PATHOGENIC AND MOLECULAR CHARACTERIZATION OF POPULATIONS OF *OROBANCHE CERNUA* LOEFL. FROM SUNFLOWERS IN SPAIN

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Abstract

Populations (38) of *Orobanche cernua* collected in the main Spanish sunflower growing areas from 1989-1994, were characterized by phenotypic pathotyping. Of them, 23 were used to explore further characterization by RAPD-derived genetic fingerprinting. Race-differentials were sown in a soil mixture infested with broomrape seeds at the rate of 0.01% and grown at 20-26°C and in the greenhouse for 75-90 days Disease reactions were assessed by the number of broomrapes developed at 15-day intervals. None of *O. cernua* populations from 1989-1991 overcame resistance genes Or_2 and Or_5, but most of them were pathogenic to Or_1. In 1992-1993, most populations were pathogenic to Or_1 and Or_3, but two from Cuenca (Central Spain) overcame Or_5 and/or Or_2. Ten populations from Cuenca (9) and southern Spain (1) in 1994 overcame Or_1 and Or_3, of which seven were also pathogenic to Or_2 and/or Or_4. Broomrape total DNA was extracted from a single stem apex of a population and amplified with commercial 10-mer primers in PCR reactions. Of 32 primers used with 17 broomrape populations, eight amplified DNA bands which were informative concerning the geographical origin of the population, but none was related to specific pathogenicity. When three of the former and six new populations were analysed with some of the above or with new primers, eight primers produced identical or nearly identical fingerprintings for populations which shared geographical origin and virulence pattern. In some cases, fingerprintings of a population provided by given primers changed between experiments, indicating that genetic diversity may occur within a population.

Keywords: Broomrape races, *Helianthus annuus*, RAPD-PCR fingerprinting

Introduction

Sunflower broomrape (*Orobanche cernua* Loefl.) is a holoparasitic angiosperm that severely attacks sunflower crops in several European countries. Control of the disease is mainly by the use of resistant cultivars, the efficacy of which is counteracted by development of new pathogenic variants in the pathogen population. Races and "race complexes" of *O. cernua* have been described in the former USSR and some southern European countries during this century, and the breakdown of broomrape resistance in sunflower cultivars widely used has taken place respeatedly (PUSTOVOIT 1976, SKORIC 1988). In Spain, the pathogen was known to severely affect confectionary sunflower mainly (GONZÁLEZ TORRES et al. 1982). However, *O. cernua* has recently become widespread in oilseed cultivars and currently is the main threat for the crop in the country. This prompted the screening of

parental lines from our sunflower breeding programme for resistance against the pathogen (MELERO VARA et al. 1989).
Broomrape "race complexes" A, B and M were first described in the USSR. Later, five "races" namely A-E, were established in Romania. These races can be phenotypically characterized by means of their differential interactions with sunflower lines AD-66, Kruglik A-41 (KA-41), Jdanov 8281 (J-8281), Record, S-1358 and P-1380-2. Resistance in "KA-41", "J-8281", "Record", "S-1358" and "P-1380-2" is conferred by dominant alleles of genes Or_1, Or_2, Or_3, Or_4 and Or_5 respectively, which provide "cumulative" resistance to races A through E (VRÂNCEANU et al. 1981). Such a race pattern is not valid for the pathogenic characterization of broomrape populations in Spain, as "Record" and "S-1358" are susceptible to all our populations (MELERO-VARA et al. 1989). The aim of this study was to further phenotypically characterize field populations of *O. cernua* collected from sunflower crops in Spain, and to explore the possibility of their characterization by means of genetic fingerprinting.

Materials and methods

Phenotypic characterization. Thirty eight field populations of sunflower broomrape were collected in provinces of the main growing areas of the crop in Central [Cuenca (CU)] and southern Spain [Córdoba (CO) and Sevilla (SE)] from 1987 through 1994. Broomrape seeds were cleaned free from debris and stored at room temperature until used.
Sunflower lines, including broomrape race differentials and inbred lines HA-99 and RHA-273, were artificially inoculated with the 38 broomrape populations in a series of experiments. Inoculum consisted of aliquots of 25 mg of *O. cernua* seeds. The inoculum was thoroughly mixed with 250 g of a soil mixture (sand: silt, 1:1, v/v) and a pot was filled with the infested soil (PANCHENKO, 1975). Germinated sunflower seeds were sown in the infested soil (one per pot). Plants were grown at 20-26°C and a 12-h photoperiod of 216 $\mu E.m^{-2}. s^{-1}$ for 15 days. Thereafter, plants were transplanted to 4-L pots filled with a fertilized (Osmocote 9g/pot) potting soil mixture (sand:silt:peat, 2:1:2, by volume) and grown in the greenhouse for additional 60-75 days. Disease reactions were assessed by the number of emerged broomrapes at 15 days intervals. There were ten replicated pots in a complete randomized block design for each broomrape population-race differential combination.

Genetic fingerprinting. Twenty three broomrape populations were used for the studies. Seventeen populations were first used in a preliminary approach to establish a suitable methodology. Of them, seven were collected from sunflower crops at Sevilla in 1992 (SE-392, -592, -692 and -792), 1993 (SE-193, -293) and 1994 (SE-194), and 10 did from crops at Cuenca in 1992 (CU-292) and 1994 (CU-194, -294, -394, -494, -594, -694, -794, -894 and -994). For each population, a single stem was harvested from pathotyping experiments disregarding the host-parasite combination being sampled. Information on the final outcome of the pathotyping experiments was withheld, so that studies on genetic fingerprinting with those broomrape samples were carried out as "blind tests".
Unless otherwise stated, total broomrape DNA was extracted from the apex of a stem by

the method of CENIS (1992) with minor modifications. This method, which lacks phenolization steps and was developed for quick purification of fungal DNA, has been adapted for use with plant tissues. Appropriate concentrations of purified broomrape total DNA was directly used for RAPD analyses.
A total number of 32 10-mer primers from kits H(OPH-06,-07,-08,-10,-11,-12,-14,-15,-16,-17,-18 and -19), I(OPI-01 through OPI-18) and F(OPF-09, -11) were first tested with standardised DNA amplification conditions. Amplification was performed in a Pharmacia LKB Gene ATAQ Controller. Controls lacking template DNA were included. Reaction products (the whole volume, 25μl) were resolved by electrophoresis on 2% agarose gels in TAE buffer and stained with ethidium bromide.
Six new broomrape field populations collected from sunflowers at Córdoba in 1991 (CO-191) and 1992 (CO-192), Cuenca in 1993 (CU-193), and Sevilla in 1989 (SE-289), 1990 (SE-390) and 1991 (SE-891) together with populations SE-193 and -194, and CU-394, were sampled from a new pathotyping experiment and used for RAPD analyses. For each population, 10 to 20 stems were sampled from any of sunflower lines HA-99, J-8281, KA-41 and RHA-273 on which they developed. Thus, both inter and intra population comparisons could be done with results from RAPD analyses. Total broomrape DNA was extracted and amplified using some of the former (OPH-07, -15; OPI-02, -07, -09 and -14; and OPF-09, and -11) or new (OPF-01, -05; and KS) primers in a Perkin Elmer Gene Amp. PCR System 2400. In order to determine genetic differences amongst broomrape populations, a single stem apex was used from each population. That stem was selected from those that had developed on the susceptible differential line that best differentiates a given *O. cernua* population at the time of sampling (i.e. SE-289 and -390/HA-99; CO-191 and -192, and SE-891,-193, and -194/RHA-273; and CU-193, and -394/J-8281).

Results and Discussion

Phenotypic characterization. Broomrape populations collected from southern Spain in 1990 were not pathogenic to inoculated race differentials (Table 1). Populations (11) collected from the same region in 1991 were not pathogenic to "J-8281" and "P-1380-2". All of them shared the same disease reaction pattern, except for SE-191 which was not pathogenic to all differential used, and SE-291 and -391 which differed in disease reactions to "KA-41" and "Record" (Table 1).
Pathotyping of populations collected in 1992 was determined on two different sets of differentials (Tables 2 and 3). Populations from Sevilla were pathogenic to "KA-41" and "Record" except for SE-292 which was weakly virulent to "KA-41", but nonpathogenic to "J-8281" (Tables 1 and 2). In contrast, population CU-192 fron Central Spain was moderately virulent to "P-1380-2", suggesting a breakdown of resistance conferred by Or_5. (Table 1). Populations collected in 1993 showed similar virulence pattern, except for CU-193 which was moderately virulent to "J-8281" (Table 2).
Populations collected in Central Spain in 1994 were all pathogenic to "KA-41", "Record" and "S-1358", except for CU-594 and -694 to which "S-1358" was resistant (Table 3). However, four of the tested populations were also pathogenic to "J-8281" carrying Or_2, thus indicating a trend to increased pathogenicity (Table 3).

Finally, broomrape populations from an ample variety of locations and years were compared in a further experiment. While all inocula tested were pathogenic to "KA-41" and "HA-99", only CU-394 overcame resistance in "J-8281", and hence confirmed previous results on increased pathogenicity (Table 4).

Genetic fingerprinting. Of the 32 primers used in a preliminary approach, all of them except OPH-07; OPI-02,-06,-07,-08,-09 and -18; and OPF-09, amplified identical fingerprintings with all 17 broomrape stems studied. Primer OPH-07 amplified two bands of 1.0 and 1.6 kb which showed specific for the broomrape sampled from population SE-194. Primer OPI-02 amplified a band of 0.6 kb which was lacking in population SE-194 only, and a band of 3.5 kb which was present in populations from Cuenca province only. Similarly, primer OPI-07 amplified a band of 1.0 kb that was present in all populations except SE-194, and bands of 1.1 and 1.4 kb which were lacking in all populations studied except SE-194. On the contrary, primer OPI-09 amplified a band of 1.8 kb which was present in populations from Cuenca only, and primer OPI-18 amplified a band of 2.0 kb which was present in populations from Sevilla only. However, none of the fingerprintings obtained so far would be useful for the characterization of populations differing in specific pathogenicity.
When six new broomrape populations, together with populations SE-193, -194 and CU-384 were analyzed with some of the above primers or with new primers, identical fingerprintings were obtained for all populations with primers OPF-01, -05 and -09. However, all other eight primers produced identical amplification patterns for populations from Cuenca (CU-193, -394), which were pathogenic to all differential lines inoculated (except for a 0.65 kb band amplified by OPI-02 from CU-193). This pattern was close only to that obtained for population SE-390 which was not pathogenic to "J-8281". Similarly, the eight primers produced nearly identical fingerprinting for populations SE-891, -193, and -194 from Sevilla, which showed same virulence to race differentials and were more virulent than SE-390. On the contrary, populations from Córdoba, which were similarly virulent showed fingerprintings which were either close to populations from Cuenca (CO-192) or from Sevilla (CO-191).
When results with primers and broomrape populations common to the two experiments (SE-193, -194, and CU-394) were compared, amplification patterns were same for SE-194, but differed for SE-193 (OPH-07, OPI-07) and CU-394 (OPI-02). This indicates that genetic differences may exist within a broomrape population.

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Table 1. Disease reactions of race differential lines of sunflower to inoculation with populations of *Orobanche cernua*

Broomrape population and year		Sunflower differentials			
		KA-41	J-8281	Record	P-1380-2
SE-490	1990	L[x]	L	R	R
SE-590	1990	L	L	L	R
SE-191	1991	R	R	R	R
SE-291	1991	M	R	M	R
SE-391	1991	L	R	S	R
SE-491	1991	S	R	S	R
SE-591	1991	S	R	S	R
SE-691	1991	S	L	S	R
SE-791	1991	S	R	S	R
SE-891	1991	S	R	S	R
SE-991	1991	S	L	S	R
CO-191	1991	S	R	S	R
CO-291	1991	S	R	S	R
SE-192	1992	S	L	S	R
SE-292	1992	L	R	S	R
CU-192	1992	S	M	S	M

[x] Disease reaction: R=Highly resistant, L=Resistant, M=Moderately susceptible, S=Susceptible.

Table 4. Disease reactions of race differential and inbred lines of sunflower to inoculation with populations of *Orobanche cernua*

Broomrape population and year		Sunflower differentials			
		KA-41	J-8281	HA-99	RHA-273
SE-194	1994	S[x]	R	S	S
SE-289	1989	S	L	S	R
SE-891	1991	S	R	S	S
CU-394	1994	S	S	S	S
SE-390	1990	S	R	S	L
SE-193	1993	S	R	S	S
CO-191	1991	S	R	S	S
CO-192	1992	S	R	S	M
CU-193	1993	S	L	S	S

[x] Disease reaction: R=Highly resistant, L=Resistant, M=Moderately susceptible, S=Susceptible.

Table 2. Disease reactions of race differential and inbred lines of sunflower to inoculation with populations of *Orobanche cernua*

Broomrape population and year		Sunflower differentials			
		KA-41	J-8281	Record	HA-99
SE-392	1992	S[x]	R	S	M
SE-492	1992	M	R	M	L
SE-592	1992	S	L	S	M
SE-692	1992	S	R	S	M
SE-792	1992	S	R	S	S
SE-292	1992	S	R	S	S
SE-193	1993	S	R	S	S
SE-293	1993	S	L	S	S
CU-193	1993	S	M	S	S
CU-293	1993	S	R	S	S

[x] Disease reaction:R=Highly resistant, L=Resistant, M=Moderately susceptible, S=Susceptible.

Table 3. Disease reactions of race differential lines of sunflower to inoculation with populations of *Orobanche cernua*.

Broomrape population and year		Sunflower differentials			
		KA-41	J-8281	Record	S-1358
CU-194	1994	S[x]	L	S	S
CU-294	1994	S	R	S	S
CU-394	1994	S	S	S	S
CU-494	1994	S	M	S	S
CU-594	1994	S	L	S	R
CU-694	1994	S	R	S	R
CU-794	1994	S	S	S	S
CU-894	1994	S	S	S	S
CU-994	1994	S	R	S	S

[x] Disease reaction:R=Highly resistant, L=Resistant, M=Moderately susceptible, S=Susceptible.