Phytopathologia Mediterranea (2017), 56, 3, 421–430
DOI: 10.14601/Phytopathol_Mediterr-20839

RESEARCH PAPERS

Genetic diversity and race composition of sunflower broomrape populations from Tunisia

Malek JEBRI[1,2], Mekki BEN KHALIFA[2,3], Hatem FAKHFAKH[2,3], Begoña PÉREZ-VICH[1] and Leonardo VELASCO[1]

[1] Institute for Sustainable Agriculture (IAS-CSIC), Córdoba, Spain
[2] Laboratory of Molecular Genetics, Immunology and Biotechnology, Faculty of Science of Tunis, University of Tunis El Manar, Tunisia
[3] Faculty of Sciences of Bizerte, Carthage University, Zarzouna, Tunisia

Summary. Sunflower broomrape (*Orobanche cumana* Wallr.) was detected in 2010 for the first time in sunflower fields of Tunisia, in the Béja region. No information is available on the race composition and genetic diversity of the broomrape populations parasitizing sunflower in that area. Plant tissue and seeds were collected from nine populations in Béja Sud (ORD, ORE), Amdoun (ORF, ORG), and Béja Nord (ORH to ORL) areas of the Béja region. Virulence studies on populations ORD, ORH and ORK revealed that the ORD population was essentially race E, whereas race G individuals were present in ORH and ORK poulations, with greater frequency in the ORK population. Cluster analysis of inter-population relatedness indicated that Tunisian populations were more related to Eastern European than to Spanish populations, with the Béja Nord populations clustering separately from the rest of Tunisian populations. Analysis at the intra-population level of the ORD, ORG, ORH and ORK populations revealed the existence of two gene pools (GP1 and GP2), that were present in all populations at different frequencies. GP2 was at a lower frequency (1/14) in ORD and ORG but at higher frequencies in ORH (5/15) and ORK (8/14). This indicates that GP1 was probably an initial introduction of a race E population, while GP2 is most likely a later introduction of a race G population, introduced initially in Béja Nord area but now spreading to other areas in the region. The risk of a generalized expansion of race G of broomrape in this sunflower cultivation area is discussed.

Key words: gene flow, parasitic weed, SNP markers, virulence, plant introductions.

Introduction

Sunflower broomrape (*Orobanche cumana* Wallr.) is naturally distributed in a broad area between South-eastern Europe and Central Asia, parasitizing wild Compositae species, mainly in the genera *Artemisia* L., *Xanthium* L. and *Carelinia* (Pall.) Less. (Beck-Mannagetta, 1930; Novopokrovsky and Tzvelev, 1955). Parasitization of sunflower crops was first observed in the Voronezh region of Russia in 1866 (Antonova, 2014). The parasite rapidly expanded across sunflower cultivation areas of Russia (Antonova, 2014). Later, it was observed in other countries around the Black Sea. It was first observed in 1935 in Bulgaria (Batchvarova, 2014), in 1940–41 in Romania (Pacureanu, 2014), in the second half of 1940s in Turkey (Demirci and Kaya, 2009), and in 1951 in Serbia (Miladinovic *et al.*, 2014). In Spain, where sunflower broomrape is not found parasitizing wild host species, it was initially observed in sunflower crops in 1958 in Central Spain and later in the Guadalquivir Valley in the south of that country (Alonso *et al.*, 1996). Sunflower infestations have also been observed from 1979 in China (Ma and Jan, 2014) and, more recently, from 2007 in France (Jestin *et al.*, 2014) and from 2010 in Tunisia (Amri *et al.*, 2012).

The genetic interaction between broomrape and sunflower has been reported in most cases as governed by a gene-for-gene interaction, characterized by the existence of physiological races of the parasite

Corresponding author: L. Velasco
E-mail: lvelasco@ias.csic.es

www.fupress.com/pm
Firenze University Press
ISSN (print): 0031-9465
ISSN (online): 1593-2095

and dominant monogenic resistance mechanisms in the host plants (Fernández-Martínez *et al.*, 2015). Vrânceanu *et al.* (1980) identified the existence of five races, named as A to E, and developed a set of differential lines to identify these races carrying, respectively, the resistance genes *Or1* to *Or5*. New races appeared in several countries from the mid 1990's onwards. These have been designated as races F, G and H, although the relationships between populations from different geographic areas have not been studied (Fernández-Martínez *et al.*, 2015). Recently, a nomenclature based on the identification of the race and the geographic area of collection, e.g. G_{TK} to designate race G from Turkey, has been proposed (Martín-Sanz *et al.*, 2016).

In the wild, sunflower broomrape has been described as a polymorphic species (Novopokrovsky and Tzvelev, 1955). A comparison of genetic diversity between populations parasitizing wild species and sunflower in Eastern Bulgaria revealed a significant loss of genetic diversity in populations parasitizing this host (Pineda-Martos *et al.*, 2014a). Several studies conducted on sunflower broomrape populations from crop fields in its natural distribution area have concluded that there is low genetic diversity within populations and small degree of differentiation between populations (Gagne *et al.*, 1998; Ciuca *et al.*, 2004; Atanasova *et al.*, 2005; Guchetl *et al.*, 2014; Molinero-Ruiz *et al.*, 2014). In Spain, however, where this species is not found in the wild, two well-differentiated gene pools have been described in Central Spain and the Guadalquivir Valley, with extremely low genetic diversity within each gene pool. This has been attributed to a founder effect after separate introduction events (Pineda-Martos *et al.*, 2013).

There is no information on the genetic diversity of sunflower broomrape populations from other regions out of its natural distribution area. In Tunisia, sunflower broomrape was first observed in the Béja region during the 2009–2010 agricultural season (Amri *et al.*, 2012). The objective of the present study was to determine race composition and genetic diversity of sunflower broomrape populations from sunflower fields of the Béja region of Tunisia.

Materials and methods

Sunflower broomrape populations

Plant tissue of individual plants from nine sunflower broomrape populations was collected at seven locations of Béja Governorate (Figure 1), the only area of Tunisia where infestations of this parasitic plant have been reported (Amri *et al.*, 2012). The populations were designated ORD to ORL. Populations ORD and ORE were collected in Béja Sud delegation. Populations ORF and ORG were collected in Amdoun delegation. The populations ORH to ORL were collected in Béja Nord delegation, with populations ORJ, ORK, and ORL collected in very closely associated fields within one area.

Mature seeds were also collected from all the populations except population ORG, but enough seeds for evaluation of race composition using differential lines were only available for populations ORD, ORH, and ORK. Germination capacity of the seeds of these three populations was measured using root exudates of susceptible sunflower line B117. Eighteen seeds of B117 were sown and the plants maintained for 3 weeks on sterile perlite in a growth chamber at 25/20°C (day/night) with 16 h photoperiod and photon flux density of 300 μmol m^{-2} s^{-1}. Plants received Hoagland's nutrient solution twice per week. The plants were then removed from the perlite and the roots were carefully washed and immersed for 3 d in 0.5 L capacity flasks containing distilled water in groups of six plants per flask, to release root exudates. After 3 d, the plants were removed, and the roots were dried and weighed. The root solution from each flask was diluted with distilled water to achieve a ratio of 0.10 g of fresh weight of root per mL of water. The root solution was used to determine seed germination of the three sunflower broomrape populations on filter paper discs in Petri dishes. Three replications were used, each corresponding to one root extract collection flask. Germinating seeds were maintained in the dark at 25°C for 2 weeks. After that period, the percentage of germinated seeds was determined in four areas of 2.25 cm^2 each in each Petri dish.

Twenty-three sunflower broomrape populations from several countries, previously described by Martín-Sanz *et al.* (2016), were used to evaluate molecular genetic similarity of Tunisian populations with those from other areas. They included four race F populations from the Guadalquivir Valley in Southern Spain (CO-02, SE-10, EK-23, and SP), four race G populations from the same area (BR-24, BR-25, BR-27, and BR-28), four populations from Central Spain (IASCum-4, CU-05, CU-07, CU-12), one from Serbia (Boro-9), one from Romania (Boro-10), one from Israel (Boro-11), two from Turkey (Boro-14, Boro-15), and six populations from Bulgaria (Boro-16 to Boro-19, Boro-21, and Boro-22). In all cases, seeds were collected in the respective

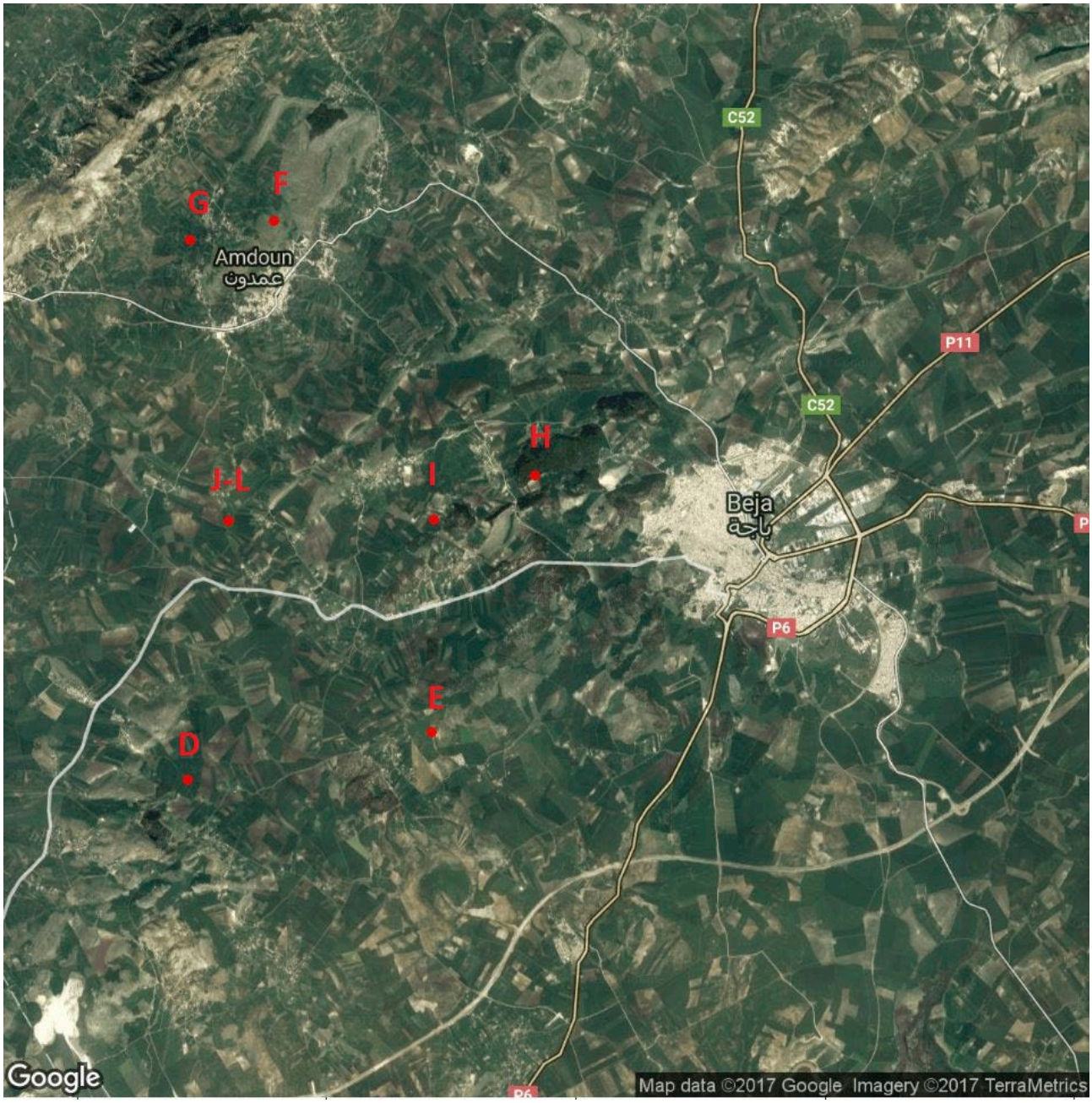

Figure 1. Locations where sunflower broomrape populations were collected. Letters D to L represent populations ORD to ORL.

areas and tissue from 20 individual plants growing on the sunflower susceptible line B117 was collected for DNA extraction. Equal amounts of DNA of the plants from each population were pooled and used as a template for PCR amplification.

Race composition

Race composition was studied in three broomrape populations (ORD, ORH, and ORK) for which sufficient amount of seed was available, using eight differ-

ential inbred lines. These were: B117, susceptible to all races; J8281resistant to race B, Record to race C, S1358 to race D, and P1380 resistant to race E (Vrânceanu *et al.*, 1980); LP2, resistant to race F and susceptible to race G populations from the Guadalquivir Valley and Eastern Europe; P96, resistant to race F and race G populations of the Guadalquivir Valley and susceptible to race G populations from Eastern Europe (Martín-Sanz *et al.*, 2016); and DEB2, resistant to race F and race G populations from the Guadalquivir Valley and Eastern Europe (Martín-Sanz *et al.*, 2016).

Because of the limitation in the amount of seed available, evaluation of the inbred lines for reaction to the three sunflower broomrape populations was conducted in small pots containing 40 cm^3 of infested substrate in a multi-pot tray. Two replications of ten plants each were used for each combination on inbred line and broomrape population. The substrate consisted of a mixture of sand and peat (1:1 by volume) to which sunflower broomrape seeds were added at an approximate concentration of 100 seeds (approx. 0.15 mg) per cm^3 of soil. The mixture was carefully shaken in a plastic bag to obtain an homogeneously infested substrate. Sunflower seeds were germinated on moistened filter paper and planted in the pots. The plants were grown in a growth chamber at 25/20°C (day/night) with a 16-h photoperiod and photon flux density of 300 μmol m^{-2} s^{-1} for 60 d, after which they were uprooted for counting the number of sunflower broomrape attachments in the roots (Rodríguez-Ojeda *et al.*, 2013a). The plants were watered as required, and not fertilized. Statistically significant differences for the numbers of broomrape attachments per plant were assessed by one-way ANOVA and Tukey's test ($P<0.05$), using IBM SPSS Statistics version 20 (IBM Corp.).

DNA extraction, SSR and SNP analyses

Tissue of individual sunflower broomrape plants was lyophilized and ground to a fine powder using a laboratory ball mill. DNA was extracted from individual plants using the protocol described in Malek *et al.* (2017).

For the interpopulation diversity study, equal amounts of DNA of 14 broomrape plants from each population were pooled and used as a template for PCR amplification. SSR analyses were conducted following Pineda-Martos *et al.* (2014b), using 27 high-quality, polymorphic SSR primer pairs reported in that study. These were: Ocum-003, Ocum-006, Ocum-011, Ocum-023, Ocum-040, Ocum-041, Ocum-052, Ocum-056, Ocum-059, Ocum-063, Ocum-070, Ocum-074, Ocum-075, Ocum-081, Ocum-085, Ocum-087, Ocum-091, Ocum-092, Ocum-108, Ocum-122, Ocum-136, Ocum-141, Ocum-151, Ocum-160, Ocum-174, Ocum-196, and Ocum-197. Amplification products were separated by gel electrophoresis using 3% Metaphor agarose (BMA) in 1× TBE buffer and SaveView Nucleic Acid Stain (NBS Biologicals Ltd.), and viewed under UV light. A 100 bp DNA ladder (Solis BioDyne) was used as a standard molecular weight marker. Bands were scored manually with the support of Quantity One® 1-D Analysis Software (Bio-Rad Laboratories Inc.). Amplified fragments were scored for the presence (1) or absence (0) of homologous bands and compiled into a binary data matrix.

For the intrapopulation diversity study, DNA from each of 14 plants per population was used. A set of 164 unpublished SNP markers, provided by Biogemma (France) were used. Individual plants were genotyped for these SNPs using the KASP™ genotyping assay service provided by LGC.

Analysis of interpopulation relatedness

Interpopulation relatedness was evaluated on the nine broomrape populations from Tunisia and the set of 23 populations from other areas, applying Cluster analysis to the binary matrix developed from SSR markers. For this, the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was applied to a dissimilarity matrix produced by transformation of the original binary matrix with DICE dissimilarity index, using NTSYSpc ver. 2.21q (Applied Biostatistics Inc.).

Analysis of intrapopulation diversity

Intrapopulation genetic diversity was evaluated for populations ORD, ORG, ORH, and ORK, with a set of 164 SNP markers. These populations were selected based on cluster analysis of interpopulation relatedness, in which no differences between ORD and ORE, ORF and ORG, ORH and ORI, and ORJ, ORK and ORL were observed. The following parameters were computed using GenAlEx ver. 6.5: percentage of polymorphic loci (P), observed and expected heterozygosity (Ho and He), and Shannon's diversity index (I). Pairwise genetic distances

Table 1. Mean percentage of susceptible plants (%S) and average number of sunflower broomrape shoots in susceptible plants (Xs, mean ± standard deviation) in eight differential inbred lines of sunflower inoculated with three broomrape populations collected from Tunisia (ORD, ORH and ORK).

Line/Hybrid[a]	Sunflower broomrape populations[b]					
	ORD		ORH		ORK	
	%S	Xs	%S	Xs	%S	Xs
B117	100	28.0±9.5b	100	24.8±7.0ab	100	20.2±5.9a
J8281	0	-	65	2.4±2.3a	100	8.5±5.2b
Record	100	4.1±2.5a	100	5.0±3.3ab	100	6.7±4.0b
S1358	100	4.8±4.1a	100	10.4±3.9b	100	17.0±6.8c
P1380	0	-	35	1.1±0.6a	100	4.7±8.0b
P96	0	-	0	-	0	-
LP2	0	-	10	1.0±0.3a	100	3.2±1.9b
DEB2	0	-	0	-	0	-

[a] B117 is susceptible to all races; J8281 is resistant to race B; Record is resistant to race C; S1358 is resistant to race D; P1380 is resistant to race E; LP2 is resistant to race F and susceptible to race G populations from the Guadalquivir Valley and Eastern Europe; P96 is resistant to race F and race G populations of the Guadalquivir Valley and susceptible to race G populations from Eastern Europe; DEB2 is resistant to race F and race G populations from the Guadalquivir Valley and Eastern Europe.

[b] Xs values with different letters within the same row are statistically different ($P<0.05$)

between populations were calculated as the genetic distance coefficient GST, using 1,000 random permutations to assess significance. The matrix of GST pairwise distances was used as input for a principal co-ordinate analysis (PCoA).

The average number of pairwise differences between individuals of each population (PiX) was computed as an estimate of intra-population genetic diversity. Analysis of molecular variance (AMOVA) was conducted on the distance matrix to separate total variance into variance attributable to differences between individuals within a population and variance attributable to differences between populations. Both analyses were computed with Arlequin ver. 3.5.2.2 (L. Excoffier, CMPG, University of Bern, Switzerland).

Results

Race classification of the sunflower broomrape populations

Population ORD did not parasitize line J8281 resistant to race B, parasitized lines Record resistant to race C and S1358 resistant to race D, and did not parasitize lines P1380 resistant to race E or the other lines resistant to more virulent populations (Table 1). Population ORD was accordingly classified as race E. Population ORH had a similar pattern to ORD but contained some individuals with ability to parasitize lines J8281, P1380, and LP2. Since the level of parasitization on P1380 resistant to race E, and LP2 resistant to race F, was small and similar, the results suggest that the ORH population was composed of race E individuals with a very low proportion of race G individuals. Conversely, the ORK population was more virulent on lines P1380 and LP2, which were 100% susceptible, suggesting a greater proportion of race G individuals. Since race classification in sunflower is conventionally made considering the most aggressive individuals present in the population (Martín-Sanz *et al.*, 2016), both ORH and ORK populations should be considered as race G populations. The observed differences between the three populations cannot be attributed to differences in germination, as the three populations showed no significant ($P<0.05$) differences in mean germination percent-

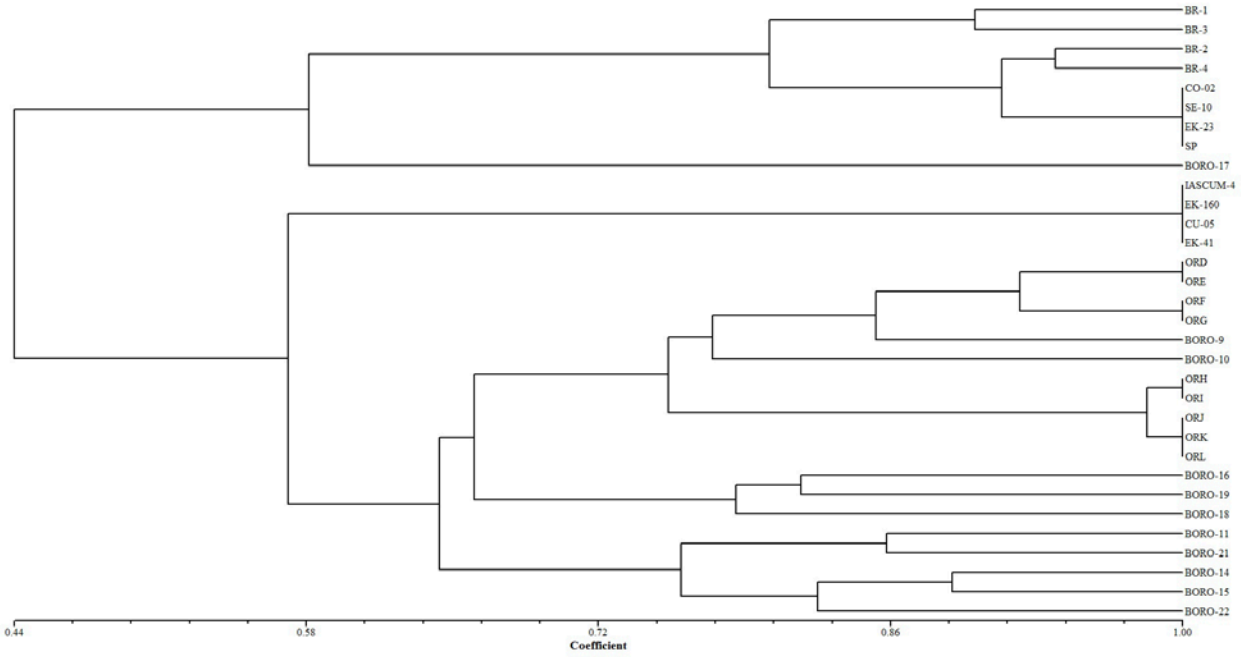

Figure 2. Tree plot of the nine broomrape populations studied (ORD through ORL), together with eight reference populations from the Guadalquivir Valley in Southern Spain classified as race F (populations CO-02, SE-10, EK-23, and SP) and race G (BR-24, BR-25, BR-27, and BR-28), four populations from Central Spain (IASCum-4, CU-05, CU-07, CU-12), one from Serbia (Boro-9), one from Romania (Boro-10), one from Israel (Boro-11), two from Turkey (Boro-14, Boro-15), and six populations from Bulgaria (Boro-16 to Boro-19, Boro-21, and Boro-22).

age: 42.11 ± 3.89 for ORD, 40.97 ± 5.35 for ORH, and 38.97 ± 4.03 for ORK.

Inter-population relatedness

Cluster analysis on bulked DNA of the populations showed that Tunisian populations fell into a cluster which included most of the populations from Eastern Europe. The exception was Boro-17 from Bulgaria, which clustered closer to populations from the Guadalquivir Valley of Spain (Figure 2). Tunisian populations formed a separate subcluster together with populations Boro-9 from Serbia, and Boro-10 from Romania. No differences for the SSR marker scores were observed between populations ORD and ORE, between ORF and ORG, between ORH and ORI, and between ORJ, ORK, and ORL. Populations ORD and ORE from Béja Sud grouped together with populations ORF and ORG from Amdoun. This cluster was closer to populations Boro-9 and Boro-10 than to the five populations from Béja Nord, which clustered together (Figure 2).

Intra-population diversity

Populations ORD and ORG were similar for all the genetic diversity parameters, as was the case for populations ORH and ORK. The latter group differed clearly from the former for the expected heterozygosity (He), Shannon Index (I), and average number of pairwise differences between individuals (PiX) (Table 2). For these parameters, populations ORH and ORK showed greater values than populations ORD and ORG, indicating greater intra-population diversity. This was more clearly seen in the Principal Coordinate Analysis, which revealed the existence of two gene pools in the populations clearly separated along Coordinate 1, that explained 96.30% of the total variation (Figure 3). The four populations contained individuals of both gene pools, but at different frequencies: 1/14 in ORD and ORG, 5/14 in ORH, and 8/14 in ORK. AMOVA analysis confirmed that differences within populations (76.65% of total variance) was greater than differences between populations (25.35%).

Table 2. Genetic diversity parameters of four sunflower broomrape populations (ORGD, ORG, ORH, and ORK) from Tunisia.

Population	P[a]	H_0 (±SE)	He (±SE)	I (±SE)	PiX
ORD	45.73	0.00	0.06 ± 0.01	0.12 ± 0.01	10.02
ORG	45.73	0.00	0.06 ± 0.01	0.12 ± 0.01	10.01
ORH	46.34	0.00	0.21 ± 0.02	0.30 ± 0.03	34.28
ORK	46.95	0.00	0.23 ± 0.02	0.32 ± 0.03	36.94

[a] P = percentage of polymorphic loci; Ho, observed heterozygosity; He, expected heterozygosity; I, Shannon's diversity index; PiX, average number of pairwise differences between individuals within each population.

Table 3. Analysis of molecular variance in a set of four populations of sunflower broomrape populations (ORD, ORH, ORG, ORK) collected in Tunisia.

Source of variation	Sum of squares	Variance components	% Variation	*P* value
Among populations	359.49	3.87	25.35	< 0.01
Within populations	1231.82	11.41	74.65	< 0.01

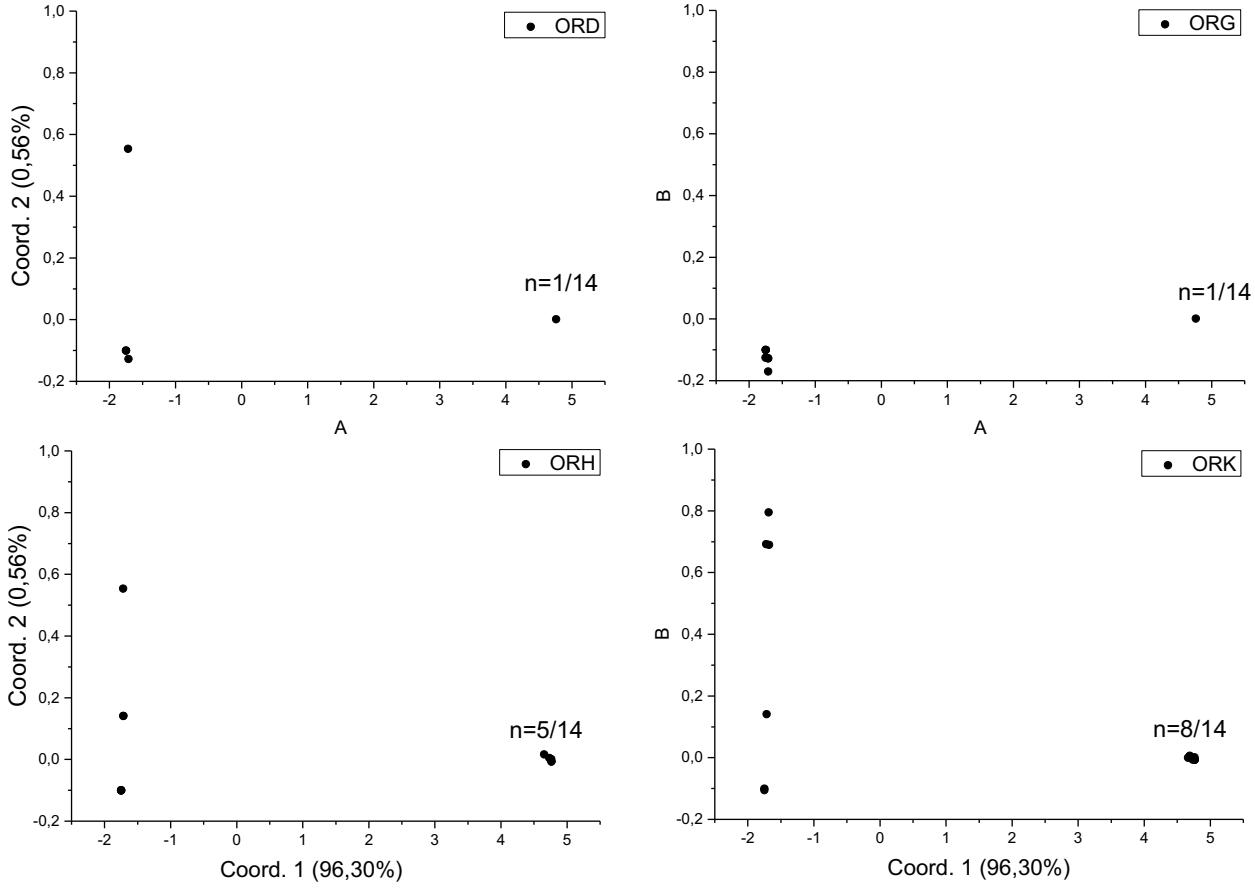

Figure 3. Principal coordinates analysis of four sunflower broomrape populations (ORD, ORH, ORG, ORK) collected in Tunisia. The analysis was carried out for the four populations together, but they are represented in separate graphs.

Discussion

The results of this study showed that sunflower broomrape populations from Tunisia are composed by individuals of two distinct gene pools, that we have designated as GP1 (predominant in populations ORD and ORG), and GP2 (predominant in population ORK). The populations mainly differed in the frequencies of individuals of both pools. There was an association between the frequency of GP2 individuals and the level of virulence of the populations. Population ORD, with 1/14 of GP2 individuals, was classified as race E, whereas ORH, with 5/14 individuals of GP2, formed nodules on 35% of race-E resistant P1380 plants, and ORK, with 8/14 individuals of GP2, formed nodules on 100% of P1380 plants. This suggests, as a preliminary hypothesis, that GP1 plants have no capacity to overcome the *Or5* gene, which confers resistance to sunflower broomrape race E, whereas GP2 plants probably have capacity to parasitize plants having resistance genes against races E and F, as 100% of plants of line LP2, resistant to race F, showed also parasitization nodules. Such distant gene pools are probably the result of separate broomrape introductions in Tunisia, probably from Eastern Europe. Considering the collection locations of the populations, GP1 was probably a first introduction of a race E population that has subsequently been widely distributed throughout the Béja region, while GP2 is most likely a more recent introduction of a race G population, initially introduced in Béja Nord area but that is spreading to other areas. The fact that GP1 and GP2 show marked genetic differences supports that they may have been the result of two separate introduction events. Studies in Spain, which is also outside the natural broomrape distribution area, revealed the existence of two distant gene pools probably corresponding to two different introduction events (Pineda-Martos *et al.*, 2013; Molinero-Ruiz *et al.*, 2014). ORD, which was classified as race E because it parasitized differential lines Record and S1358, did not parasitize the race B resistant line J8281 (Table 1). A similar situation was reported by Melero-Vara *et al.* (1989) for populations from Spain, which were able to parasitize the race C resistant line Record but not on race B resistant line J8281. Molinero-Ruiz and Domínguez (2014) concluded that the set of differential lines developed by Vrânceanu *et al.* (1980) for Romanian broomrape populations have limitations for accurate race classification of Spanish populations. The situation may be similar for populations of new areas such as Tunisia, but this should be examined with more detail. The set of differential lines has been used in the present study because it is the only publicly available set of differential lines for sunflower broomrape race classification.

The observed heterozygosity was zero in the four populations evaluated at the intra-population level, despite the presence of individuals of both gene pools in all populations. In Spain, Pineda-Martos *et al.* (2013) and Martín-Sanz *et al.* (2016) characterized the presence of heterozygous individuals in populations containing the two gene pools present in that country. The reason for not observing heterzygotes in the populations from Tunisia may be that a very recent introduction of one of the gene pools has occurred, probably the most virulent one, GP2. Another possibility is that environmental conditions are not favourable for cross pollination. Sunflower broomrape is essentially self-pollinated (Satovic *et al.*, 2009; Pineda-Martos *et al.*, 2014a). However, it has been demonstrated that cross-pollination may be as greater as around 30% under certain conditions, and small insects of the Halictidae family were involved in cross pollination of sunflower broomrape plants in Spain (Rodríguez-Ojeda *et al.*, 2013b). Therefore, the abundance of appropriate pollinators may also be an important factor determining the heterozygosity of sunflower broomrape populations that contain more than one gene pool.

Selection pressure is the main driving force for racial evolution in the case of sunflower broomrape populations heterogeneous for virulence (Molinero-Ruiz *et al.*, 2008). In these populations, the use of sunflower hybrids resistant to the predominant gene pool with low virulence may result in a drastic increase of frequencies of another gene pool that initially was present at a lower frequency in the seed bank (Alonso, 2014). This is probably the situation that is currently occurring in the Béja region of Tunisia. This study identified individuals of GP2 in all populations examined at the intrapopulation level. If our hypothesis that GP2 is associated with race G virulence is true, then there is a risk of generalized distribution of the race G population through this sunflower producing area if sunflower hybrids resistant to race E and/or race F are used. The use of sunflower genotypes combining several resistance mechanisms (Pérez-Vich *et al.*, 2013), or the combined use of genetic resistance to broomrape and for herbicide tolerance (Alonso, 2014), have been suggested

as strategies to avoid drastic changes is the race composition of sunflower broomrape populations.

Acknowledgments

This study was been funded by research project AGL2014-53886-P of the Spanish Ministry of Economy and Innovation, co-funded with EU FEDER Funds, and the Tunisian Ministry of Higher Education and Research by a grant awarded to M. Jebri. The authors are grateful to Dr M. Amri for his assistance in the collection of sunflower broomrape populations, and to Biogemma for providing access to their proprietary SNP markers.

Literature cited

Alonso L.C., 2014. Syngenta's integrated sunflowers broomrape management program. In: *Proceedings of the 3rd International Symposium on Broomrape* (Orobanche spp.) *in Sunflower*. International Sunflower Association, Paris, France, 237–252.

Alonso L.C., J. Fernández-Escobar, G. López, M.I. Rodríguez-Ojeda, and F. Sallago, 1996. New highly virulent sunflower broomrape (*Orobanche cernua* Loefl.) pathotype in Spain. In: *Advances in Parasitic Plant Research. Sixth International Parasitic Weed Symposium* (M.T. Moreno, M.T., J.I. Cubero, D. Berner, D. Joel, L.J. Musselman and C. Parker, eds), Junta de Andalucia, Sevilla, Spain, 639–644.

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

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.

Atanasova R., R. Batcharova, E. Todorovska and A. Atanassov, 2005. Molecular study of broomrape (*Orobanche* spp.) by RAPD analyses. *Biotechnology & Biotechnological Equipment* 19, 51–60.

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, Germany, 348 pp.

Ciuca M., M. Pacureanu and M. Iuora, 2004. RAPD markers for polymorphism identification in parasitic weed *Orobanche cumana* Wallr., *Romanian Agriculture Research* 21, 29–32.

Demirci M. and Y. Kaya, 2009. Status of *Orobanche cernua* Loefl. and weeds in sunflower production in Turkey. *Helia* 51, 153–160.

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

Gagne G., P. Roeckel-Drevet, B. Grezes-Besset, P. Shindrova, P. Ivanov, C. Grand-Ravel, F. Vear, D. Tourvieille de Labrouhe, G. Charmet and P. Nicolas, 1998. Study of the variability and evolution of *Orobanche cumana* populations infesting sunflower in different European countries. *Theoretical and Applied Genetics* 96, 1216–1222.

Guchetl S.Z., T.S. Antonova and T.A. Tchelustnikova, 2014. Genetic similarity and differences between the *Orobanche cumana* Wallr. populations from Russia, Kazakhstan, and Romania revealed using the markers of simple sequence repeat. *Russian Agricultural Sciences* 40, 326–330.

Jestin C., V. Lecomte and F. Duroueix, 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.

Ma D.T. and C.C. Jan, 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., L. del Moral, J. Fernández-Escobar, B. Pérez-Vich and L. Velasco, 2017. Racial characterization and genetic diversity of sunflower broomrape populations from Northern Spain. *Phytopathologia Mediterranea* 56, 70–76.

Martín-Sanz A., J. Malek, J.M. Fernández-Martínez, B. Pérez-Vich and L. Velasco, 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.

Melero-Vara, J.M., J. Domínguez and J.M. Fernández-Martínez, 1989. Evaluation of differential lines and a collection of sunflower parental lines for resistance to broomrape (*Orobanche cernua*) in Spain. *Plant Breeding* 102, 322–326.

Miladinović D., S. Jocić, B. Dedić, S. Cvejić, A. Dimitrijević, I. Imerovski and G. Malidža, 2014. Current situation of sunflower broomrape in Serbia. In: *Proceedings of the 3rd International Symposium on Broomrape* (Orobanche spp.) *in Sunflower*. International Sunflower Association, Paris, France, 33–38.

Molinero-Ruiz M.L. and J. Domínguez, 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., B. Pérez-Vich, R. Pineda-Martos and J.M. Melero-Vara, 2008. Indigenous highly virulent accessions of the sunflower root parasitic weed *Orobanche cumana*. *Weed Research* 48, 169–178.

Molinero-Ruiz M.L., A.B. García-Carneros, M. Collado-Romero, S. Raranciuc, J. Domínguez and J.M. Melero-Vara, 2014. Pathogenic and molecular diversity in highly virulent populations of the parasitic weed *Orobanche cumana* from Europe. *Weed Research* 54, 87–96.

Novopokrovsky I.V. and N.N. Tzvelev, 1955. Orobanchaceae Lindl., in *Flora SSSR, Volume XXIII* (B.K. Schischkin, ed.), Izdatelstvo Akademii Nauk SSSR, Moskva-Leningrad, 19–117.

Pacureanu M.J., 2014. Current situation of sunflower broomrape (Orobanche cumana Wallr.) in Romania. In: *Proceedings of the 3rd International Symposium on Broomrape* (Orobanche spp.) *in Sunflower*. International Sunflower Association, Paris, France, 39–43.

Pérez-Vich B., L. Velasco, P.J. Rich and G. Ejeta, 2013. Marker-assisted and physiology-based breeding for resistance to root parasitic Orobanchaceae. In: *Parasitic Orobanchaceae* (D.M. Joel, J. Gressel, and L.J. Musselman, eds.), Springer, New York, USA, 369–391.

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

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

Pineda-Martos R., L. Velasco and B. Pérez-Vich. 2014b. Identification, characterization, and discriminatory power of microsatellite markers in the parasitic weed *Orobanche cumana*. *Weed Research* 54, 120–132.

Rodríguez-Ojeda M.I., R. Pineda-Martos, L.C. Alonso, J. Fernández-Escobar, J.M. Fernández-Martínez, B. Pérez-Vich and L. Velasco, 2013a. A dominant avirulence gene in *Orobanche cumana* triggers *Or5* resistance in sunflower. *Weed Res.* 53, 322–327.

Rodríguez-Ojeda M.I., J.M. Fernández-Martínez, L. Velasco and B. Pérez-Vich, 2013b. Extent of cross-fertilization in *Orobanche cumana* Wallr. *Biologia Plantarum* 57, 559–562.

Satovic Z., D.M. Joel, D. Rubiales, J.I. Cubero and B. Román, 2009. Population genetics in weedy species of *Orobanche*. *Australasian Plant Pathology* 38, 228–234.

Vrânceanu A.V., V.A. Tudor, F.M. Stoenescu and N. Pirvu, 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.

Accepted for publication: November 24, 2017