

# INHERITANCE OF RESISTANCE TO BROOMRAPE IN SUNFLOWER INBRED LINE LIV-17

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## Abstract

**Abstract:** Genetic resistance to broomrape (*Orobanche cumana* Wallr.) in sunflower is the most effective way to control the parasite. The objective of this study was to determine the inheritance and gene action for broomrape resistance in sunflower inbred line LIV-17. This line was first tested and found to be resistant to broomrape, in heavily infested locations in Spain and Turkey, where races F and G were predominant. Resistant line was crossed with susceptible line HA-26-PR and F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> generation were developed. All plants in F<sub>1</sub> generation were susceptible, indicating recessive inheritance. The segregation ratios in F<sub>2</sub> generation had a goodness of fit to the expected ratio 3(S):1(R), indicating that broomrape resistance was controlled by a single recessive gene. Testing of the F<sub>3</sub> generation gave a more precise evaluation and enabled differentiation between homozygous and heterozygous susceptible plants. The respective F<sub>3</sub> families segregated, as expected, in 1 (homozygous S): 2 (heterozygous S): 1 (homozygous R) ratio, confirming that this line is resistant to broomrape when gene was present in homozygous recessive condition. From the breeding perspective, recessive nature of resistance to broomrape in LIV-17 highlights the necessity to introduce resistance genes into both parental lines in order to obtain resistant hybrid.

Key words: *sunflower, broomrape, inheritance, recessive gene*

## Introduction

The parasitic weed *Orobanche cumana* (sunflower broomrape) is an obligatory and non-photosynthetic root parasitic plant of the sunflower (*Helianthus annuus* L.) and is a substantial threat in Europe, especially in countries around the Black Sea and in Spain (Molinero-Ruiz et al., 2013, Louarn et al., 2016). Under favourable conditions, it infects the roots of sunflower plants and connects to the vascular tissue, thus depleting the nutrients and affecting host growth and yield (Heide-Jorgensen, 2008; Molinero-Ruiz et al., 2015). Broomrape seeds are very small and individual plants can produce an impressive number of seeds that remain viable in the soil for up to 20 years. They are widely disseminated by water, wind, animals, humans, machinery, or through attachment to sunflower seeds (Parker, 2013).

A major difficulty for the breeders is the fast development of new races of the parasite, which overcome the resistance of sunflower genotypes. To the present day, more than seven races of sunflower broomrape have been identified (Kaya 2014). Vranceanu et al. (1980) identified five races of *O. cumana*, designated as A, B, C, D and E. Later on, more virulent race F was identified in Spain (Alonso et al., 1996; Molinero-Ruiz et al., 2008, Martin-Sanz et al. 2016), Romania (Păcureanu-Joita et al., 1998), Turkey (Kaya et al., 2004) and some other countries. Presence of more virulent broomrape races, designated G and H, has been also reported (Shindrova and Penchev, 2012, Antonova et al., 2014, Kaya, 2014). Current racial situation of broomrape in the main infested areas is unclear, since there is a lack of information on whether races under the same

name reported in different countries are the same or differ in terms of virulence (Fernández-Martínez et al., 2012; Molinero-Ruiz et al., 2015, Martin-Sanz et al., 2016).

Breeding for genetic resistance appears to be the most appropriate and reliable measure to control the parasite. Resistance to *O. cumana* in sunflower is primarily vertical, that is, monogenic, dominant, and race specific (Škorić et al., 2010; Molinero-Ruiz et al., 2015). Therefore, *Or*<sub>1</sub>, *Or*<sub>2</sub>, *Or*<sub>3</sub>, *Or*<sub>4</sub> and *Or*<sub>5</sub>, conferring resistance to races A, B, C, D and E, respectively, are single dominant genes (Vranceanu et al., 1980). Resistance to the race F could be controlled by a single dominant gene, *Or*<sub>6</sub> (Păcureanu-Joita et al., 1998; Pérez-Vich et al., 2002), two recessive genes (Akhtouch et al., 2002) or two partially dominant genes (Velasco et al., 2007), depending on origin sunflower origin. Velasco et al. (2012) showed that the resistance (from *H. debilis* subsp. *tardiflorus*) to the race G was dominant and controlled by a single locus in their population, while Imerovski et al. (2015) found that resistance to races higher than F in a newly identified resistance source is controlled by a single recessive gene.

The changes in broomrape race composition have forced sunflower breeders to continuously search for resistance genes to new races and study their genetic control. The objective of this study was to determine the inheritance and gene action for broomrape resistance in sunflower inbred line LIV-17, which was first tested and found to be resistant to broomrape, in heavily infested fields, where races F and G were predominant.

### Materials and methods

Sunflower inbred line LIV-17, was chosen from the gene pool of Institute of Field and Vegetable Crops, Novi Sad. It originates from population developed from interspecific hybridization with *Helianthus tuberosus*. This line was found to be resistant to broomrape in consecutive trials in the field where virulent races, overcoming F, appear (Cvejić et al., 2012, 2014).

For genetic studies, LIV-17 were crossed with susceptible line HA-26-PR, which is completely susceptible to broomrape (Cvejić et al., 2014), and F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> progenies were developed. Parents, F<sub>1</sub> and F<sub>2</sub> generation were planted in the naturally infested field in Northern Serbia. To validate field results, F<sub>3</sub> generation was grown in greenhouse. Screening for resistance in greenhouse was done using modified method by Panchenko (1975). Broomrape plants were collected from hybrids resistant to race E across the heavily infested sunflower field. Broomrape plants were dried, minute seed was separated using mesh and stored on 4°C until use. Surface sterilised sunflower seed was sown in 9 dm<sup>3</sup> elongated pots containing equal quantity of sand, perlite and substrate (Klasmann-Deilmann Substrat 1). Approximately 8 mg of broomrape seed per dm<sup>3</sup> of mixture was added. Ten plants per pot in two replications were grown for seven weeks under temperature regime of 24/18°C and 16 h photoperiod. Presence of broomrape was evaluated after careful uprooting of sunflower plants and counting of - nodules and stalks. Plants were considered resistant (R) when no broomrape nodules or stalk was found within the complete pot; and susceptible (S) when plants were infested (even one broomrape nodule or/and stalk per plant). The  $\chi^2$  analyses were performed to test the goodness of fit of observed to expected ratios.

### Results and discussion

In the naturally infested field, the resistant line LIV-17 was uniformly resistant while susceptible line HA-26-PR was completely susceptible. All F<sub>1</sub> progenies from HA-26-PRxLIV-17 crosses had more than five broomrape stalks per plant when tested in the infested field, which indicates their susceptibility (Table 1). The susceptibility of F<sub>1</sub>s suggested the recessive nature of broomrape resistance. Broomrape resistance controlled by recessive genes has also been reported by Akhtouch et al. (2002) and Rodríguez-Ojeda et al. (2001) for race F, as well as for races overcoming race F (Imerovski et al., 2014). Although recessive resistance is not as exploited in breeding as dominant resistance, it appears to be generally very durable (Lecoq et al., 2004; Ssali et al., 2013). The barley

*mlo* allele, associated with resistance to powdery mildew, remains effective more than 30 years after its introduction (Lyngkjaer et al., 2000).

Table 1. Segregation of broomrape resistance in F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> progenies of HA-26-PRxLIV-17

Pedigree	Generation	Total	Observed no. of plants/families		Hypothesized ratio		$\chi^2$	P value	
			Resistant	Susceptible (heterozygous)	Observed	Theoretical			
Ha-26-PR	P <sub>1</sub>	20	0		20				
LIV-17	P <sub>2</sub>	17	17		0				
HA-26-PRxLIV-17	F <sub>1</sub>	18	0		18				
	F <sub>2</sub>	99	26		73	1:2.8	1:3	0.220	0.65
	F <sub>3</sub>	89	26	46	17	1:1.7:0.6	1:2:1	0.130	0.75

In F<sub>2</sub> generation, segregation ratio of 3(S):1(R) was observed, indicating that resistance to broomrape in LIV-17 was controlled by a single recessive gene (Table 1). In order to deduct the genotypes of F<sub>2</sub> individual plants, their respective F<sub>3</sub> families were tested for broomrape resistance in the greenhouse. F<sub>3</sub> families segregated, as expected, in 1(homozygous S):2(heterozygous S):1 (R) ratio (Table 1). Some of previous studies revealed similar mechanisms of inheritance depending on the used material and racial composition. Păcureanu-Joița et al. (1998) reported single dominant gene (*Or<sub>6</sub>*) in inheritance of race F in Romania in differential line LC-1093. Perez-Vich et al. (2002) crossed inbred line (J1) derived from interspecific population BR4 with a susceptible genotype and found that the line had a single dominant gene for resistance to race F in the segregating generations. Furthermore, monogenic control was also reported in resistance to broomrape overcoming race F. Velasco et al. (2012) found single dominant inheritance of broomrape resistance to population classified as race G in a resistant sunflower accession of *Helianthus debilis* subsp. *trandiflorus*. However, inheritance study on F<sub>2</sub> generation with resistance donor line HA-267 showed segregation ratio 1(R): 3(S) (Imerovski et al., 2014). This new line was found to be resistant in fields where broomrape races F and G were detected and has resistance higher than *Or<sub>6</sub>*. This study is in agreement with our findings of single recessive mode of inheritance of resistance to races overcoming F in inbred line LIV17.

In conclusion, results pointed out that line LIV17 had a resistant reaction to broomrape to races overcoming F when gene was present in homozygous recessive condition. The present information will be further clarified using molecular markers in identifying *Or* genes. From the breeding perspective, recessive nature of these inbred lines highlights the necessity to introduce resistance genes into both parental lines in order to obtain resistant hybrids, but pyramidization of these two genes in both parental lines could provide better and more reliable protection to broomrape in heavily infested areas.

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### References

- Akhtouch B, Munoz-Ruz J, Melero-Vara J, Fernandez-Martinez J, Dominguez J (2002) Inheritance of resistance to race F of broomrape in sunflower lines of different origins. *Plant Breed* 121: 266–268.
- Alonso LC, Fernandez-Escobar J, Lopez G, Sallago F (1996) New highly virulent sunflower broomrape (*Orobancha cernua* Loefl.) pathotypes in Spain. In *Proceedings of 6<sup>th</sup>*

- International Parasitic Weed Symposium, Seville, Spain, 3-6 June 1996. Seville, Spain: Direccion General de Investigacion Agraria, Consejería de Agricultura y Pesca: 639-644.
- Cvejić S, Dedić B, Jocić S, Miladinović D, Miklič V (2012) Broomrape resistance in newly developed sunflower inbred lines. In: Proc. 18<sup>th</sup> Int Sunfl Conf, Mar del Plata, Argentina, Int Sunflower Assoc, Paris, France: 1037-1042.
- Cvejić S, Jocić S, Dedić B, Radeka I, Imerovski I, Miladinović D (2014) Determination of resistance to broomrape in newly developed sunflower inbred lines. In: Proc. 3<sup>rd</sup> Int Symp on Broomrape (*Orobanch* spp.) in Sunflower, Córdoba, Spain. Int Sunflower Assoc, Paris, France: 184-188.
- Fernández-Martínez J, Velasco L, Pérez-Vich B (2012) Progress in research on breeding for resistance to broomrape. In Proceedings of 18<sup>th</sup> International Sunflower Conference, Mar del Plata, Argentina, Feb 27-March 1 2012. Paris, France: International Sunflower Association: 1111-1114
- Heide-Jorgensen H (2008) Parasitic flowering plants. The Netherlands: Koninklijke Brill NV.
- Imerovski I, Dimitrijević A, Miladinović D, Dedić B, Jocić S, Cvejić S (2014) Preliminary SSR analysis of a novel broomrape resistance source. In Proceedings of 3<sup>rd</sup> International Symposium on Broomrape (*Orobanch* spp.) in Sunflower, Cordoba, Spain, 3-6 June 2014. Paris, France: International Sunflower Association: 214-218.
- Imerovski I, Dimitrijević A, Miladinović D, Dedić B, Jocić S, Kočiš Tubić N, Cvejić S (2015) Mapping of a new gene for resistance to broomrape races higher than *F. Euphytica* 209(2), 281-289.
- Kaya Y (2014) Current situation of sunflower broomrape around the world. Proceedings of 3<sup>rd</sup> International Symposium on Broomrape (*Orobanch* spp.) in Sunflower, Cordoba, Spain, Int Sunflower Assoc, Paris, France: 9-18.
- Kaya Y, Evci G, Pekcan V, Gucer T (2004) Determining new broomrape-infested areas, resistant lines and hybrids in Trakya region of Turkey. *Helia* 27: 211-218.
- Lecoq H, Moury B, Desbiez C, Palloix A, Pitrat M (2004) Durable virus resistance in plants through conventional approaches: a challenge. *Virus Res.* 100: 31-39.
- Louarn J, Boniface M-C, Pouilly N, Velasco L, Pérez-Vich B, Vincourt P, Muñoz S (2016) Sunflower resistance to broomrape (*Orobancha cumana*) is controlled by specific QTLs for different parasitism stages. *Front. Plant Sci.* 7: 590.
- Lyngkjær MF, Newton AC, Atzema JL, Baker SJ (2000) The barley *mlo*-gene: an important powdery mildew resistance source. *Agronomie* 20: 745-756.
- Martín-Sanz A, Malek J, Fernández-Martínez JM, Pérez-Vich B, Velasco L (2016) Increased Virulence in Sunflower Broomrape (*Orobancha cumana* Wallr.) Populations from Southern Spain Is Associated with Greater Genetic Diversity. *Front. Plant Sci.* 7:589.
- Molinero-Ruiz L, Delavault P, Pérez-Vich B, Păcureanu-Joita M, Bulos M, Altieri E, Dominguez J (2015) History of the race structure of *Orobancha cumana* and the breeding of sunflower for resistance to this parasitic weed: A review. *Spanish J. Agric. Res.* 13:4.
- Molinero-Ruiz L, García-Carneros AB, Collado-Romero M, Raranciuc S, Domínguez J, Melero-Vara JM (2013) Pathogenic and molecular diversity in highly virulent populations of the parasitic weed *Orobancha cumana* (sunflower broomrape) from Europe. *Weed Res.* 54: 87–96.
- Molinero-Ruiz ML, Pérez-Vich B, Pineda-Martos R, Melero-Vara JM (2008) Indigenous highly virulent accessions of the sunflower root parasitic weed *Orobancha cumana*. *Weed Res.* 48: 169-178.
- Păcureanu-Joita M, Vranceanu AV, Seare G, Marinescu A, Sandu I (1998) The evaluation of the parasite-host interaction in the system *Helianthus annuus* L. – *Orobancha cumana* Wallr. in Romania. In: Proc. 2<sup>nd</sup> Balkan Symposium on Field Crops, Novi Sad, Yugoslavia: 153-158.

- Panchenko AY (1975) Early diagnosis of broomrape resistance in breeding and improving seed production. *Vestnik S-X, Nauki*, 2: 107-115
- Parker C (2013) The Parasitic Weeds of the Orobanchaceae, In *Parasitic Orobanchaceae*, Springer, Berlin, Germany: 313-344
- Perez-Vich B, Akhtouch B, Munoz-Ruz J, Fernandez-Martinez J, Jan CC (2002) Inheritance of resistance to a highly virulent race F of *Orobanche cumana* Wallr. in a sunflower line derived from interspecific amphiploids. *Helia*, 25: 137-144.
- Rodriguez-Ojeda MI, Fernandez-Escobar J, Alonso LC (2001) Sunflower inbred line (KI-374) carrying two recessive genes for resistance against a highly virulent Spanish population of *Orobanche cernua* Loefl./*Orobanche cumana* Wallr. race F. In *Proceedings of the 7th International Parasitic Weed Symposium* (Eds. A Fer, P Thalouarn, DM Joel, LJ Musselman, C Parker & JAC Verkleij) Faculte des Sciences, Universite de Nantes, Nantes, France: 208–211.
- Shindrova P, Penchev E (2012) Race composition and distribution of broomrape (*Orobanche cumana* Wallr.) in Bulgaria during 2007-2011. *Helia* 35: 87-93.
- Škorić D, Pacureanu-Joila M, Sava E (2010) Sunflower breeding for resistance to broomrape (*Orobanche cumana* Wallr.). *An. I. N. C. D. A. Fundulea* 78 (1): 63-79.
- Ssali, RT, Kiggundu A, Lorenzen J, Karamura E, Tushemereirwe W, Viljoen A (2013) Inheritance of resistance to *Fusarium oxysporum* f. sp. *cubense* race 1 in bananas. *Euphytica* 194, 425-430.
- Velasco L, Perez-Vich B, Jan CC, Fernandez-Martinez JM (2007) Inheritance of resistance to broomrape (*Orobanche cumana* Wallr.) race F in a sunflower line derived from wild sunflower species. *Plant Breed* 126: 67–71.
- Velasco L, Perez-Vich B, Yassein AAM, Jan CC, Fernandez-Martinez JM (2012) Inheritance of resistance to sunflower broomrape (*Orobanche cumana* Wallr.) in an interspecific cross between *Helianthus annuus* and *Helianthus debilis* subsp. *tardiflorus*. *Plant Breed* 131: 220-221.
- Vranceanu AV, Tudor VA, Stoenescu FM, Pirvu N (1980) Virulence groups of *Orobanche cumana* Wallr. differential hosts and resistance sources and genes in sunflower. In: *Proc 9<sup>th</sup> Int Sunfl Conf*, Torremolinos, Spain. *Int Sunfl Assoc Paris, France*: 74-80.