Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Virulence, genetic diversity, and putative geographical origin of sunflower broomrape populations in Morocco

Abdelghani NABLOUSSI[1], Begoña PÉREZ-VICH[2], Leonardo VELASCO[2,*]

[1] *Research Unit of Plant Breeding and Plant Genetic Resources Conservation, National Institute of Agricultural Research, Regional Center of Meknes, Morocco*
[2] *Institute for Sustainable Agriculture (IAS-CSIC), Córdoba, Spain*
*Corresponding autor. E-mail: lvelasco@ias.csic.es

Summary. Sunflower broomrape (*Orobanche cumana* Wallr.) was detected for the first time parasitizing sunflower in Morocco in 2016. Seeds of three broomrape populations from two separate areas of Morocco, Souk Al Arbaa (populations SA1 and SA2) and Meknès (Population MK1) were collected. The populations' virulence, genetic diversity, and putative area of origin were examined. Race classification using a set of sunflower differential lines showed that MK1 was a race-E population, while SA1 and SA2 were race-G populations. The analysis with 192 SNP markers showed that SA1 and SA2 populations are genetically similar and very distant from the MK1 population. The three populations exhibited low intrapopulation diversity. Comparisons with populations from other areas showed that MK1 was introduced from a race-E population from the Guadalquivir Valley gene pool in Southern Spain, probably before 1988. Populations SA1 and SA2 showed close relationships with a population from Russia, although more exact knowledge of the origin of these populations requires further investigation. Since the SA and MK populations were collected from areas located approx. 100 km apart, the risks of mixing and recombining both gene pools to produce more virulent variants must be considered.

Keywords. Genetic diversity, parasitic weeds, SNP markers, virulence, plant introductions.

OPEN ACCESS

Citation: A. Nabloussi, B. Pérez-Vich, L. Velasco (2023) Virulence, genetic diversity, and putative geographical origin of sunflower broomrape populations in Morocco. *Phytopathologia Mediterranea* 62(1): 65-72. doi: 10.36253/phyto-13948

Accepted: February 28, 2023

Published: May 8, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Maurizio Vurro, National Research Council, (CNR), Bari, Italy.

ORCID:
AN: 0000-0002-6125-1490
BP-V: 0000-0002-7085-5173
LV: 0000-0003-4998-9406

INTRODUCTION

Sunflower broomrape (*Orobanche cumana* Wallr.) is a parasitic plant naturally distributed in South-eastern Europe and Central Asia, where it parasitizes wild Compositae species (Beck-Mannagetta, 1930). Broomrape was first observed on sunflower in Russia in 1866 (Antonova, 2014), from where the parasite spread to sunflower crops in the Black Sea area, where it was first observed in 1935 (Batchvarova, 2014). By the middle 1950s, the main sunflower-producing areas in Russia, Ukraine, Kazakhstan, Moldavia, Romania, Bulgaria, Serbia, and Turkey had become infested by broomrape seeds, and sunflower production became dependent on the development of

resistant cultivars (Antonova, 2014; Kaya, 2014). The parasite was detected in 1958 in Spain (Molinero-Ruiz and Domínguez, 2014), in 1979 in China (Ma and Jan, 2014), in 2007 in France (Jestin *et al*., 2014), in 2010 in Tunisia (Amri *et al*., 2012), in 2016 in Morocco (Nabloussi *et al*., 2018), and in 2017 in Portugal (González-Cantón *et al*., 2019).

Sunflower broomrape is largely a self-pollinated plant, although it has some cross-pollination estimated to be up to 29% (Rodríguez-Ojeda *et al*., 2013). In eastern Europe, where sunflower broomrape occurs in the wild and has been parasitizing sunflower since the introduction of this crop, intrapopulation diversity is typically large (Gagne *et al*., 1998; Cvejić *et al*., 2020). Broomrape plants produce thousands of seeds that are readily dispersed naturally by wind, water, and agricultural machinery and can be accidentally introduced into new areas as contaminants of sunflower seed (Fernández-Martínez *et al*., 2015; Parker, 2016). Molecular genetic analyses of populations collected in Tunisia showed that some were most likely introduced from Eastern Europe (Jebri *et al*., 2017). In Spain, two separate gene pools were initially detected in Central Spain and the Guadalquivir Valley in the south. Low genetic diversity in both indicated two separate introduction events and a founder effect (Pineda Martos *et al*., 2013).

We have identified and collected sunflower broomrape populations from two separate locations in Morocco: Souk Al Arbaa (Rabat-Salé-Kénitra region) and Meknès (Fès-Meknès region), separated by approx. 100 km. The objective of the present research was to assess the virulence of the populations against a set of differential lines, evaluate their genetic variability with a set of molecular markers, and compare them with populations from other areas. This was to provide information on the introductions' putative origin(s).

MATERIALS AND METHODS

Sunflower broomrape populations

Seeds from two sunflower broomrape populations (designated as SA1 and SA2) were collected in two sunflower fields in Souk Al Arbaa, Rabat-Salé-Kénitra region of Morocco in 2016. These populations were named SA1 and SA2. Seeds from a third population (designated MK1) were collected in 2019 in a sunflower field of Meknès, Fès-Meknès region.

Several populations were used as controls, to test the putative areas of origin of the populations found in Morocco. These were populations OC94, SP, EK147, and EK21, with race E or race F virulence, and IN201, with race G virulence, from the Guadalquivir Valley in Southern Spain; INA, EK37, and EK43, with race E or race F virulence, from the Cuenca province in Central Spain (Pineda-Martos *et al*., 2013); OC1, with race E virulence, from Serbia; OC2, with race F virulence, from Romania; OC14, with race G virulence, from Russia; Boro-14, with race G virulence, from Turkey; Boro-19, with race F virulence, from Bulgaria (Pineda-Martos *et al*., 2014a); and ORD, ORG, ORH, and ORK from Béja Governorate in Tunisia (Jebri *et al*., 2017).

Sunflower differential lines

Seeds of the three Moroccan broomrape populations were tested against a set of eight differential sunflower lines: B117, with no resistant genes; J8281, resistant to broomrape races A and B; Record, resistant to races A to C; S1358, resistant to races A to D; NR5, resistant to races A to E; P96 and LP2, resistant to races A to F; and DEB2, resistant races A to G. B117 was developed from a confectionery landrace collected in Spain (Martín-Sanz *et al*., 2016). J8281, Record, and S1358 were reported by Vranceanu *et al.* (1980). NR5 was a selection from line P-1380-2 reported by Vranceanu *et al.* (1980). P96 was developed by Fernández-Martínez *et al.* (2004). LP2 is a line containing the *Or7* gene isolated from the commercial sunflower hybrid PR64A95. DEB2 is a line developed by Velasco *et al.* (2012).

Phenotypic evaluation

Evaluation of the broomrape populations against the set of sunflower differential lines was conducted in two different experiments, because the broomrape populations were identified and collected in different years. Populations SA1 and SA2, for which seed availability was low, were evaluated in 2017 in multi-pot trays with pot volumes of 0.04 L, using two replications of ten plants for every combination of broomrape population and differential line. The experimental conditions were as described by Nabloussi *et al.* (2018). The pots were filled with a soil mixture of sand and peat (1:1 by volume) inoculated with broomrape seeds at 0.28 mg per g of soil. The evaluation was conducted in a growth chamber at 25/20°C (light/dark) and 16 h photoperiod. Population MK1 was evaluated in 2020 in a greenhouse with no temperature control using 6 L capacity pots and 12 plants per sunflower genotype. Sunflower seeds were germinated on moistened filter paper at 25°C in the dark for 48 h, then planted into pots (7 × 7 × 7 cm) filled with a mixture of sand and peat, each containing

approximately 30 mg of broomrape seeds. After 4 weeks in a growth chamber at 25ºC/20ºC (light/dark) and 16 h photoperiod, the plants were transplanted to 6 L capacity pots that each contained a mixture of sand, silt, and peat (2:1:1). In the multi-pot tray experiment, underground and emerged sunflower structures were counted as described by Nabloussi *et al.* (2018). In the pot experiment, emerged broomrape shoots were counted. ANOVA with Tukey's post hoc tests to compare means was carried out for data from each broomrape population, using IBM SPSS Statistics version 29.

Plant genotyping and diversity analyses

Apical tissues from 40 broomrape shoots parasitizing the susceptible line B117 were collected for each of the three Moroccan populations studied. The tissues were initially frozen at -80°C, then lyophilized and ground in a laboratory ball mill. DNA was extracted following an adaption of the protocol described by Pérez-Vich *et al.* (2004). For the other broomrape populations (controls), DNA from 15 to 48 individual plants was used, previously extracted and maintained at -80°C.

Genotyping of individual broomrape plants from the populations collected in Morocco and those used as controls was conducted with a set of 192 *O. cumana* SNP markers reported and mapped by Calderón-González *et al.* (2019), and KASP genotyping assays (LGC Biosearch Technologies).

Data were analyzed using GenAlEx ver. 6.5. The following parameters of intrapopulation diversity were calculated: percentage of polymorphic loci (P), observed heterozygosity (Ho), expected heterozygosity (He), and Shannon's diversity index (I). Genetic distances between populations were estimated using Nei's unbiased genetic distance between pairs of populations (uNeiP), to provide a preliminary indication of the putative geographic areas of origin of the three populations found in Morocco.

The matrix of GST pairwise distances was used as an input for principal coordinates analysis (PCoA). To simplify the graph, the populations used to evaluate the relatedness of the Moroccan populations with other geographical areas were assessed in the following groups: Guadalquivir Valley races E and F, Guadalquivir Valley race G, Cuenca Province, Eastern Europe, and Tunisia. The Guadalquivir Valley populations were separated into two groups because a previous study (Martín Sanz *et al.*, 2016) showed that the race G populations exhibited increased intrapopulation diversity.

RESULTS

Race classification of the sunflower broomrape populations

Populations SA1 and SA2, collected in Souk Al Arbaa, showed a similar virulence pattern against the differential sunflower lines. They parasitized all lines with resistance to races A to E, producing from 5.7 to 9.8 nodules/shoots per plant for SA1, or from 6.7 to 17.6 nodules/shoots per plant for SA2. Parasitization of sunflower lines P96 and LP2 with resistance to race F was much less, respectively 0.3 and 0.8 nodules/shoots per plant for SA1 and 1.7 and 2.7 nodules/shoots per plant for SA2. However, no parasitization on sunflower line DEB2 (resistant to races A to G) was observed (Table 1). These results indicated that both populations are classified as race G. The virulence pattern of population MK1 was different. This population parasitized the differential lines Record (resistant to races A-C) and S1358 (resistant to races A-D) but not the lines J8281 (resistant to races A-B), NR5 (resistant to races A-E), P96, and LP2 (resistant to races A-F), and DEB2 (resistant to races A-G). Accordingly, the population is classified as race E. The absence of parasitization on line J8281 will be discussed below.

Table 1. Mean (numbers (± standard errors) of broomrape nodules/shoots[1] for three broomrape populations collected in Morocco evaluated with a set of differential sunflower lines.

Differential line	Resistant to broomrape races	Sunflower broomrape populations[2]		
		SA1	SA2	MK1
B117	None	13.2±1.5 d	17.2±2.2 e	24.6±2.6 c
J8281	A-B	8.8±0.7 bc	12.7±1.7 de	0.1±0.1 a
Record	A-C	5.7±0.7 b	6.7±0.9 bc	4.2±0.7 ab
S1358	A-D	7.3±0.9 bc	9.6±1.0 cd	8.9±1.9 b
NR5	A-E	9.8±1.1 cd	17.6±2.0 e	0.0 a
P96	A-F	0.3±0.1 a	1.7±0.4 ab	0.0 a
LP2	A-F	0.8±0.2 a	2.7±0.4 ab	0.0 a
DEB2	A-G	0.0 a	0.0 a	0.0 a

[1] Populations SA1 and SA2 were collected in 2016 and evaluated in an experiment in multi-pot trays in a growth chamber. The plants were uprooted, and nodules and developing shoots were assessed. Population MK1 was collected in 2019 and evaluated in a greenhouse experiment in 6 L capacity pots, where only emerged shoots were assessed.

[2] Values followed by the same letter within each column are not statistically significant at α=0.05 based on Tukey's post hoc test.

Table 2. Genetic diversity parameters of the sunflower broomrape populations examined in this study. The populations were: SA1 and SA2 from Souk Al Arbaa, Rabat-Salé-Kénitra region, Morocco; MK1 from Meknès, Fès-Meknès region, Morocco; OC94, SP, EK147, EK21, and IN201 from the Guadalquivir Valley in Southern Spain; INA, EK37, and EK43, from the Cuenca province in Central Spain; OC1 from Serbia; OC2 from Romania; OC14 from Russia; Boro-14 from Turkey; Boro-19 from Bulgaria; ORD, ORG, ORH, and ORK from Béja Governorate, Tunisia.

Population	P	H_0 (±SE)	He (±SE)	I (±SE)
SA1	0.66	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SA2	0.66	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MK1	0.66	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
OC94	2.65	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
SP	0.66	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
EK147	1.99	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
EK21	0.66	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
IN201	50.33	0.05 ± 0.01	0.12 ± 0.01	0.54 ± 0.03
INA	0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
EK37	0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
EK43	0.66	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
OC1	17.88	0.01 ± 0.00	0.06 ± 0.01	0.89 ± 0.02
OC2	66.89	0.02 ± 0.00	0.20 ± 0.01	0.89 ± 0.02
OC14	52.32	0.02 ± 0.01	0.16 ± 0.01	0.86 ± 0.03
Boro-14	17.88	0.01 ± 0.01	0.02 ± 0.00	0.82 ± 0.04
Boro-19	67.55	0.01± 0.01	0.17 ± 0.01	0.93 ± 0.02
ORD	47.68	0.00 ± 0.00	0.06 ± 0.01	0.00 ± 0.00
ORG	47.68	0.00 ± 0.00	0.06 ± 0.01	0.00 ± 0.00
ORH	48.34	0.00 ± 0.00	0.22 ± 0.02	0.00 ± 0.00
ORK	49.01	0.00 ± 0.00	0.24 ± 0.02	0.00 ± 0.00

P = percentage of polymorphic loci; Ho = observed heterozygosity; He = expected heterozygosity; I = Shannon's diversity index.

Intrapopulation diversity and relatedness to broomrape populations from other areas

Within the three broomrape populations SA1, SA2, and MK1, all the indexes of intrapopulation diversity (P, Ho, He), and Shannon's diversity index (I), indicated the absence of intrapopulation diversity (Table 2). Nei's unbiased genetic distance (uNeiP) between populations was zero between SA1 and SA2, and between MK1 and the four populations of the Guadalquivir Valley of races E and F. The genetic distance between SA1 and SA2, and MK1, was uNeiP = 0.71. The closest population to SA1 and SA2 was OC14 from Russia (uNeiP = 0.16), followed by OC2 from Romania (uNeiP = 0.43).

Relatedness of the Moroccan populations to those from other geographical areas is shown in the biplot of PCo1 and PCo2 (Figure 1), which explained 30.96% and 24.15%, respectively of the total variation. It can be observed how SA1 and SA2 are grouped together due to their null genetic distance. The same occurred for MK1 and the populations of races E and F of the Guadalquivir Valley.

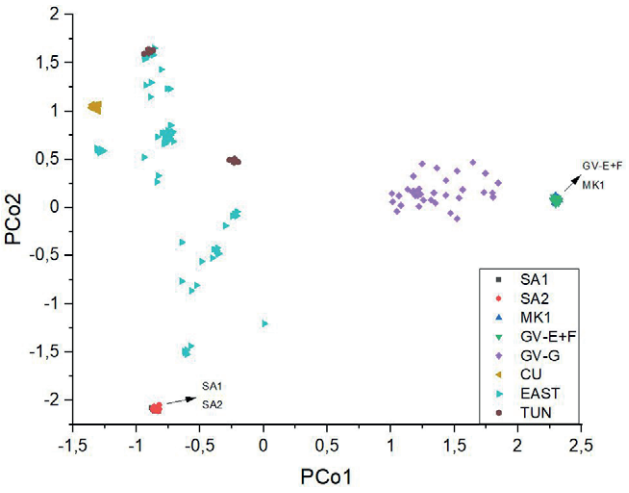

Figure 1. Principal coordinates analysis of four *Orobanche cumana* populations SA1, SA2, and MK1 collected in Morocco together with reference populations from the Guadalquivir Valley in Southern Spain, with virulence E and F (GV-E+F) or G (GV-G), Cuenca in Central Spain (CU), Eastern Europe (EAST) and Tunisia (TUN). The percentage of variation explained by each axis is given in parentheses.

DISCUSSION

This study has shown that the broomrape populations collected from sunflower in Morocco belong to two distant gene pools, which indicates two separate introduction events. The population MK1 collected in Meknès belongs to the classical gene pool of the Guadalquivir Valley in Southern Spain. Populations of this gene pool are genetically distant from the populations of Eastern Europe, and they are characterized by low intrapopulation genetic diversity (Pineda-Martos *et al.*, 2013). These Guadalquivir Valley populations were initially of race E until the second half of the 1990s, when a new race F emerged as a result of a point mutation, i.e., without alteration of the genetic structure of the populations (Pineda-Martos *et al.*, 2013). The mutation leading to race F virulence spread rapidly, due to selection pressure produced by the generalized cultivation of race-E resistant sunflower hybrids (Molinero-Ruiz and Domínguez, 2014). Molinero-Ruiz *et al.* (2008) found that all populations collected in the Guadalquivir Valley area in 1988 and 1989 already contained varying proportions of race-F individuals, and all these populations were infective to the sunflower differential line NR5. This line carries the *Or5* gene that confers resistance to race E but not to race F.

In the present research, population MK1 did not parasitize sunflower NR5, indicating that MK1 is a race E population. This, in turn, suggests that the broomrape seeds that founded this population were most likely introduced to Morocco before the generalized spread of race F in the Guadalquivir Valley, i.e., before 1988. This asks the question of why the population remained unobserved until 2016, when we detected it on broomrape plants in Meknès. There is no clear answer to this question, but it is important to note that an identical phenomenon occurred in the Castilla y León region in Northern Spain, where broomrape on sunflower was first observed in 2008 (Fernández-Escobar *et al.*, 2009). Evaluation of six populations collected in Castilla y León showed that three were race E of the Guadalquivir Valley gene pool (Malek *et al.*, 2017), like the MK1 population. Fernández-Escobar *et al.* (2009) suggested that the adaptation of populations to a new environment may provide an explanation for the long period that broomrape remained undetected, with low numbers of individuals each sunflower growing season.

The reaction of population MK1 with the set of differential lines requires further discussion. This population was avirulent on the race B resistant line J8281, while it was virulent on the race C differential line Record and the race D differential line S1358. This is a common behavior of race E and race F populations from the Guadalquivir Valley gene pool (Melero-Vara *et al.*, 2000). This is because the set of sunflower differential lines for races A to E was developed in Romania (Vranceanu *et al.*, 1980), and the gene pool of sunflower broomrape from the Guadalquivir Valley is genetically distant from the populations from eastern Europe (see Figure 1). Therefore, it is unsurprising that line S1358 has contrasting responses to sunflower broomrape populations from Romania and southern Spain. Despite this, we continue using this line in all studies on broomrape race classification to maintain a universal set of differential lines for use with broomrape populations from all geographical areas.

Populations SA1 and SA2 had similar virulence patterns, exhibiting race G virulence because they parasitized race F resistant sunflower lines P96 and LP2. The degree of attack of these populations on the host lines was low, which could be attributed to incomplete resistance of both lines to race G populations (Martín-Sanz *et al.*, 2016), and probably also to a low proportion of race G genotypes in the broomrape populations. SA1 and SA2 populations parasitizing line P96 indicates that they were most probably introduced from Eastern Europe, since the race G population from the Guadalquivir Valley has been shown to be avirulent on P96 (Martín-Sanz *et al.*, 2016). This was further supported by the Nei's unbiased genetic distances (Table 3) and PCoA analysis (Figure 1), which showed that SA1 and SA2 populations were distant from to the Guadalquivir Valley gene pool, and were closer to some populations from Eastern Europe, particularly to population OC14 from Russia. The objectives of the present study did not include unequivocal identification of the origin of broomrape populations in Morocco. To do that for populations SA1 and SA2, it would be necessary to include many populations from several countries, which was beyond the scope of this study. The objective was to provide preliminary indication of the putative area of origin, and to conduct detailed studies in further research. For the MK1 population, the results indicated that the area of origin was the Guadalquivir Valley. For SA1 and SA2, the results indicate that these populations originated from Eastern Europe, most likely Russia, but this should be confirmed by expanding comparative analysis to a broad set of populations from that area. The origin of these populations in Russia or surrounding countries is not unexpected, since Russian sunflower cultivars such as Peredovik, or other cultivars developed from Russian or Ukrainian germplasm, are cultivated in Morocco (Nabloussi *et al.*, 2011). Therefore, broomrape seeds may have been introduced associated with imported sunflow-

Table 3. Nei's unbiased genetic distances (uNeiP) between the Moroccan broomrape populations SA1, SA2 and MK1 and other populations used in this study from diverse geographical areas.

Population and provenance	SA1	SA2	MK1
SA2	0.00		
MK1	0.71	0.71	
OC94 (Guadalquivir Valley, Spain)	0.70	0.70	0.00
SP (Guadalquivir Valley, Spain)	0.70	0.70	0.00
EK147 (Guadalquivir Valley, Spain)	0.70	0.70	0.00
EK21 (Guadalquivir Valley, Spain)	0.70	0.70	0.00
IN201 (Guadalquivir Valley, Spain)	0.52	0.52	0.12
INA (Cuenca, Spain)	0.60	0.60	0.85
EK37 (Cuenca, Spain)	0.60	0.60	0.85
EK43 (Cuenca, Spain)	0.60	0.60	0.85
OC1 (Serbia)	0.66	0.66	0.66
OC2 (Romania)	0.43	0.43	0.62
OC14 (Russia)	0.16	0.16	0.57
Boro-14 (Turkey)	0.59	0.59	0.91
Boro-19 (Bulgaria)	0.50	0.50	0.60
ORD (Tunisia)	0.68	0.68	0.66
ORG (Tunisia)	0.68	0.68	0.66
ORH (Tunisia)	0.60	0.60	0.55
ORK (Tunisia)	0.60	0.60	0.54

er seed, which is one of the main modes of international broomrape dispersion (Fernández-Martínez *et al.*, 2015). Most relevant is that SA1 and SA2 populations were race G, which indicated that, unlike MK1, seed introduction was recent, as race G populations were not reported in Russia until 2013 (Antonova *et al.*, 2013).

Sunflower broomrape populations in Eastern Europe generally contain large intrapopulation diversity (Pineda-Martos *et al.*, 2014b; Bilgen *et al.*, 2019). This is partly due to co-existence in some areas of broomrape populations parasitizing sunflower crops and wild host species. Pineda-Martos *et al.* (2014b), documented gene flow between both types of populations, which contributed to increased genetic diversity in populations parasitizing on sunflower. Conversely, populations in areas where broomrape is not found in the wild, (e.g. Morocco and Spain) can exhibit no genetic diversity. This was the case for populations in Cuenca province in Central Spain and populations of races E and F from the Guadalquivir Valley in Southern Spain, which has been attributed to founder effects (Pineda-Martos *et al.*, 2014b). A similar situation in populations SA1 and SA2 in Morocco, where of numbers of seeds from a population from Eastern Europe has resulted in genetically homogeneous populations. For MK1, this population reproduces the absence of genetic variability of the original population from the Guadalquivir Valley.

Sunflower broomrape was considered to be an autogamous plant (Gagne *et al.*, 1998). However, several studies have shown cross fertilization in this species (Rodríguez-Ojeda *et al.*, 2013; Pineda-Martos *et al.*, 2013; Pineda-Martos *et al.*, 2014b; Martín-Sanz *et al.*, 2016). The appearance of race G populations in the Guadalquivir Valley area of Southern Spain was suggested to be due to mixture and subsequent genetic recombination between individuals of the two gene pools present in Spain (Martín-Sanz *et al.*, 2016). A similar situation may occur in Morocco if the individuals of the two gene pools identified in the present study come into contact and hybridize. Control measures are therefore required in Morocco to prevent introduction of new broomrape populations and expansion of existing ones, to limit the area infested by the parasite and to avoid creation of new populations with increased virulence, as has occurred in Southern Spain.

ACKNOWLEDGMENT

This study was funded by research project PID2020-117286RB-I00 of the Spanish Ministry of Economy and Innovation, and was co-funded with EU FEDER Funds.

LITERATURE CITED

Amri M., Abbes Z., Youssef S.B., Bouhadida M., Salah H.B., Kharrat M., 2012. Detection of the parasitic plant, *Orobanche cumana* on sunflower (*Helianthus annuus* L.) in Tunisia. *African Journal of Biotechnology* 11: 4163–4167. https://doi.org/10.5897/AJB11.3031

Antonova T., 2014. The history of interconnected evolution of *Orobanche cumana* Wallr. and sunflower in the Russian Federation and Kazakhstan. In: *Proceedings of the 3rd International Symposium on Broomrape* (Orobanche spp.) *in Sunflower*, International Sunflower Association, Paris, France, 57–64.

Antonova T.S., Araslanova N.M., Strelnikov E.A., Ramazanova S.A., Guchetl S.Z., Chelyustnikova T.A., 2013. Distribution of highly virulent races of sunflower broomrape (*Orobanche cumana* Wallr.) in the southern regions of the Russian Federation. *Russian Agricultural Sciences* 39: 46–50. https://doi.org/10.3103/S1068367413010023

Batchvarova R., 2014. Current situation of sunflower broomrape in Bulgaria. In: *Proceedings of the 3rd International Symposium on Broomrape* (Orobanche spp.) *in Sunflower*, International Sunflower Association, Paris, France, 51–54.

Beck-Mannagetta G., 1930. Orobanchaceae. In: *Das Pflanzenreich Vol. IV* (A. Engler, ed.), Verlag von Wilhelm Engelmann, Leipzig, 1–348.

Bilgen B.B., Barut A.K., Demirbaş S., 2019. Genetic characterization of *Orobanche cumana* populations from the Thrace region of Turkey using microsatellite markers. *Turkish Journal of Botany* 43: 3. https://doi.org/10.3906/bot-1807-71

Calderón-González A., Pouilly N., Muños S., Grand X., Coque M., Velasco L., Pérez-Vich B., 2019. A SSR-SNP linkage map of the parasitic weed *Orobanche cumana* Wallr. including a gene for plant pigmentation. *Frontiers in Plant Science* 10: 797. https://doi.org/10.3389/fpls.2019.00797

Cvejić, S., Radanović A., Dedić B., Jocković M., Jocić Miladinović D., 2020. Genetic and genomic tools in sunflower breeding for broomrape resistance. *Genes* 11: 152. https://doi.org/10.3390/genes11020152

Fernández-Escobar J., Rodríguez-Ojeda M.I., Fernández-Martínez J.M., Alonso L.C., 2009. Sunflower broomrape (*Orobanche cumana* Wallr.) in Castilla-León, a traditionally non-broomrape infested area in Northern Spain. *Helia* 51: 57–64. https://doi.org/10.2298/HEL0951057F

Fernández-Martínez J.M., Pérez-Vich B., Akhtouch B., Velasco L., Muñoz-Ruz J., Melero-Vara J.M., Domínguez J., 2004. Registration of four sunflower

germplasm lines resistant to race F of broomrape. *Crop Science* 44: 1033–1034. https://doi.org/10.2135/cropsci2004.1033

Fernández-Martínez J.M., Pérez-Vich B., Velasco L., 2015. Sunflower broomrape (*Orobanche cumana* Wallr.), in *Sunflower Oilseed. Chemistry, Production, Processing and Utilization* (E. Martínez-Force, N.T. Dunford, and B.K. Salas, eds.), AOC Press, Champaign, IL (USA), 129–156.

Gagne G., Roeckel-Drevet P., Grezes-Besset B., Shindrova P., Ivanov P., … Nicolas P., 1998. Study of the variability and evolution of *Orobanche cumana* populations infesting sunflower in different European countries. *Theoretical and Applied Genetics* 96: 1216–1222. https://doi.org/10.1007/s001220050859

González-Cantón E., Velasco-Sanchez A., Velasco L., Martin-Sanz A., 2019. First report of sunflower broomrape (*Orobanche cumana* Wallr.) in Portugal. *Plant Disease* 103: 2143. https://doi.org/10.1094/PDIS-10-18-1723-PDN

Jebri, M., Ben Khalifa M., Fakhfakh H., Pérez-Vich B., Velasco L., 2017. Genetic diversity and race composition of sunflower broomrape populations from Tunisia. *Phytopathologia Mediterranea* 56, 421–430. https://doi.org/10.14601/Phytopathol_Mediterr-20839

Jestin C., Lecomte V., Duroueix F., 2014. Current situation of sunflower broomrape in France. In: *Proceedings of the 3rd International Symposium on Broomrape* (Orobanche spp.) *in Sunflower*, International Sunflower Association, Paris, France, 28–31.

Kaya Y., 2014. Current situation of sunflower broomrape around the world. In: *Proceedings of the 3rd International Symposium on Broomrape* (Orobanche spp.) *in Sunflower*, International Sunflower Association, Paris, France, 9–18.

Ma D.T., Jan C.C., 2014. Distribution and race composition of sunflower broomrape (*Orobanche cumana* Wallr.) in Northern China. In: *Proceedings of the 3rd International Symposium on Broomrape* (Orobanche spp.) *in Sunflower*, International Sunflower Association, Paris, France, 65–69.

Malek J., del Moral L., Fernández-Escobar J., Pérez-Vich B., Velasco L., 2017. Racial characterization and genetic diversity of sunflower broomrape populations from Northern Spain. *Phytopathologia Mediterranea* 56: 70–76. https://doi.org/10.14601/Phytopathol_Mediterr-19163

Martín-Sanz A., Malek J., Fernández-Martínez J.M., Pérez-Vich B., Velasco L., 2016. Increased virulence in sunflower broomrape (*Orobanche cumana* Wallr.) populations from Southern Spain is associated with greater genetic diversity. *Frontiers in Plant Science* 7: 589. https://doi.org/10.3389/fpls.2016.00589.

Melero-Vara J.M., Domínguez J., Fernández-Martínez J.M., 2000. Update on sunflower broomrape situation in Spain: Racial status and sunflower breeding for resistance. *Helia* 33: 45–56. https://doi.org/10.1515/helia.2000.23.33.45

Molinero-Ruiz M.L., Domínguez J., 2014. Current situation of sunflower broomrape in Spain. In: *Proceedings of the 3rd International Symposium on Broomrape (Orobanche spp.) in Sunflower*, International Sunflower Association, Paris, France, 19–27.

Molinero-Ruiz M.L., Pérez-Vich B., Pineda-Martos R., Melero-Vara J.M., 2008. Indigenous highly virulent accessions of the sunflower root parasitic weed *Orobanche cumana*. *Weed Research* 48: 169–178. https://doi.org/10.1111/j.1365-3180.2007.00611.x

Nabloussi A., Fernández-Cuesta A., El-Fechtali M., Fernández-Martínez J.M., Velasco L., 2011. Performance and seed quality of Moroccan sunflower varieties and Spanish landraces used for confectionery and snack food. *Helia* 55: 75–82. https://doi.org/10.2298/HEL1155075N

Nabloussi A., Velasco L., Assissel N., 2018. First report of sunflower broomrape, *Orobanche cumana* Wallr, in Morocco. *Plant Disease* 102, 457. https://doi.org/10.1094/PDIS-06-17-0858-PDN

Parker C., 2016. *Orobanche cumana* (sunflower broomrape). In: *Invasive Species Compendium*. Wallingford, UK: CAB International. www.cabi.org/isc.

Pérez-Vich B., Akhtouch B., Knapp S.J., Leon A.J., Velasco L., Fernández-Martínez J.M., Berry S.T., 2004. Quantitative trait loci analysis of broomrape (*Orobanche cumana* Wallr.) resistance in sunflower. *Theoretical and Applied Genetics* 109: 92–102. https://doi.org/10.1007/s00122-004-1599-7

Pineda-Martos R., Velasco L., Fernández-Escobar J., Fernández-Martínez J.M., Pérez-Vich B., 2013. Genetic diversity of sunflower broomrape (*Orobanche cumana*) populations from Spain. *Weed Research* 53: 279–289. https://doi.org/10.1111/wre.12022

Pineda-Martos R., Velasco L., Pérez-Vich B., 2014a. Identification, characterisation and discriminatory power of microsatellite markers in the parasitic weed *Orobanche cumana*. *Weed Research* 54: 120–132. https://doi.org/10.1111/wre.12062

Pineda-Martos R., Pujadas-Salvà A.J., Fernández-Martínez J.M., Stoyanov K., Velasco L., Pérez-Vich B., 2014b. The genetic structure of wild *Orobanche cumana* Wallr. (Orobanchaceae) populations in Eastern Bulgaria reflects introgressions from weedy populations. *Scientific World Journal* 150432. https://doi.org/10.1155/2014/150432

Rodríguez-Ojeda M.I., Fernández-Martínez J.M., Velasco L., Pérez-Vich B., 2013. Extent of cross-fertilization in *Orobanche cumana* Wallr. *Biologia Plantarum* 57: 559–562. https://doi.org/10.1007/s10535-012-0301-1

Velasco, L., Pérez-Vich B., Yassein A.A.M., Jan C.C., Fernández-Martínez J.M., 2012. Inheritance of resistance to sunflower broomrape (*Orobanche cumana* Wallr.) in an interspecific cross between Helianthus annuus and *H. debilis* subsp. *tardiflorus*. *Plant Breeding* 131: 220–221. https://doi.org/10.1111/j.1439-0523.2011.01915.x

Vrânceanu A.V., Tudor V.A., Stoenescu F.M., Pirvu N., 1980. Virulence groups of *Orobanche cumana* Wallr., differential hosts and resistance source genes in sunflower. In: *Proceedings of the 9th International Sunflower Conference*, International Sunflower Association, Paris, France, 74–82.