EVOLUTION OF *OROBANCHE CUMANA* WALLR. IN INTENSIVE SUNFLOWER CULTIVATION IN REGIONS OF RUSSIAN FEDERATION

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ABSTRACT

The intensification of sunflower cultivation over the last three decades with reduced crop rotation in regions of the Russian Federation has led to accelerated development of its obligate parasitic plant *Orobanche cumana* Wallr. Annual monitoring of broomrape seed infestation of fields over the last 15 years shows that despite the widespread distribution of race G, seeds of other, weaker races E and F remain in many fields. Seed reemergence of these races in the fields is probably due to the continued cultivation of susceptible sunflower varieties-populations. A highly aggressive parasite biotype (future race H) that overcomes the resistance of the sunflower differentiator line RG, which has immunity to race G, has so far been identified in small numbers in some fields in the Krasnodar, Stavropol, Rostov, Voronezh, Samara, Saratov, and Orenburg regions. Obviously, the continuation of intensive sunflower cultivation in these fields will lead to a rapid spread of race H in these regions. In addition to the racial diversity, frequent changes in plant habitus (bushy forms) were observed in some representatives of race G. For the first time, we have shown an overgrowth of the haustorial-tubercle area of the parasite and the formation of multiple stems from a single tubercle. This creates an advantage in the competition for food between adjacent broomrape individuals on the same sunflower root and ultimately accelerates and increases the seed production of the plant. Thus, the rapid evolution of *O. cumana* during the intensification of sunflower cultivation is expressed not only in the formation of new physiological races, but also in an accelerated increase in the seed productivity of the parasite by changing the habitus of the plants, including the haustorial-tubercle area.

Keywords: Sunflower, broomrape, evolution, race, haustorial area, root thickening, habitus change.

INTRODUCTION

Broomrape (*Orobanche cumana* Wallr.) is of the higher plant taxon and is an obligate parasite that is widespread in most sunflower growing countries and it continues to cause significant losses in sunflower yields (Kaya, 2014; Ma et Jan, 2014; Molinero-

Ruiz et al., 2015; Martin-Sanz et al., 2016; Risnoveanu et al., 2016; Maklik et al., 2018; Dedic et al., 2022; Duca et al., 2022; Zhang X.et al., 2018; Antonova et al., 2022).

In the Russian Federation, sunflower is the main oil crop, highly profitable and therefore attractive for cultivation. Over the last three decades, the sunflower crop acreage in the Russian Federation has increased steadily each year, reaching 10,032,800 ha in 2022. Initially (more than 30 years ago), this crop occupied a total area of 2,322,000 ha throughout the former Soviet Union (Antonova, 2014). This continued rapid increase in crop acreage was only possible when crop rotation was reduced from the scientifically based 8-10 years to 1-3 years. This ongoing phenomenon of short sunflower rotations continues to pose a challenge to broomrape control.

The reduction in crop rotation promoted the acceleration of the mutation process in broomrape, the rapid increase in the frequency of emergence of its new generations, and the expansion of their spread areas. This process led to an increase in the frequency of emergence of new broomrape biotypes capable of overcoming resistance to it in the cultivated sunflower varieties, i.e., accelerated the emergence of new physiological races of the parasite. It also accelerated the formation of new, hereditary fixed traits, which caused an acceleration of the more powerful development of the parasite individual, an increase in its seed productivity and survival under the influence of the human desire to destroy this harmful weed. It should be emphasized that not only coevolution of broomrape and sunflower is observed, but also rapid, hereditary fixed changes in the parasite individuals, which improve their resistance to unfavourable habitat conditions arising under the influence of man.

The aim of our work was to identify the racial origin of broomrape seeds from fields of sunflower growing regions of the Russian Federation and to describe new botanical characteristics in representatives of the most widespread race G of the parasite.

MATERIAL AND METHOD

Identification of racial origin of broomrape seeds collected annually for breeding purposes was carried out using the following sunflower genotypes: hybrid NK Brio (resistant to 5 broomrape races from A to E), line LC 1093 (resistant to race F of the Romanian type, including previous races), line P 96 (resistant to race F of the Spanish type and all previous races), hybrid Tunka (resistant to races A to G) and line RG (resistant to race G and all previous races), developed in the immunity laboratory of V.S. Pustovoit All-Russian Research Institute of Oil Crops. Sunflower variety VNIIMK 8883, which was susceptible to all races of broomrape, was used as a control variant.

The seeds of each broomrape sample were mixed with soil-sand mixture (3 :1) at a rate of at least 200 mg per 1 kg of this mixture and placed in plastic boxes of 50x20x20 cm. Seeds of the above sunflower genotypes were sown in these boxes and placed in the climate chamber Biotron-5. They were grown at a temperature of 25-27 °C, with a 16-hour photoperiod and appropriate lighting.

The seedlings were watered moderately when the topsoil dried out. After 25 days from the seedling emergence, the plants were dug up and the roots were washed with water. The number of tubercles and broomrape shoots on the roots of 20 plants of each differentiator was counted, the average degree of plant affection was calculated and compared with the affection of the control variant (Antonova et al., 2019).

Seeds of broomrape pure race G were collected on sunflower hybrids of foreign breeding containing the *Or*$_7$ gene. The presence of this gene in only one of the parent lines of the hybrid (usually in the paternal form) confers incomplete resistance to broomrape race G. Some degree of affection of hybrid plants is observed. The broomrape seeds collected from such affected plants, when identified, always represented race G. (Antonova et al., 2020).

Stereoscopic microscopy was used to observe the early stages of tubercle and shoot formation on the roots of plants of a race G susceptible sunflower variety grown on an infectious background produced from seeds of this race.

RESULTS AND DISCUSSION

Annual monitoring of the racial origin of broomrape seeds collected from sunflower sowings in different regions of the Russian Federation (Samara, Voronezh, Rostov, Orenburg, Volgograd, Saratov, Belgorod, Krasnodar and Stavropol regions) shows that race G is currently widespread. At the same time, however, there are still fields where only races E or F predominate, or where there is a mixture of two or three races with the predominance of one of them. Table 1 shows sample data for selected fields (25 in total) from six regions of the Russian
Federation for the period 2020 - 2022. In general, and for the other regions mentioned above but not listed in the table, the picture is similar to the data presented. As can be seen from the
Table, race G is now dominant in many fields and its seed admixture is already present where other races predominate.

In the sample of fields for the last three years shown in the Table, the least virulent race E dominates in seven of them. In general, analyses of the racial origin of broomrape seed samples collected in the period 2020-2022 in different regions showed considerable heterogeneity in virulence. Therefore, monitoring the racial origin of broomrape seeds from different fields in the sunflower growing regions of the Russian Federation remains relevant and is a necessary condition for the correct placement of cultivated sunflower varieties, which can slow down the formation of highly virulent biotypes of the parasite in each particular field.

There are several reasons for the current presence of the weaker races E and F in the fields. First, it has long been known that germination of broomrape seeds can persist for up to 20 years under field conditions (Molinero-Ruiz et al., 2008). Secondly, some farms are acting sensibly by trying to maintain a long crop rotation, thus slowing the emergence and spread of more virulent biotypes of the parasite. Thirdly, in Russia, in addition to hybrids, sunflower varieties are traditionally grown on which weaker races of broomrape can reproduce new generations of seeds, thus prolonging their existence in agrocenoses.

However, in each of these regions, there are already fields where the even more virulent biotype H, which has overcome the resistance of the line RG, is accumulating. In total, half of the fields shown in the Table contain this broomrape biotype. It is only a matter of time before such problem fields will accumulate enough seeds of this biotype so to be collected to create a highly infectious background for the selection of resistant sunflower genotypes in immunity breeding.

We observed early stages of tubercle formation in a continuation of our earlier studies on artificial inoculation of sunflower plants with broomrape in an infectious background developed from race G seeds.

Table 1. Degree of affection* of sunflower resistance differentiators by broomrape collected in selected fields of six regions of the Russian Federation in 2020 - 2022.

Broomrape seeds collection area	Susceptible control VNIIMK 8883	Differentiators, resistant to races:					Dominant broomrape race in the seed sample	Admixture of other races in the seed sample
		A-E (NK Brio)	A-F (LC1093)	A-F (R 96)	A-G (Tunka)	A-G (RG)		
Rostov region								
Azovsky	30	12	20	5	6	0	G	E
Zernogradsky	42	41	10	4	3	0.7	F	G, H
Oktyabrsky	38	12	11	3	1.8	0	E	G
Matveevo-Kurgansky	55	48	59	31	15	6,0	G	H
Bokovsky	53	59	36	23	2	0.9	F+ G	H
Voronezh region								
Kalachevsky	41	20	22	9	9	0	G	E
Pavlovsky	36	11	10	5	2	0.7	E	G, H
Novousmansky	31	30	21	16	4	2	F+ G	H
Kashirsky	33	13	6	0	1	0.3	E	F, G, H
Samara region								
Bolsheglushitsky	30	13	21	2	5	0.4	G	E, H
Volzhsky	42	18	28	4	6	0.5	G	F, H
Neftegorsky	34	9	9	5	4	0	E	G
Orenburg region								
Oktyabrsky	30	31	15	0.3	4	0	F+ G	
Plemanovsky	29	28	25	2	3	1	G	H
Buguruslansky	32	0,6	0.4	0.5	2	0	E	G

Kurmanaevsky	51	43	5.5	0.1	2	0	E	F, G
Stavropol region								
Kochubeevsky	38	29	14	9	4	0	E	G
Sovetsky	56	39	23	7	11	1	E+ F+ G	H
Krasnodar region								
Primorsko-Akhtarsky	32	23	22	3	6	05	G	E
Krylovskoy	34	33	30	7	3	0.8	G	H
Vyselkovsky	49	38	30	16	8	0	G	E, F
Brukhovetsky	33	37	18	14	0	4	F+ G	H
Kanevskoy	28	36	22	8	0.8	0	G	F
Tbilissky	38	23	24	4	3	0	G	E
Giaginsky	36	31	39	2	1,5	4	G	H

* Degree of affection is a number of broomrape specimens per affected sunflower plant

The stereomicroscopic study of the early stages of tubercle and shoot development of race G, when susceptible sunflower genotypes are infected, showed that the stage of thickening of the host plant root in the haustorial area of the parasite, described previously (Antonova et al., 2022), is consistently observed with a fairly high frequency when evaluating the resistance of breeding material on an infectious background developed from broomrape seeds of this race. We continue to observe thickenings (as well as their absence) of the haustorial area of the parasite in the roots of sunflower plants under developing tubercles (Fig. 1 a-i). Furthermore, a tubercle with a thickening underneath and a tubercle without a thickening can be found side by side on the same root of the host plant (Fig. 1 h, i). This proves that the stimulation of the formation of such a thickening is a property of some individuals *O. cumana*. In Fig. 1 h, the large tubercle on the left feeds simultaneously on two roots of the host plant and both haustorial areas are thickened (indicated by arrows). On the right side of this figure, there is no thickening under two other tubercles located on different roots.

Usually, numerous growth meristems are formed in the tubercle, under which there is a thickening of the host plant root, from which multiple shoots develop (Fig. 2 a, b, c, d). When they reach the soil surface, we observe bushy forms (Fig. 3 a, b) of individual broomrape plants, which have become a common phenomenon in sunflower sowings in fields infested with the seeds of broomrape race G. The habitus of an individual broomrape plant has noticeably changed from 1-2-stemmed to multi-stemmed, although both forms will coexist in sunflower sowings for a long time.

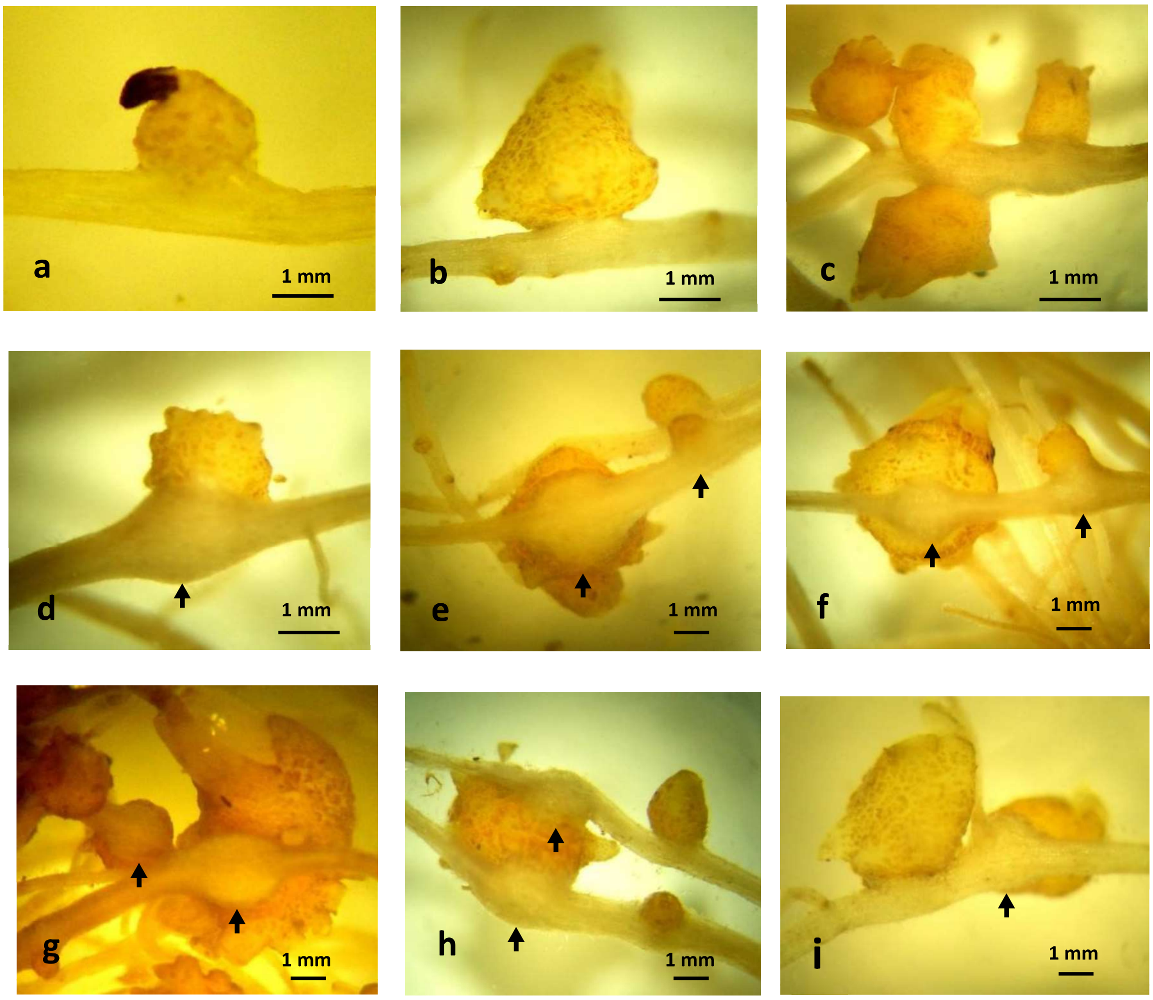

Fig. 1 a-i. The area of connection of the broomrape tubercle with the sunflower root: **a, b, c** - absence of root thickening under the tubercles; **d, e, f, g** - presence of thickenings (indicated by arrows); **h, i** - tubercles next to each other on the same root without thickenings under them and with thickenings (indicated by arrow); **h** - a large tubercle (left) has haustorial areas in two roots of the host plant and both are thickened (indicated by arrows). On the right, there are no thickenings under two other tubercles on different roots.

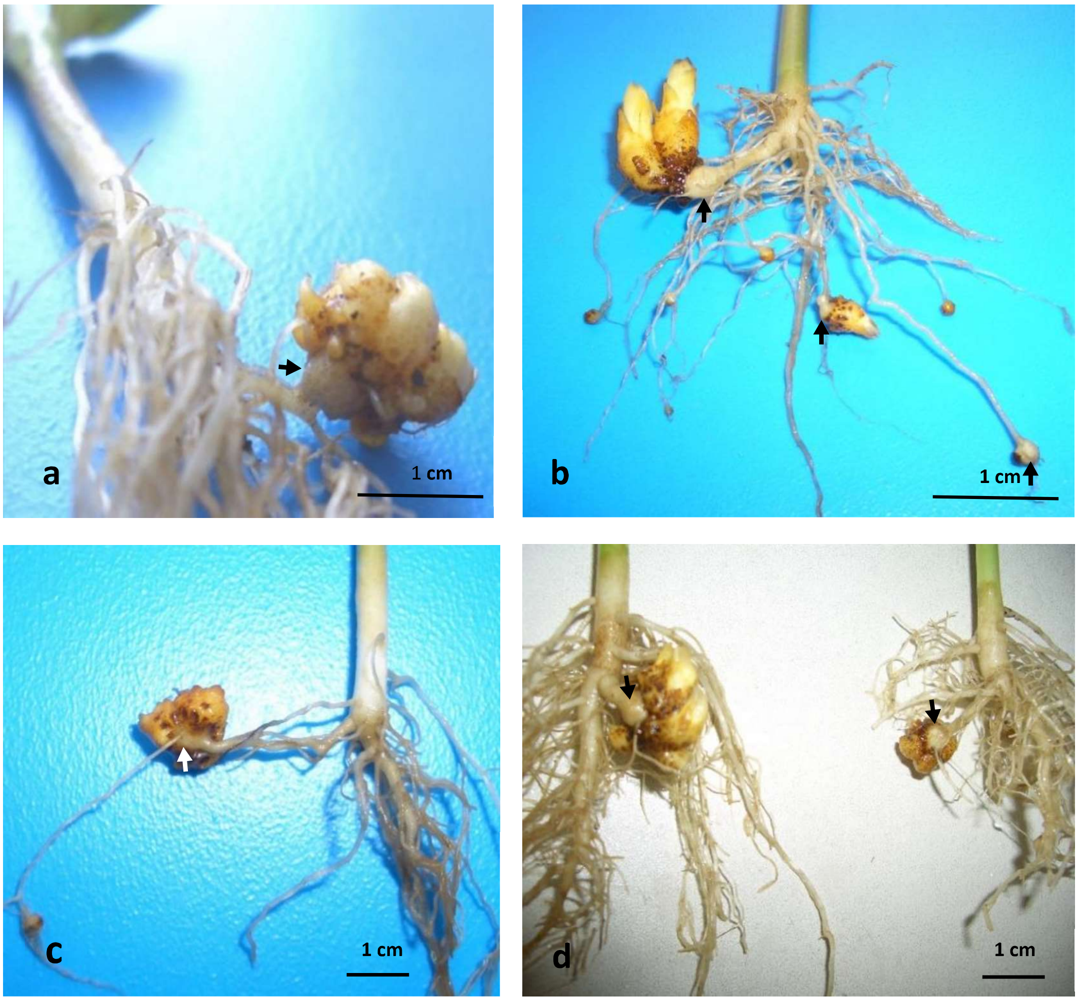

Fig. 2 a-d. Multiple shoots developing from *Orobanche cumana* tuber with thickening of the sunflower root underneath (**a, c, d**); **b** - thickening of the root under broomrape shoots and tubercles that are just beginning to develop.

We have also observed a phenomenon where the tubercle as such is partially or completely absorbed by the overgrown thickening of the haustorial area in the sunflower root, when its lower part is hidden by the root tissues. In this case, multiple shoots usually develop from the tubercle (Fig. 4 a, b). This suggests that, in the future the tubercles of *Orobanche cumana* will be immersed in sunflower root tissues in a gradual transformation process. From the point of view of the advantage of this location of the tubercle in relation to the external one for the broomrape specimen, parasitizing on sunflower, it is an evolutionary more advanced trait - the tubercle is protected from mechanical damage when inter-row weeding is carried out. Actually, the tubercle, as a basic stage of the formation of the future stem of the parasite, fulfils the function of accumulating in its cells the nutrients necessary for the formation and development of the apical meristem (one or more) and the beginning of shoot growth. It does not need to be outside the root of the host plant to perform this function.

Fig. 3 a, b. Bushiness is a new habitus found in plants of race G of *Orobanche cumana* on sunflower: **a** - mature bush; **b** - flowering bushes of some individuals (indicated by arrows) on the same host plant.

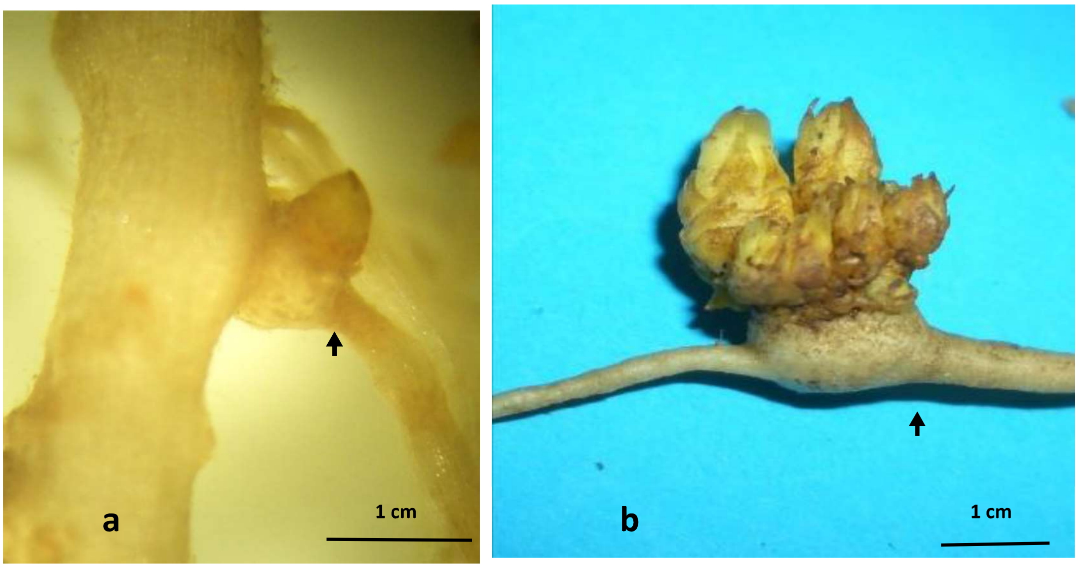

Fig. 4 a, b. Thickenings (indicated by arrows) of the haustorial area in the sunflower root, hiding the broomrape tubercle: **a** – complete absorption of the tubercle, the broomrape shoot that has started to develop emerges directly from the thickening in the sinus of the additional root; **b** - partial absorption of the tubercle, its lower part is hidden by the root tissue, and strong multiple shoots develop from its upper part.

The growth of the parasite's storage tissues and their filling with nutrients inside the sunflower root can ensure the initial development of a larger number of shoots, stems and, eventually, the production of a larger number of seeds in one broomrape specimen. It is possible that the strengthening of the whole structure, which we have called the thickening of the sunflower root in the haustorial area of the parasite, is facilitated by the accelerated formation of secondary tissues in the developing root of the host plant. It is also possible that broomrape has learned to stimulate the accelerated formation of secondary tissues in the root of host plant to protect and strengthen its own cells and tissues in the haustorial-tubercle area. This issue needs to be studied more closely. It is necessary to make an anatomo-histochemical study of such thickenings and to differentiate between parasite and host tissues within them. This should be the subject of further research.

CONCLUSIONS

Broomrape race G is dominant in the sunflower growing regions of the Russian Federation. However, the identification of the racial origin of broomrape seeds from different fields in six regions in the period 2020-2022 showed that there are fields with only races E or F or an equal (unequal) mixture of biotypes E, F, G. Half of the surveyed fields also contain seeds of the most virulent biotype H, which is not yet widespread enough to be called a new race.

It is shown that there are two types of specimens in populations of race G of *O. cumana*: a) - with thickening of the sunflower root in the haustorial area of the parasite and b) - without thickening. Forms with absorption of the tubercle by such a thickening, when the sunflower root tissue hides the tubercle, were revealed. It is shown that parasite specimens that stimulate the development of sunflower root thickening under the forming tubercle usually develop multiple shoots from it, which further form a bush on the soil surface. The bushy habitus characterizes a new morphotype of *O. cumana* specimens, which differs from the typical 1-2 stem form. Both morphotypes of *O. cumana* are present in the fields of the studied regions of the Russian Federation, which are infested with seeds of broomrape race G. The haustorial area of a broomrape specimen in the sunflower root plays a crucial role in the success of its parasitic lifestyle. Therefore, it is important to carry out an anatomo-histochemical study of the described thickenings of the haustorial area in the sunflower root under the tubercle of the parasite.

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