populations of Córdoba and Mengibar (Table 3) suggests that some samples from the population of Córdoba are more similar to the population of Mengibar than to the samples of their own population and vice versa. In the case of the Israeli populations, the molecular differentiation among populations is significant and higher than in the Spanish ones (11.76%; $\phi_{st} = 0.118$; $P < 0.001$). Differences on the population levels found in the present study for Israeli *O. crenata* may indicate that different genotypes exist in this region. We may speculate that each one originates from another source that invaded the country separately and then established in agricultural fields. Lack of significant differences among the Spanish populations of *O. crenata* may indicate that only a single source gave rise to the Andalusian populations of this species.

Previous isoenzymatic studies in *O. crenata* populations from Syria (28) and Spain (29) and RAPD analysis of two *O. crenata* populations from Israel (13) also described a clear genetic variation at the intrapopulation level and only little differentiation among populations. Nevertheless, the differences we found among populations are higher than those obtained for three Egyptian *O. crenata* populations (31). However, in this latter case, similarities might have been overestimated because each population was represented by a bulk of seeds and consequently band frequencies of individual genotypes were not analyzed.

When the spatial distance between populations increases, a higher genetic differentiation is usually expected (24). The fixation index (0.243) estimated with AMOVA between Andalusia and Israel was higher than the indexes among populations within each region (0.001 and 0.118, respectively), indicating that geographic distance provides a substantial barrier to gene exchange among populations promoting the appearance of genetic differentiation between both regions.

The use of molecular markers may be a suitable method for the identification of pathogenic groups in parasite populations (6). The exploration of molecular diversity within parasite species

Fig. 1. Interpopulation distance (ϕ_{st}) among Spanish and Israeli *Orobancha crenata* populations. Unweighted pair-group method with arithmetic average tree for the six *O. crenata* populations analyzed with pairwise comparisons derived from analysis of molecular variance. Significant values of ϕ_{st} (proportion of the total variance that is partitioned between two populations) are given in Table 3.

TABLE 3. Interpopulation distance matrix ϕ_{st} for the six *Orobancha crenata* populations^a

Population	Israel C	Israel S	Israel N	Spain M	Spain	Spain C
Israel C	...	0.0000	0.0000	0.0000	0.0000	0.0000
Israel S	0.0542	...	0.0000	0.0000	0.0000	0.0000
Israel N	0.1097	0.1812	...	0.0000	0.0000	0.0000
Spain M	0.2881	0.3009	0.3034	...	0.4815	0.4166
Spain PP	0.2708	0.2792	0.2935	0.0002	...	0.3896
Spain C	0.2898	0.3005	0.3071	-0.0022	0.0047	...

^a Lower matrix diagonal indicates ϕ_{st} value, proportion of the total variance partitioned between two populations. Upper matrix diagonal indicates corresponding P values.

may allow us to characterize races rather than populations and eventually to obtain molecular markers for these broomrape races. Cubero and Moreno (3) and Radwan et al. (21) did not find evidence supporting the existence of races in *O. crenata*. This might be due to the lack of genetic resistance available in commercial cultivars of grain and forage legume hosts. Although races are more likely to develop within highly inbred species or in geographically diverse species with little gene flow, there is the risk that the widely diverse *O. crenata* populations could be selected for virulence when challenged by the widely extensive use of a highly resistant cultivar as has recently been suggested in Israel (11) where a virulent population seems to have been selected by the frequent culture of a resistant vetch.

From an evolutionary point of view, the high genetic variability of *O. crenata* occurring on various legume crops, which are genetically uniform, is difficult to explain. Apparently the maintenance and reinforcement of the genetic variability is regulated by a "permissive" host environment. In natural vegetation, this interaction between the genetic diversity of host and parasite populations has probably led to a certain level of tolerance in the host plant, precluding a total eradication by the parasite (19). If similar genetic interaction occurs between the parasite and the crop species, one might expect a sharp decrease in the amount of genetic variation that can be counterbalanced by other forces such as gene flow in the form of high seed dispersal or large seed bank. The aggressiveness of weedy parasites to their host crop is in general very high. This could be explained by the fact that for its survival in agrosystems a parasite does not necessarily depend on the survival of its host (19).

Comparative studies among *Orobanch* biotypes in natural habitats and crop fields should be considered for a better understanding of the evolution from wild parasitic plants into aggressive parasitic weeds. The occurrence of *O. crenata* as a native species is rare or unknown and in most areas of its range of distribution it has probably been introduced. The situation is similar in *Striga hermonthica*, a parasitic plant that only attacks cultivated species. On the other hand, *S. asiatica* is found on crops but also parasitizing wild hosts. The mode of reproduction may determine these facts since an autogamous species such as *S. asiatica* has higher probability of survival and maintenance in isolation than allogamous species such as *Striga hermonthica* because it does not have the seed production ensured. The mating system as well as the facilities of fixation of mutation in autogamous species could explain the origin, diversity, and diffusion of autogamous parasitic species such as *S. asiatica* (30) and the exclusive appearance of allogamous species like *O. crenata* in crop fields. The existence of *O. foetida* in faba bean fields in Tunisia (14) is of relevant importance because this species only parasitized wild species until its recent appearance in crop legume fields. In Andalusia, *O. foetida* attacks wild legumes such as *Astragalus lusitanicus*, *Scorpius muricatus*, *Trifolium angustifolium*, *Lotus* spp., and *Melilotus* spp. (20). Molecular studies considering both *O. foetida* genotypes should be considered.

ACKNOWLEDGMENTS

We thank D. Zohar from the Israeli Ministry of Agriculture for help in collecting some *O. crenata* samples in Israel, V. Portnoy and Y. Danin-Poleg for helpful discussions, and E. Leshzeshen for technical assistance. Z. Satovic is grateful to E.T.S.I.A.M., Córdoba, Spain and to UNESCO (UNESCO Biotechnology Action Council Programme, Short-Term Fellowship in Biotechnology, 1999) for financial support.

LITERATURE CITED

- Bartlett, M. S. 1937. Some examples of statistical methods of research on agriculture and applied biology. *J. R. Stat. Soc. Suppl.* 4:137-170.
- Cubero, J. I. 1983. Parasitic diseases in *Vicia faba* L. with special reference to broomrape (*Orobanch crenata* Forsk.). Pages 257-277 in: *The Faba Bean*. P. B. Hebblethwaite, ed. Butterworth, London, UK.
- Cubero, J. I., and Moreno, M. T. 1979. Agronomical control and sources of resistance in *Vicia faba* to *Orobanch crenata*. Pages 41-80 in: *Some Current Research on Vicia faba in Western Europe*. D. A. Bond, G. T. Scarascia-Mugnozza, and M. H. Poulsen, eds. Comm. Eur. Communities, Luxembourg.
- Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction sites. *Genetics* 131:479-491.
- Fulton, T. M., Chunwongse, J., and Tanksley, S. D. 1995. Micropep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Mol. Biol. Rep.* 13:207-209.
- Gagne, G., Roeckel-Drevet, P., Grezes-Besset, B., Shindrova, P., Ivanov, P., Grand-Ravel, C., Vear, F., Tourvieille de Labrouhe, D., Charmet, G., and Nicolas, P. 1998. Study of the variability and evolution of *Orobanch cumana* populations infesting sunflower in different European countries. *Theor. Appl. Genet.* 96:1216-1222.
- Gilbert, J. E., Lewis, R. V., Wilkinson M. J., and Caligari, P. D. S. 1999. Developing an appropriate strategy to assess genetic variability in plant germplasm collections. *Theor. Appl. Genet.* 98:1125-1131.
- Godwin, I. D., Aitken, E. A. B., and Smith, L. W. 1997. Application of inter simple sequence repeat (ISSR) markers to plant genetics. *Electrophoresis* 18:1524-1528.
- Gustine, D. L., and Huff, D. R. 1999. Genetic variation within and among white clover populations from managed permanent pastures of the northeastern USA. *Crop Sci.* 39:524-530.
- Huff, D. R. 1997. RAPD characterisation of heterogeneous perennial ryegrass cultivars. *Crop Sci.* 37:557-564.
- Joel, D. M. 2000. A new virulent race of *Orobanch crenata*. *Haustorium* 37.
- Katzir, N., Leshzeshen, E., Tzuri, G., Reis, N., Danin-Poleg, Y., and Paris, H. S. 1998. Relationships among accessions of *Cucurbita pepo* based on ISSR analysis. *Cucurbitaceae* 98:331-335.
- Katzir, N., Portnoy, V., Tzuri, G., Castejón-Muñoz, M., and Joel, D. M. 1996. Use of random amplified polymorphic DNA (RAPD) markers in the study of the parasitic weed *Orobanch*. *Theor. Appl. Genet.* 93:367-372.
- Kharrat, M., Halila, M. H., Linke, K. H., and Haddar, T. 1992. First report of *Orobanch foetida* Poiret on faba bean in Tunisia. *FABIS Newsl.* 30:46-47.
- Lassner, M. W., Peterson, P., and Yoder, J. I. 1989. Simultaneous amplification of multiple DNA fragments by polymerase chain reaction in the analysis of transgenic plants and their progeny. *Plant Mol. Biol. Rep.* 7:116-128.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27:209-220.
- Paran, I., Gidoni, D., and Jacobsohn, R. 1997. Variation between and within broomrape (*Orobanch*) species revealed by RAPD markers. *Heredity* 78:68-74.
- Parker, C., and Riches, C. A. 1993. *Parasitic Weeds of the World: Biology and Control*. CAB International, Wallingford, UK.
- Pieterse, A. H., and Verkleij, J. A. C. 1989. Genetic variability in *Orobanch* (broomrape) and *Striga* (witchweed) and its implications for host crop resistance breeding. Pages 290-302 in: *Ecological Responses to Environmental Stresses*. J. Rozema and J. A. C. Verkleij, eds. W. Junk Publisher, Amsterdam, The Netherlands.
- Pujadas, A. 1999. Species of the family *Orobanchaceae* parasitic of cultivated plants and its relatives growing on wild plants, in the south of the Iberian Peninsula. Pages 187-193 in: *Resistance to Orobanch: The State of the Art*. J. I. Cubero, M. T. Moreno, D. Rubiales, and J. Sillero, eds. Junta de Andalucía, Spain.
- Radwan, M. S., Abdalla, M. M. F., Fischbeck, G., Metwally, A. A., and Darwish, D. S. 1988. Variation in reaction of faba bean lines to different accessions of *Orobanch crenata* Forsk. *Plant Breed.* 101:208-216.
- Román, B., Rubiales, D., Torres, A. M., Cubero, J. I., and Satovic, Z. 2001. Genetic diversity in *Orobanch crenata* populations from Southern Spain. *Theor. Appl. Genet.* 103:1108-1114.
- Salimath, S. S., de Oliveira, A., Godwin, I. D., and Bennetzen, J. L. 1995. Assessment of genome origins and genetic diversity in the genus *Eleusine* with DNA markers. *Genome* 38:757-736.
- Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* 47:264-279.
- Stewart, C. N., and Excoffier, L. 1996. Assessing population structure and variability with RAPD data: Application to *Vaccinium macrocarpon* (American cranberry). *J. Evol. Biol.* 9:153-171.
- Torres, A. M., Weeden, N. F., and Martín, A. 1993. Linkage among isozyme, RFLP and RAPD markers in *Vicia faba*. *Theor. Appl. Genet.* 85:937-945.

27. Tsumura, Y., Ohba, K., and Strauss, S. H. 1996. Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas-fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). *Theor. Appl. Genet.* 92:40-45.
28. Verkleij, J. A. C., Egbers, W. S., and Pieterse, A. H. 1991. Allozyme variation in populations of *Orobancha crenata* from Syria. Pages 304-317 in: *Proc. Int. Workshop Orobancha Res.* K. Wegmann and L. J. Musselman, eds. Tübingen, Germany.
29. Verkleij, J. A. C., Koevoets, P., López-Granados, F., Egbers, W. S., García-Torres, L., and Pieterse, A. H. 1991. Genetic variability in populations of *Orobancha crenata* from Spain. Pages 462-469 in: *Proc. Int. Symp. Parasitic Weeds*, 5th. J. K. Ransom, L. J. Musselman, A. D. Worsham, and C. Parker, eds. Nairobi, Kenya.
30. Verkleij, J. A. C., and Pieterse, A. H. 1994. Genetic variability of *Orobancha* (broomrape) and *Striga* (witchweed) in relation to host specificity. Pages 67-79 in: *Proc. Int. Workshop Orobancha Related Striga Res.*, 3rd. A. H. Pieterse, J. A. C. Verkleij, and S. J. ter Borg, eds. Royal Tropical Institute, Amsterdam, The Netherlands.
31. Zeid, M., Madkour, M., Koraïem, Y., Nawar, A., Soliman, M., and Zaitoun, F. 1997. Molecular studies on *Orobancha*. *J. Phytopathol.* 145:351-355.
32. Zietkiewicz, E., Rafalski, A., and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20:176-183.