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Original article

Allelic Interaction of OR_7 Gene of Broomrape Resistance in Sunflower

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Abstract

Broomrape (*Orobanche cumana* Wallr.) causes severe yield losses in sunflower (*Helianthus annuus* L.). The emergence and rapid spread of highly virulent races of the parasite have been observed. The dominant Or_7 ($HaOr_7$) gene is used in breeding to control the widely spread broomrape race G. The question of the dominance degree in conditions of severe infection of different genotypes remains open. Twelve sunflower genotypes with different allelic state of the gene Or_7 were used. The plant resistance was assessed in a climatic chamber at optimal conditions for sunflower and broomrape growth. Molecular marker system was applied to confirm the allelic state of Or_7 gene. Resistant homozygous Or_7Or_7, heterozygous Or_7or_7 and susceptible homozygous or_7or_7 genotypes shown infection rate of broomrape at 1.81, 6.58 and 19.00 tubercles per plant accordingly. Partial dominance degree of h/d = -0.44 and the dose effect of a dominant Or7 allele were obvious. For the first time a change of dominance was noted in a cross combination in which the broomrape resistance trait was completely recessive. Development of a homozygous hybrid for Or_7 gene can solve the problem of low broomrape resistance of heterozygous high yield hybrids in breeding programs.

Keywords: Sunflower; Broomrape; Gene; Dominance; Molecular Marker; Hybrid

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INTRODUCTION

Broomrape (*Orobanche cumana* Wallr.), an obligate parasitic plant of the family Orobanchaceae, presents a significant biotic threat to sunflower cultivation in all major sunflower-growing regions, except for North and South America (Kaya et al., 2004; Molinero-Ruiz et al., 2015; Martín-Sanz et al., 2016; Risnoveanu et al., 2016). Yield losses due to broomrape infestations range from 50 to 100% (Shi and Zhao, 2020). The intensification of sunflower production, driven by its profitability, has led to the emergence and rapid spread of new, highly virulent races of the parasite. These races have overcome the resistance mechanisms of previously immune sunflower varieties and hybrids (Fernández-Melero et al. 2023). Currently, eight broomrape races, designated by the Latin alphabet letters from A to H, are known, with each successive letter representing increased virulence. Annual monitoring of broomrape race distribution in sunflower-growing regions across Russia (Samara, Saratov, Voronezh, Rostov, Orenburg, Volgograd, Belgorod, Krasnodar, and Stavropol) indicates an increasing distribution of the virulent H biotype, although race G remains predominant race across most areas. Furthermore, morphological changes in *O. cumana* have been observed: a thickening in the haustorial region, frequently seen in race G, leading to a bushy growth habit in contrast to the typical single-stemmed form (Antonova et al., 2024).

Measures to control the spread of broomrape primarily include crop rotation and application of ALS-inhibiting herbicides. However, a case of herbicide resistance in broomrape has also been documented (Kaundun et al., 2024). Breeding for genetic resistance remains the most effective method for managing this harmful parasite.

Extensive research has been conducted on the race structure of broomrape populations worldwide, as well as on the genetic mechanisms determined sunflower resistance to different races of *O. cumana*. It has been found that sunflower resistance to broomrape is predominantly governed by vertical resistance mechanisms. For example, resistance to broomrape races A, B, C, D, and E is controlled by a series of major dominant genes, designated from Or_1 to Or_5 (Vrânceanu et al., 1980; Sukno et al., 1999; Fernández-Martínez et al., 2008). The $HaOr_5$ gene provides resistance to sunflower broomrape race E by preventing the connection of *O. cumana* to the root vascular system (Pubert et al. 2024). It has been reported that a single dominant gene controls broomrape races that overcome Or_5 resistance, such as Or_6, which confers resistance to race F from Romania (Pacureanu-Joita et al. 2004; Pérez-Vich et al., 2004), and $HaOr_7$, which controls race F from Spain, as well as other more virulent races (Duriez et al., 2019; Martín Sanz et al., 2020). In some sunflower genotypes, resistance to race F is controlled either by recessive alleles at two independent loci (Akhtouch et al., 2002; Fernández-Martínez et al., 2004) or by the dominant-recessive epistasis of two loci (Akhtouch et al., 2016).

Resistance to the G_{TK} race of broomrape has been transferred into cultivated sunflower from *Helianthus debilis* subsp. *tardiflorus*, and is conferred by a single dominant gene, Or_{Deb2} (Fernández-

Aparicio et al., 2022). Quantitative resistance to races F and G is attributed to a partially dominant gene, Or_{SII} (Martín Sanz et al., 2020). The gene $or_{ab\text{-}vl\text{-}8}$, which controls resistance to races higher than F, exhibits recessive inheritance (Imerovski et al. 2016). A new gene, Or_{Anom1}, derived from wild *H. anomalus*, provides late resistance to race G after the parasite has attached to the host roots, and the resistant trait showed monogenic dominant inheritance (Fernández-Melero et al. 2024). In Russia, resistance to broomrape race G_{RU} has been found to be governed by a single dominant gene Or_7 (Guchetl et al., 2019), which is identical to $HaOr_7$ (Savichenko et al., 2024). In addition to these major qualitative genes, quantitative trait loci (QTL) have been identified, which play a role in reducing the number of broomrape tubercles attached to sunflower roots (Pérez-Vich et al., 2004; Louarn et al., 2016; Imerovski et al., 2019; Cvejic et al., 2020).

From breeding point of view different genetic control of broomrape resistance determine the strategy of F_1 hybrid development. For dominant traits one-parent basis are available whereas in case of recessive genetic control both parent lines have to carry appropriate recessive alleles. However, the question arises as to how to proceed in the case of partial dominance or intermediate inheritance. Will one Or_7 allele of resistance in a heterozygote of F_1 sunflower plants provide a sufficient level of broomrape control? The answer to this question was the aim of our research.

MATERIALS AND METHODS

Sunflower Plant Material. The research material includes 12 sunflower genotypes with differences in both the status of the broomrape resistance gene Or_7 and genetic backgrounds: OP variety VNIIMK 8883 (or_7or_7), hybrid Fakel (or_7or_7), hybrid Nataly (or_7or_7), inbred line VK678 (or_7or_7), inbred line VK551 (or_7or_7), hybrid SY Chester (Or_7or_7), hybrid Fakel BR (Or_7or_7), hybrid VA760×VK305BR (Or_7or_7), hybrid VA760×VK301BR (Or_7or_7), hybrid BA761×VK301BR (Or_7or_7), hybrid Taizar Plus (Or_7Or_7) and inbred line VK551 BR (Or_7Or_7).

Phenotyping of Broomrape Resistance. The plant resistance was assessed in a climatic chamber in the stage of six leaves (BBCH-16) in 30 days after sunflower seed emergence. A box with peat soil for growing plants with the addition of 0.2 g of broomrape seeds per one kg of soil was engaged. LED lamps with a photoperiod of 16 h day and 8 h night with corresponding temperature of 25/23 °C were used. The experiment was carried out in 2 replicates, each with 15 plants per genotype. Plants with well-developed roots were assessed. Infection rate was calculated as the mean of tubercles per a root for all estimated plants. Dominance degree (h/d) of a quantitative trait was calculated as standard procedure according Mather and Jinks (1982).

DNA Extraction. DNA was extracted from sunflower leaves and purification was performed by the MagnoPrime® FITO kit (NextBio, Russia) employing the Auto-pure 96 automated nucleic acid

extraction and purification system (Allsheng, China). The quality of the isolated DNA was determined using a Nano-300 microspectrophotometer (Allsheng, China).

Marker System Description and Genotyping. A codominant marker system, developed by the authors, was employed to assess genotypes based on the allelic state of the Or_7 (*HaOr*$_7$) gene, utilizing the genetic markers RORS1 and SORS9. The RORS1 marker was developed to detect the dominant allele of the *Or7* gene, which is associated with resistance to race G_{RU} of *O. cumana*, and it amplified a specific DNA fragment of 168 bp. Conversely, the SORS9 marker identified the recessive allele linked to susceptibility, amplifying a fragment of 217 bp. Multiplex PCR was utilized for the analysis, enabling the simultaneous amplification of both markers in a single reaction. Resistant genotypes were identified by the presence of the amplified RORS1 fragment, while susceptible genotypes were characterized by the amplification of the SORS9 fragment. In hybrid plants derived from crosses between susceptible and resistant lines, both markers were amplified, indicating a heterozygous state for Or_7. The efficacy of the marker system was validated using a sample of 90 sunflower lines and hybrids, as well as 57 F_2 plants. The results demonstrated the high accuracy of this system in identifying genotypes based on the Or_7 gene, confirming its suitability for use in this study (Savichenko et al. 2024). To determine the presence or absence of the partially dominant Or_{SII} gene, proprietary DNA markers linked to this gene were employed (Hassen et al. 2008). The sunflower hybrid P64LE25 (Pioneer Hi-Bred) served as a PCR control, as it carries this gene according to information from the breeding company.

PCR Amplification. The PCR was performed using 25 µl of the following reaction mixture containing 1×PCR-buffer-B for Taq DNA polymerase (Syntol, Russia); 0.2 mM of each deoxyribonucleoside triphosphates (dNTPs); 10 pmol of each primer; 10-30 ng of genomic DNA and 1 U of SynTaq DNA polymerase (Syntol, Russia). PCR was performed in a MiniAmp™ Nucleic Acid Amplifier (Thermo Fisher Scientific, USA). Amplification conditions: initial denaturation - 3 min at 94 °C, then 35 cycles: denaturation at 94 °C - 30 sec, annealing at 60 °C for 40 sec, extension - 40 sec at 72 °C, final extension - 5 min. PCR using TaqMan probes for the marker of the Or_{SII} gene was performed on the QuantStudio™ 5 Real-Time PCR system.

Electrophoresis and Visualization. Electrophoresis of PCR amplification products was performed in an agarose gel (1.5 % agarose, 1×SB-buffer) using the SE.2 horizontal electrophoresis chamber (Helicon, Russia) for 1 h at a current of 50-58 mA and a voltage of 80-100 V. PCR products were stained with ethidium bromide and visualized. The results of electrophoresis were documented using a GenoSens 2200 gel documentation video system (Clinx, China). The size of DNA fragments was determined using Image Lab Software (Bio-Rad, USA) relative to a 100–1000 bp molecular weight marker (DNA Ladder) (Syntol, Russia).

Data Analysis. The significance of differences in sunflower genotypes resistance to broomrape was assessed using R statistical software version 4.2.3 (R Core Team 2024). Analysis of variance (ANOVA) was conducted using the *aov* function, applying a log(mu + 1) transformation to account for zero values. Estimated marginal means (EMMs), standard errors (SE), and confidence limits (CL) were calculated using the *emmeans* function from the «emmeans» package (Lenth et al. 2024). A confidence level of 0.95 was used, and all values, including EMMs, were back-transformed from the log (mu + 1) scale. Pairwise comparisons among different factor levels were performed using the *cld* function. P-values for multiple comparisons were adjusted using the Sidak method across 66 tests, with a significance level of alpha = 0.05. Contrasts were maintained on the log (mu + 1) scale. Data were visualized with the *ggplot* function from the «ggplot2» package.

RESULTS AND DISCUSSION

Five homozygous susceptible genotypes or_7or_7 showed the high infection rate ranging from 13.00 (Nataly) to 34.74 (Fakel) tubercles *per* plant. This proves optimal conditions for the development of the parasite. For two resistant homozygous genotypes Or_7Or_7, the low infection rate was 1.47 (Taizar Plus) and 2.23 (VK551 BR) tubercles per plant. Five heterozygous genotypes Or_7or_7 were characterized by a wide variation in the values of infection from 1.48 (SY Chester) to 26.10 (Fakel BR). Consequently, depending on the combination of crossing, the *Or7* gene in heterozygous form exhibited dominance, incomplete dominance, or a recessive inheritance pattern (Table 1, Figure 1).

Allelic state of both Or_7 (*HaOr*$_7$) and Or_{SII} resistant genes has been verified with the molecular markers. This also corresponds to pedigree information. None of the studied genotypes contained the resistant allele of gene Or_{SII} (Table 1). At the same time, the control sunflower hybrid P64LE25 (Pioneer Hi-Bred) showed a heterozygous state for the Or_{SII} gene, thereby validating the PCR analysis and confirming the absence of false negatives.

Among heterozygous genotypes of Or_7 gene, the hybrid SY Chester showed the lowest infection rate of broomrape at the level of two dominant homozygotes. On the other hand heterozygous hybrid Fakel BR and homozygous susceptible hybrid Fakel showed the highest infection rate of broomrape in the corresponding group of genotypes (Figure 2).

Table 1. Infection rate of broomrape on sunflower genotypes

Genotype	Mean number of tubercles per plant	Standard Error (SE)	Or_7 Allelic State	Or_{SII} Allelic State	Breeding Type
Taizar Plus	1.47^{A}	0.24	R	S	Hybrid F_1
SY Chester	1.48^{A}	0.29	RS	S	Hybrid F_1
VK551 BR	2.23^{AB}	0.34	R	S	Inbred line
VA761 × VK301 BR	3.34^{BC}	0.49	RS	S	Hybrid F_1
VA760 × VK301 BR	5.45^{CD}	0.90	RS	S	Hybrid F_1
VA760 × VK305 BR	9.20^{DE}	0.99	RS	S	Hybrid F_1
Nataly	13.00^{EF}	1,74	S	S	Hybrid F_1
VK551	13.25^{EF}	1.51	S	S	Inbred line
VK678	22.82^{FG}	4.08	S	S	Inbred line
VNIIMK 8883	25.96^{G}	2.81	S	S	OP variety
Fakel BR	26.10^{G}	3.00	RS	S	Hybrid F_1
Fakel	34.74^{G}	6.13	S	S	Hybrid F_1

Note: The letter groups above the mean number indicate the results of pairwise comparisons among factor levels. Levels sharing the same letters are not significantly different from each other ($p > 0.05$). Letter R corresponds to a resistant homozygote, S – susceptible homozygote, RS – heterozygote.

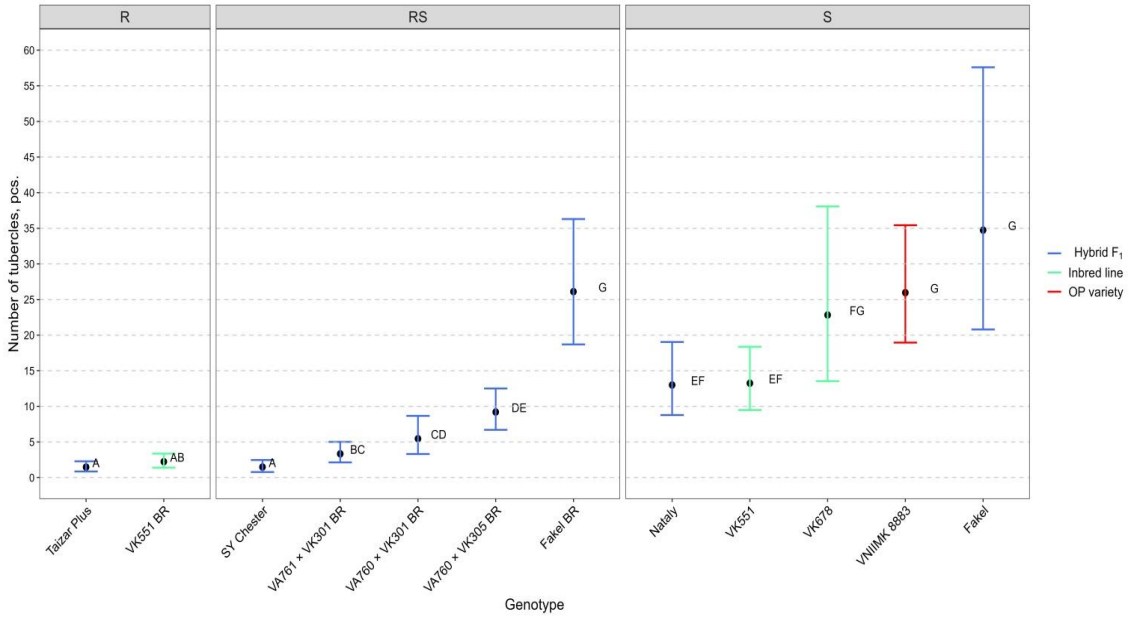

Figure 1. Ranged infection rate of broomrape within different groups of sunflower genotypes. Letter R corresponds to the Or_7Or_7 homozygote, S – or_7or_7 homozygote, RS – Or_7or_7 heterozygote

In the case of comparing the average values of broomrape damage of three groups of homo- and heterozygotes, partial dominance of the gene Or_7 was obvious with the means of infection rate of broomrape 1.81, 6.58 and 19.00 tubercles per plant (Figure 2). The dominance degree h/d of Or_7

amounts to -0.44 because of resistance is estimated as minimal value of the infection rate of broomrape damage.

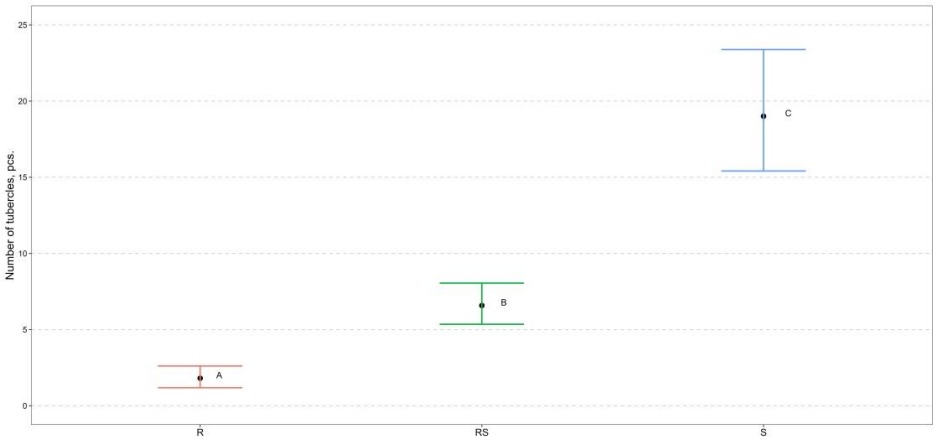

Figure 2. Combined infection rate of broomrape for homo- and heterozygotes of *Or*$_7$ gene in sunflower. Letter R corresponds to *Or*$_7$*Or*$_7$ homozygote, S – *or*$_7$*or*$_7$ homozygote, RS – *Or*$_7$*or*$_7$ heterozygote

Heterozygous for the *Or*$_7$ gene hybrid Fakel BR showed unusual results. Broomrape susceptible VK678 female line with the infection rate of 22.82 was crossed with resistant VK551 BR male line with the infection rate of 2.23 (Figure 3). The hybrid plants have been damaged with infection rate at the level of female susceptible line. Dominance degree h/d of susceptibility was 1.32, therefore the trait of broomrape resistance in this genetic background became recessive.

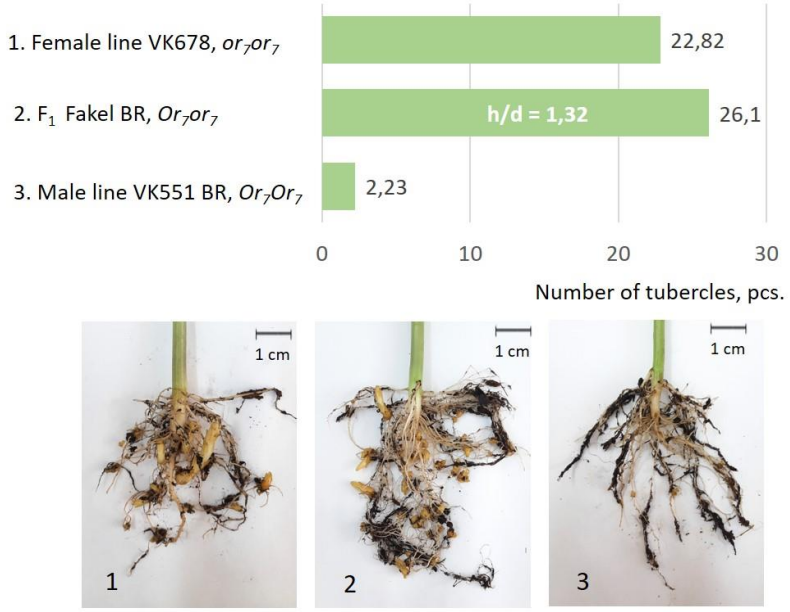

Figure 3. Inheritance of resistance to broomrape in F_1 in the cross of sunflower lines. Photo number corresponds to genotype number. Dominance degree h/d is calculated according to Mather and Jinks (1982)

Sunflower is known to have different mechanisms of resistance to broomrape from pre- to post-attachment levels, which are controlled by different corresponding genes (Molinero-Ruiz et al., 2015; Fernández-Martínez et al., 2004). Pyramiding of these resistance genes is expected to increase sunflower resistance to new virulent races of broomrape (Cvejic et al., 2020). However, it is the dominant *Or*$_7$ gene that is widely used in the breeding of sunflower hybrids in the world.

Knowledge of the type of inheritance of a trait in heterozygotes of main gene is of fundamental importance for breeding use of plant genetic resources. This information must be taken into account when constructing a hybrid based on a uniparental or biparental approaches, as well as for the reliability of selection of heterozygotes in the backcrosses when introducing desired alleles into a new breeding line.

Complete dominance of the trait allows using one parental line with the resistance gene to obtain a hybrid and to do direct selection of heterozygotes in the segregating backcross progeny. Recessive inheritance requires obtaining a hybrid which is homozygous for the resistance gene due to the homozygous state of both parental lines and to use indirect selection of heterozygotes either after evaluation by progeny or using a molecular marker in backcrossing.

Obviously in the case of intermediate inheritance of resistance, the heterozygous hybrid will not fully provide protection for sunflower crop, especially in conditions of severe broomrape infestation in the field. In our study, the evaluation of the broomrape resistance trait took place under optimal artificial conditions for the development of both sunflower and the parasite.

Allelic interaction in *Or*$_7$ gene with the type of complete dominance was noted in our research only for the SY Chester hybrid, which showed a very low value of broomrape damage of 1.48 tubercles per plant. It corresponds to the level of the homozygous Taizar Plus hybrid. Three heterozygous hybrids of VA760×VK305BR, VA760×VK301BR and BA761×VK301BR have partial dominance of *Or*$_7$ gene and probably acceptable levels of broomrape infected rate of 3.34 to 9.20 tubercles per plant.

The heterozygous hybrid of Fakel BR was characterized by an unexpected change of allelic interaction in *Or*$_7$ gene from the dominance of broomrape resistance to the level of homozygous susceptible hybrid Fakel. The increased susceptibility of the two isogenic hybrids of Fakel BR and Fakel is probably due to the identical maternal VK678 line of these hybrids, which presumably possesses genes for increased susceptibility to broomrape. Nevertheless from breeding point of view, the maintainer of sterility VK678 line has a number of valuable traits, such as good combining ability and increased oleic acid content in seed oil. The isogenic male parent lines of these hybrids VK551 BR (*Or*$_7$*Or*$_7$) and VK551 (*or*$_7$*or*$_7$) differ in the infection rate of broomrape fully expected.

The *Or*$_7$ gene, located on chromosome 7, usually shows allelic interactions of the complete or partial dominance types (Duriez et al. 2019; Guchetl et al. 2019; Cvejic et al. 2020). However, other sources

of resistance to broomrape are known in line of P-96 (Akhtouch et al. 2016) and lines of AB-VL-8, LIV-10, LIV-17 and HA267 (Cvejic et al. 2020) with recessive inheritance the trait controlled by other genes.

CONCLUSION

During development of heterozygous hybrids for the *Or*$_7$ gene, the phenomenon of intermediate inheritance in F_1 or even dominance change in some crossbreeding combinations should be taken into account. Development of a homozygous hybrid for the *Or*$_7$ gene can solve the problem of increasing resistance to broomrape in case of genetic background with high susceptibility to the parasite.

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