



**PROCEEDINGS OF  
5TH INTERNATIONAL SYMPOSIUM  
ON BROOMRAPE IN SUNFLOWER**

**1-3 NOVEMBER, 2023**

**ANTALYA, TURKEY**

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**Organized by  
Trakya University  
International Sunflower Association  
International Researchers Association**

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## WELCOME NOTES

The parasitic angiosperm broomrape (*Orobanche cumana* Wallr) causes economic damage in sunflower production in a number of countries around the world, but especially in Central and Eastern Europe, Spain, Turkey, Israel, Iran, Kazakhstan, and China. For almost a century, there has been a constant tug-of-war between sunflower breeders and *Orobanche cumana*, with frequent changes in which side has the upper hand. Almost as soon as the breeders find a source of resistance to the latest race of the pathogen, broomrape responds by evolving into another virulent race. The development of resistant cultivars as well as optimized managing strategies is a high priority in controlling this parasite, over the world.

This is the 5th specific symposium on broomrape in sunflower, after those held in Turkey in 2008, Moldova in 2011, Spain in 2014 and Romania, in 2018.

The symposium is organized by Trakya University and International Researchers Association in cooperation with the International Sunflower Association (ISA). The symposium will be held in Megasaray Westbeach Hotel, Antalya, Turkey, on November 1-3, 2023. The symposium covers all aspects related to broomrape parasitisms in sunflower, including parasite biology, physiology, parasite-host interaction, the racial status of broomrape, genetic resistance, molecular breeding, chemical control using herbicide-tolerant, and integrated management.

The symposium gathered sunflower scientists from around the world, and present their recent achievements. The organizers also invited relevant stakeholders to provide a view on the broomrape situation around the world as well as prospects to overcome the limitation for sunflower production, imposed by this parasitic weed.

There are 18 oral presentations and 8 poster presentations. There will be 146 participants from 18 countries from the world.

We would like to thank all of you for joining this conference and we would like to give also special thanks to our sponsors and collaborators for giving us a big support to organize this event.

Prof Dr Yalcin KAYA  
Head of the Organizing Committee

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## INVITED SPEAKERS

Dr Leonardo VELASCO	Broomprae resistance from wild species
Dr. Dragana MILADINOVIĆ	Broomprae resistance utilizing genomic tools
Dr Mehmet DEMIRCI	CLEARFIELD control Broomrape and weeds.

## EDITOR OF THE PROCEEDINGS ABSTRACT BOOK

Prof Dr Yalcin KAYA, Assoc Prof Dr Necmi BESER

Prof Dr Yalcin KAYA  
Head of the Organizing Committee

## 5<sup>TH</sup> INTERNATIONAL BROOMRAPE SYMPOSIUM PROGRAM

<b>WEDNESDAY, NOVEMBER 1<sup>ST</sup>, 2023</b>	
08 <sup>30</sup> - 09 <sup>50</sup>	Registration
10 <sup>15</sup> - 10 <sup>30</sup>	Opening Ceremony
10 <sup>30</sup> - 13 <sup>00</sup>	<b>OPENING SESSION:</b> Session Chair: <b>DR. VLADIMIR MIKLIC</b> , <i>Ex-President of ISA</i> , Novisad Research Inst, SERBIA
10 <sup>30</sup> - 11 <sup>10</sup>	<b>Dr Leonardo VELASCO</b> , Institute for Sustainable Agriculture CSIC, Spain “Broomrape resistance from wild species”
11 <sup>10</sup> - 11 <sup>50</sup>	<b>Prof Dr Dragana MILADINOVIĆ</b> - Novisad Research Inst, Serbia “New approaches for achieving durable resistance to broomrape in sunflower”
11 <sup>50</sup> - 12 <sup>30</sup>	<b>Dr Mehmet DEMIRCI</b> , Freelancer, Turkey - “CLEARFIELD control of broomrape parasite & weeds: A success story in sunflower”
12 <sup>30</sup> - 13 <sup>00</sup>	<b>Discussion</b>
13 <sup>00</sup> - 14 <sup>00</sup>	<b>LUNCH</b>
1 <sup>st</sup> Ses	<b>MAIN HALL Chair: Prof Dr Maria DUCA - Molecular Genetics for Broomrape Tolerance Session</b>
1 14 <sup>00</sup> - 14 <sup>30</sup>	<b>KEYNOTE SPEECH:</b> The STIGO project: Deciphering the molecular dialog of <i>O. cumana</i> seeds germination - Stephane Munos
2 14 <sup>30</sup> - 15 <sup>00</sup>	<b>KEYNOTE SPEECH:</b> Transcriptome analysis and gene mining of broomrape in sunflower-broomrape pathosystem - Ningning Yan, <u>Zhao Jun</u>
3 15 <sup>00</sup> - 15 <sup>20</sup>	Mechanism of ‘Jinmiao Target’ in Inhibiting <i>Orobanche cumana</i> Parasitism of Sunflower - <u>Zhang Jian</u> , Duan Rui, Liu Zhida, Guo Xiaoqing, Zhang Zhiwei, Zhang Wenbing, Zhao Jun
4 15 <sup>20</sup> - 15 <sup>40</sup>	Determination of resistance of oil sunflower maintainer lines to broomrape populations in the Çukurova region – Ayse Nuran Cil, Yılmaz Yaşar, Abdullah Çil
15 <sup>40</sup> - 15 <sup>50</sup>	<b>Discussion</b>
15 <sup>50</sup> - 16 <sup>10</sup>	<b>Coffee Break</b>
2 <sup>nd</sup> Ses	<b>MAIN HALL Chair: Prof Dr ZHAO JUN – Molecular Breeding and Genetics of Broomrape Session</b>
5 16 <sup>10</sup> - 16 <sup>30</sup>	DNA marker for marker-assisted selection for sunflower resistance to race G of broomrape - <u>Dmitrii Savichenko</u> , Saida Guchetl, Yakov Demurin, Yulia Chebanova, Olga Rubanova
6 16 <sup>30</sup> - 16 <sup>50</sup>	Genetic diversity analysis of broomrape ( <i>Orobanche cumana</i> ) populations in sunflower growing areas in Europe - <u>Clothilde Boubée De Gramont</u> , Sophie Bellone, Mario Hernandez, Isabelle André, Nicole Lucante, Clotilde Claudel
7 16 <sup>50</sup> - 17 <sup>10</sup>	Application of SSR markers to reveal the genetic diversity of sunflower broomrape in China - ZHANG Yu-Kuan, LIU Jin-Ping, LIU Zhida, ZHANG Zhiwei, <u>ZHANG Wenbing</u> , YANG Jia-Le, ZHANG Jian, ZHAO Jun
8 17 <sup>10</sup> - 17 <sup>30</sup>	Molecular Characterization of some Wild Sunflower Species ( <i>Helianthus spp.</i> ) and Interspecific Hybrids based on Broomrape Resistance - Havva Akar, <u>Yalçın Kaya</u> , Semra Hasançebi, Emrah Akpınar, Necmi Beşer
17 <sup>30</sup> - 17 <sup>40</sup>	<b>Discussion</b>
17 <sup>40</sup> - 18 <sup>00</sup>	<b>POSTER SESSION</b>

3 <sup>RD</sup> Ses	THURSDAY, NOVEMBER 2 <sup>ND</sup> , 2023
09 <sup>30</sup> -12 <sup>00</sup>	<b>HALL 2 Chair: Prof Dr BEGONA PEREZ VICH – Resistance Breeding and Biology of Broomrape Session</b>
9 09 <sup>30</sup> - 10 <sup>00</sup>	<b>KEYNOTE SPEECH:</b> Effect of gene dose on broomrape resistance in sunflower - <u>Yakov Demurin</u> , Yulia Chebanova, Olga Rubanova
10 10 <sup>00</sup> - 10 <sup>30</sup>	<b>KEYNOTE SPEECH:</b> Development CRISPR / CAS9 - Mediated Resistance in Sunflower against <i>O. cumana</i> - <u>Kubilay Yıldırım</u> , Ilkay Sevgen Küçük, Dragana Miladinović, Çigdem Gökcek Saraç
10 <sup>30</sup> - 10 <sup>50</sup>	<b>Discussion</b>
10 <sup>50</sup> - 11 <sup>10</sup>	<b>Coffee Break</b>
11 11 <sup>10</sup> - 11 <sup>30</sup>	A preliminary study on the identification of different sunflower varieties with the level of resistance to Race G minor species and the mechanism of resistance - <u>Bao Ting-Ting</u> , Shi Sheng-Hua, Yan Ning, Liu Zhi-Da, Yang Jia-Le, Zhang, WenBing, Zhang Jian, Zhang Zhi-Wei, Zhao Jun
12 11 <sup>30</sup> - 11 <sup>50</sup>	In the race with the broomrape - Is there a winner? <u>Milan Jocković</u> , Siniša Jocić, Sandra Cvejić, Nemanja Ćuk, Aleksandra Radanović, Vladimir Miklič, Jelena Jocković, Dragana Miladinović, Boško Dedić
13 11 <sup>50</sup> - 12 <sup>10</sup>	Determination of agricultural policy factors and their effects affecting producers' preference for production of oily sunflower: the case of Thrace region - Burak Uğur
14 12 <sup>10</sup> - 12 <sup>30</sup>	Advancing Biocontrol Strategies for Broomrape Management - Deniz İnci, <u>Emre Eren Muslu</u> , Ayşe Kökdemir, Sena Er, Seçkin Kaya, Ahmet Uludağ
15 12 <sup>30</sup> - 12 <sup>50</sup>	Evolution of <i>Orobanche cumana</i> Wallr. in intensive sunflower cultivation in regions of Russian Federation - <u>Tatiana Antonova</u> , Nina Araslanova, Maria Iwebor, Svetlana Saukova
12 <sup>50</sup> - 13 <sup>00</sup>	<b>Discussion</b>
13 <sup>00</sup> -14 <sup>00</sup>	<b>LUNCH</b>
4 <sup>th</sup> Ses	<b>HALL 2 Chair: Burak UĞUR – The Racial Status of Broomrape Session</b>
16 14 <sup>00</sup> - 14 <sup>30</sup>	<b>KEYNOTE SPEECH:</b> Recent Developments in Broomrape in Sunflower in the World – <u>Yalcin Kaya</u>
17 14 <sup>30</sup> - 15 <sup>00</sup>	<b>KEYNOTE SPEECH:</b> Recent situation of chemical control on broomrape - Maria Pacureanu
18 15 <sup>00</sup> - 15 <sup>20</sup>	Update of sunflower broomrape situation in Spain - <u>Begona Perez</u>
19 15 <sup>20</sup> - 15 <sup>40</sup>	Monitoring of <i>Orobanche cumana</i> Wallr races in sunflower fields of North East Greece - <u>Garyfalia Economou</u>
15 <sup>40</sup> - 15 <sup>50</sup>	<b>Discussion</b>
15 <sup>50</sup> - 16 <sup>20</sup>	<b>Coffee Break</b>
16 <sup>20</sup> - 17 <sup>00</sup>	<b>POSTER SESSION</b>
20 <sup>30</sup> - 23 <sup>30</sup>	<b>Gala Night (Fasil Music &amp; Turkish Dance Entertainment)</b>

3 <sup>RD</sup> Ses	FRIDAY, NOVEMBER 3 <sup>RD</sup> , 2023
10 <sup>00</sup> -12 <sup>00</sup>	<b>HALL 2 Chair: Dr Maria PACUREANU</b>
20 10 <sup>00</sup> - 11 <sup>45</sup>	<b>DISCUSSION:</b> <b>INTERNATIONAL SUNFLOWER COLLABORATION INITIATIVE</b>
11 <sup>45</sup> - 12 <sup>00</sup>	<b>CLOSING OF SYMPOSIUM</b>
13 <sup>00</sup> - 19 <sup>00</sup>	<b>SYMPOSIUM CITY TOUR</b>

## POSTER SESSION

#	TITLE AND AUTHORS
1	Climate-responsive approaches for building durable resistance of sunflower to broomrape in evolving environmental conditions - <u>Sandra Cvejić</u> , Siniša Jocić, Milan Jocković, Boško Dedić, Aleksandra Radanović, Nemanja Ćuk, Vladimir Miklič, Dragana Miladinović
2	Content and oil yield of sunflower ( <i>Helianthus annuus</i> ) - hybrid Deveda depending on the main tillage system - <u>Nina Nenova</u> , Margarita Nankova
3	Influence of broomrape on some anatomical and physiological traits in sunflower - Maria Duca, <u>Steliana Clapco</u> , Ion Burcovschi, Angela Port
4	Study the response of different interspecific sunflower forms to peg-mediated water stress - Daniela Valkova
5	Races of broomrape present in South-Eastern Romania - Florin Gabriel Anton
6	Wild <i>Helianthus</i> species as a valuable breeding source for broomrape resistance of cultivated sunflower ( <i>Helianthus annuus</i> L.) - <u>Jelena Jocković</u> , Sandra Cvejić, Aleksandra Radanović, Milan Jocković, Siniša Jocić, Dragana Miladinović, Lana Zorić, Jadranka Luković
7	"KRASELA"- The first Bulgarian sunflower hybrid, resistant to broomrape (Race H) and stable yield potential under limited moisture conditions - Nina Nenova
8	Determining the Yield Performances and the Resistance to Broomrape and Downy Mildew of IMI Type Sunflower ( <i>Helianthus annuus</i> L.) Hybrids in Different Locations - <u>Ibrahim Mehmet Yilmaz</u> , Samet Saglam, Bayram Serkan Cabar, Veli Pekcan, Ayhan Pirinc

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**KRASELA"- THE FIRST BULGARIAN SUNFLOWER HYBRID, RESISTANT  
TO BROOMRAPE (RAC DEVEDA E H) AND STABLE YIELD POTENTIAL  
UNDER LIMITED MOISTURE CONDITIONS**

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**ABSTRACT**

Plant responses to stress are an increasingly frequent subject of research investigations, especially if the stress factors are biotic and abiotic. The parasite broomrape can reduce sunflower yields to zero levels, and as climate change intensifies, droughts are getting longer, affecting agriculture and human livelihoods. In the breeding programs of Dobrudzha Agricultural Institute - General Toshevo main objective is to create drought-resistant and disease-resistant hybrids sunflower. The aim of this study is to make a complete characterization of the conventional sunflower hybrid "Krasella" and its reaction to the resistance of the parasite *Orobanche cumana* Wallr. (race H) in a particularly dry and middle wet year. Ecological experiments there are in different regions of Bulgaria /three points - Brashlen, Radnevo and Selanovtsi/ and Ukraine /2 points - Zaporozhye and Kirovograd/. The indicators "seed yield", "oil yield" compared to the relevant standard during the years of testing and the resistance of the hybrid to diseases - mildew, fhomia, fhomopsis, and the parasite *Orobanche cumana* are observed and traced. Over the years of the study, hybrid "Krasela" has shown stable yields, even in the particularly dry year – 2020 and the middle wet 2019 years.

**Key words:** sunflower, biotic and abiotic stress factors, *Orobanche cumana* Wallr.

**INTRODUCTION**

The Earth's climate is in constant change, and so has the crops development conditions. A crop moves from one phase to another in its development, as a result from reaching certain temperature sums. In the recent years we have been constantly speaking of a drastically changing climate, mainly referred to as the Global Warming. Some conclusions drawn from this research are that the rise of air temperatures during the XX century has been the highest in comparison with previous centuries, as the 1906-2005 year period, the medium air temperature has been 0.74 Co higher. From the beginning of XX century, the rain over North Europe has risen with 10 to 40%, while the rain over some regions in South Europe (Bulgaria amongst them) has declined up to 20%. The most notable drought was during the year 2000. Those regions include Dobrudzha and the south regions of northwest Bulgaria. The breeding programs of Dobrudzha Agricultural Institute - General Toshevo are include to create drought and diseases resistant sunflower hybrids. Nowadays the breeding puts into practice a large number of hybrids and varieties with high genetic potential, combined with good qualitative characters. The market proposes huge performance of sunflower hybrids. Therefore, the producer has to possess a disposable information for biological and economic qualities of every product with aim

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to establish the correct variety structure in the region, where the relevant hybrid will be planted. This is a prerequisite factor for avoiding some of the stress factors and guarantee for success. Developing of high productive sunflower hybrids is the main priority of present intensive agriculture. The present hybrids combine high seed yield, low moisture content at harvesting, resistance to stress factors as drought, high temperatures, diseases and broomrape. Their seeds are distinguished with high oil content and they are much in demand by crushing industry. Improving of qualities of commercial hybrids is strongly connected to their breeding. Many breeding programs were directed to market supply with various hybrid seeds. The main factor, determined the obtaining of high and qualitative seed yield is the correct alternative of suitable hybrids for each region. They have to insure the high yield in combination with high oil content, resistance or tolerance to the most important diseases, pests and parasites. It is very difficult or almost impossible all these characters to be included in one hybrid. This is a main purpose in the research programs of the breeding centers. In Dobrudzha Agricultural Institute - General Toshevo is increasing the amount of breeding work related to the creation of such hybrids sunflower (Encheva et al., 2002, Encheva et al., 2016 Valkova et al., 2017). We already have an extremely rich and diverse collection of parent lines, created both through the methods of classical selection and their combination with various biotechnological methods (Encheva et al., 2014; Encheva et al., 2015; Nenova et al., 2014; Nenova et al., 2016). All this is a valuable starting breeding material, which is the reason why in the last decade the institute has created a large number of highly productive lines and hybrids of sunflower, which have been officially recognized and are already distributed over large areas (Georgiev and Encheva, 2014; Georgiev et al., 2015; Georgiev, 2015; Georgiev, 2016; Georgiev, 2017; Peevska and Georgiev, 2016).

The aim of present study is to make a detailed characteristics of newly registered conventional Bulgarian hybrid of newly registered Bulgarian hybrid "Krasela" , resistance to Orobanche and the response in the particularly dry year 2020 and moderately dry year – 2019.

## **MATERIAL AND METHODS**

Hybrid "Krasela" is single cross hybrid, developed on the method of interlinear hybridization /217A x N44R/. The maternal line 217A was obtained by crossing of experimental hybrid N:72 and line 246, created from Russian cultivars, self-pollination and selection. The mother line was characterized with good general and specific combining ability. It is resistant to broomrape, race F, middle resistant to phoma, alternaria and phomopsis. During the last several years it was a maternal component of newly registered hybrids Veleka, Vokil, Linzi, Deveda and etc.

The paternal component of hybrid "Krasela" is the branched line-restorer of fertility N44R. That line was obtained using the method of induced parthenogenesis from the experimental hybrid 509x523, self-pollination, embryoculture and selection. It is resistant to downy mildew, race 731 and resistant to broomrape, race H. It is middle resistant to phomopsis and alternaria, resistant to phoma.

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**RESULTS AND DISCUSSION**

**Morphological description**

Morphological description of hybrid “Krasela” on UPOV (2002) was done. The described characters was presented on table 1.

Table 1. Morphological characteristics of sunflower hybrid “Krasela”

Traits	Expression	Degree
1. Hypocotyl:antocianic pigmentation	Absent	1
2. Hypocotyl:intensity of antocianic pigmentation	Absent	1
3. Leaf: size	Medium	5
4. Leaf: color	Medium green	4
5. Leaf: blistering	Weak	3
6. Leaf: serration	Very coarse	7
7. Leaf: share of cross section	Flat	2
8. Leaf: shape of distal part	Very strong	7
9. Leaf: auricles	Very large	2
10. Leaf: wings	Weakly present	2
11. Leaf: angle of lateral veins	Obtuse	3
12. Leaf: height of the tip of the blade compared to insertion of petiole (at 2/3 height of plant)	Present	3
13. Stem: intensity of hairiness at the top	Strong	7
14. Time of flowering	Medium to late	6
15. Ray flower number	Medium	5
16. Ray flower shape	Ovoid	2
17. Ray floret:disposition	Medium	5
18. Ray flower length	Medium	5
19. Ray flower color	Yelow	4
20. Disk flower color	Orange	2
21. Disk flower anthocyanin coloration of stigma	Absent	1
22. Disk flower: intensity of anthocyanin coloration of stigma	Absent	1
23. Disk flower presence of pollen	Present	9
24. Bract shape	Rounded	3
25. Bract length of the tip	Medium	5
26. Bract color of the external part	Medium green	5
27. Bract: attitude in relation to head	Slightly embrasing	2
28. Plant: natural height	Medium	5
29. Plant: branching	Absent	1
30. Plant: type of branching	-	-
31. Plant: natural position of closest lateral head to the central head	-	-
32. Head: posture at ripeness	Half-turned down	7
33. Head: size	Medium to Large	6

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34. Head: shape of grain side	Convex	3
35. Seed: size	Medium to large	6
36. Seed: shape	Ovoid elongated	2
37. Seed: thickness	Medium to thick	5
38. Seed: main color	Black	7
39. Seed: stripes on margin	Weakly present	2
40. Seed: color of stripes	Weakly present	2

**Values for cultivation and use (VCU) of hybrid “Krasela”**

It is a fact that climate change has direct effects on the plant productivity. Tables 3 and 4 show the results of the seed yield for the two years during which the rainfall is very low and insufficient for the vegetative period (Table 2). During the two drought years, the hybrid “Krasela” did not show its maximum productive potential, but still gave a stable yield of 320-413 kg. and this hybrid can be offered to farmers in dry years such as 2019 and 2020.

Table 2. Climatic characteristics for 2019 and 2020 for the period March – September

Month	Year	Place	Precipitation, mm/m <sup>2</sup>
March-September	2019	Selanovci	233.4
		Brashljin	279.2
		Radnevo	281.6
Average			264.7
March-September	2020	Selanovci	319.6
		Brashljin	263.2
		Radnevo	209.3
Average			264.0

Table 3. Seed yield kg/da- 2019

Hybrid	Selanovci		Brashljin		Radnevo		Average	
	kg/da	%	kg/da	%	kg/da	%	kg/da	%
Standart (NK Brio+LG5662)	485	100,0	425	100,0	357	100,0	423	100,0
NK Brio	482	99,4	473	111,1	389	109,0	448	106,0
LG5662	488+++	100,6	378+++	88,9	325	91,0	397	94,0
Krasela	403	83,1	441	103,6	396	110,9	413	97,8
GD 5%	22,86	4,71	26,56	6,24	37,62	10,53		
GD 1%	30,45	6,28	35,37	8,31	50,09	14,02		
GD 0.1%	39,68	8,18	46,10	10,84	65,29	18,28		

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Table 4. Seed yield kg/da- 2020

Hybrid	Selanovci		Brashljin		Radnevo		Average	
	kg/da	%	kg/da	%	kg/da	%	kg/da	%
Standart (NK Brio+LG5662)	392	100,0	365	100,0	302	100,0	353	100,0
NK Brio	383	97,8	391	107,1	257	84,9	343	97,3
LG5662	401	102,2	339	92,9	348++	115,1	362	102,7
Krasela	363	92,6	353	96,8	246	81,2	320	90,8
GD 5%	6,46	1,65	21,02	5,76	33,65	11,14		
GD 1%	8,64	2,20	28,10	7,70	44,98	14,89		
GD 0.1%	11,32	2,89	36,85	10,1	58,99	19,53		

**Biochemical and phytopathological characteristics of hybrid “Krasela”**

The oil content in the seeds of the Krasela hybrid is not affected by drought conditions. This is shown in Tables 5 and 6. For both years the oil content in the seeds is 45.6% and 47.6%. The excess over the average standard is 6.6% for the two years. In addition to productive indicators, breeders must also create disease- and parasite-resistant varieties and hybrids. Climate change also affects the race composition of pathogens. Plant pathologists have always considered environmental influences in their studies of plant diseases: the classic disease triangle emphasizes on the interactions between plant hosts, pathogens and the environment. The Krasela hybrid is resistant to the Orobanche - race H. This is the only Bulgarian hybrid resistant to this race and can grow to areas that are highly infected with the Orobanche pathogen. Radnevo is a region in Bulgaria where the soils are most infected with the most aggressive races Orobanche. These data show that even in drought the hybrid has given stable yields close to the standarts.

5. Oil content, % - 2019

Hybrid	Selanovci		Brashljin		Radnevo		Average	
	%	St %	%	St %	%	St %	%	St %
Average standard (NK Brio+LG5662)	44,30	100,0	43,95	100,0	48,60	100,0	45,62	100,0
NK Brio	49,10	110,8	45,90	104,4	50,10	103,1	48,37	106,0
LG5662	39,50	89,2	42,00	95,6	47,10	96,9	42,87	94,0
Krasela	46,30	104,5	48,30	109,9	48,30	99,4	47,63	104,4

Table6. Oil content, % - 2020

Hybrid	Selanovci		Brashljin		Radnevo		Average	
	%	St %	%	St %	%	St %	%	St %
Average standard (NK Brio+LG5662)	45,50	100,0	41,55	100,0	43,75	100,0	43,60	100,0
NK Brio	47,80	105,1	43,00	103,5	45,00	102,9	45,27	103,8
LG5662	43,20	94,9	40,10	96,5	42,50	97,1	41,93	96,2
Krasela	48,10	105,7	44,60	107,3	44,20	101,0	45,63	104,7

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**CONCLUSION**

Climate changes mostly affect agricultural production. Research on the climate change impact on plant disease has led to a new aim: to create a drought-resistant sunflower, highly productive hybrid with genes that control diseases, conducive to high temperatures and low soil moisture. Hybrid “Krasela” (Fig.1) is a conventional hybrid and middle early hybrid. It has a stable yield during the dry years and the content the oil in the seeds is about 47%. The hybrid is resistant to downy mildew /race 731/ and broomrape /race H/ and middle resistant to phoma and phomopsis. The hybrid needs no special requirements for cultivation.



Fig.1 Hybrid “Krasela

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**DETERMINATION OF AGRICULTURAL POLICY FACTORS AND THEIR  
EFFECTS AFFECTING PRODUCERS' PREFERENCE FOR PRODUCTION  
OF OILY SUNFLOWER: THE CASE OF THRACE REGION**

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**ABSTRACT**

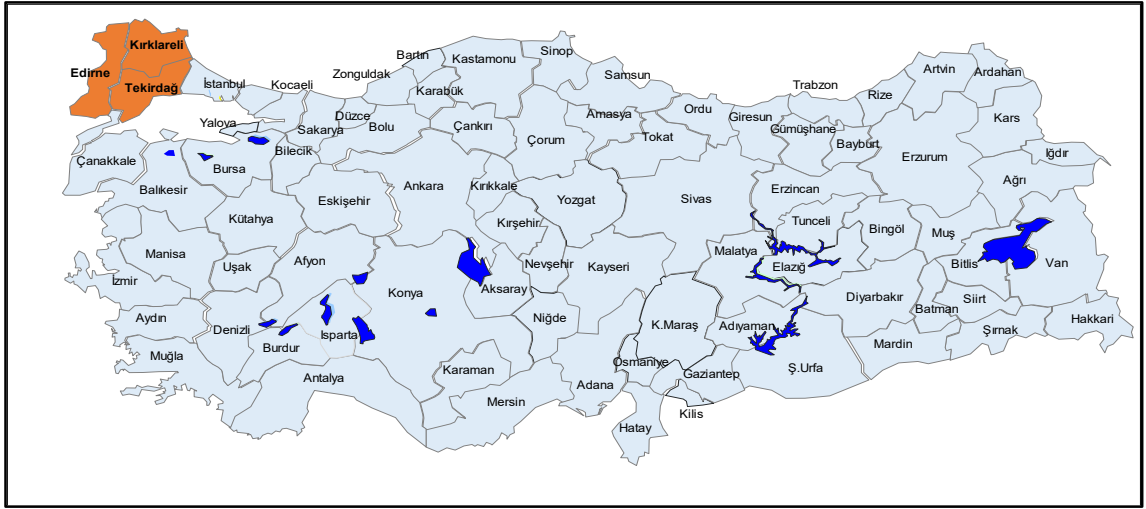
In this study, it is aimed to analyze the factors affecting the production preferences of producers of oily sunflower. It covers the provinces of Tekirdağ, Edirne and Kırklareli in the Thrace Region, which is determined as a research area, and stands out especially with sunflower production in our country. The Thrace Region refers to the geographical area where sunflower production is carried out to a large extent in our country with its sunflower cultivation area of 3.1 million. According to the results obtained in the study conducted based on micro data, the availability of the land and the suitability of the production activity and the level of profitability were determined as effective factors in the sunflower production decision of the producers. However, in the selection of sunflower seeds, price and sales channel criteria were determined as determining priority variables. In the study, the problems related to sunflower production activity are raised as high input costs and marketing issues in order of priority. In addition, increasing the supports directed to sunflower production emerges as a producer expectation in the region.

**KEYWORDS:** Sunflower, Thrace Region, Production, Producer Behavior

**INTRODUCTION**

Our research area covers the whole of the Thrace Region, especially Tekirdağ, Edirne and Kırklareli provinces. This region, which is located in the most North West region of our country, is one of the important regions of our country in terms of plant production. It is possible to come across very different morphological units in our Thrace Region, which is located in the southeastern part of the Balkan peninsula. To list the mainly, it is possible to observe mountains and hills with different elevations, as well as plateaus with less elevation and different large and small plains.

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Source: Anonymous 2019.

Map 1. Location and location of the Thrace Region in Turkey

## LITERATURE REVIEW

### Investigation of Sunflower Plantations in the Thrace Region

When the sunflower cultivation areas of Tekirdağ, one of the provinces of our Thrace Region, are examined as the last three years, it is possible to see a wavy cultivation area. In 2018, a total of 1,481,286 sunflowers were planted, while in 2019, this area decreased to 1,365,350. When it comes to 2020, our last evaluation year, we can see that the cultivation area has reached 1,424,669, approaching 2018. In our province of Tekirdağ, where producer preference varies according to different factors, the spread of canola production, especially in competition with sunflower, in some years causes fluctuations in the cultivation areas.

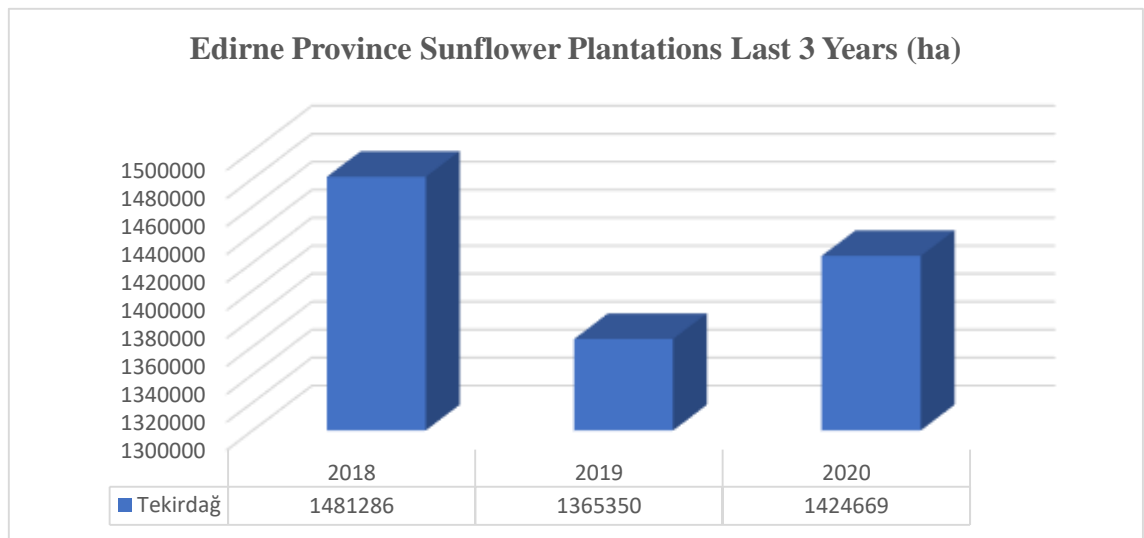


Figure 1. Tekirdağ Province Sunflower Plantations Last 3 Years (ha) (Source: TurkStat, 2020)

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When our Edirne Region, which is another province of our Thrace Region, is examined, we see that while similar cultivation areas were formed in 2018 and 2019, this area decreased in 2020. The sunflower species, which reached 954,512 cultivation areas in 2018, almost protected its area in 2019 and had 950,498 cultivation areas. When we look at 2020, our last review year, it is possible to say that there is a cultivation area that has declined to 909,155. Here, we can say that the fact that the grain group in particular is more intensively cultivated in the region is a big factor.



Figure 2. Edirne Province Sunflower Plantations Last 3 Years (ha)( Source: TurkStat, 2020)

When we look at our province of Kırklareli, which is one of the important provinces of our Thrace Region, we can say that although it has a parallel cultivation area in 2018 and 2019, there is a significant increase in 2020. Our province, which had a total sunflower planting area of 750,021 in 2018, had 740,511 sunflower planting area in 2019. In 2020, with the increase, it is seen that 778,064 sunflower planting areas are realized in this province.

If the sunflower cultivation areas of our Thrace Region in general are examined, we can say that the highest planting area was realized in 2018. The Thrace Region, which has a sunflower cultivation area of 3,185,809, has not been able to reach this figure again in the last three years. The Thrace Region, which created a sunflower planting area of 3,056,359 with a serious decrease in 2019, reached 3,111,888 sunflower planting area in 2020. These fluctuations in the sunflower planting area can be interpreted as the different gains that alternative species to sunflower cultivation provide to the producers.

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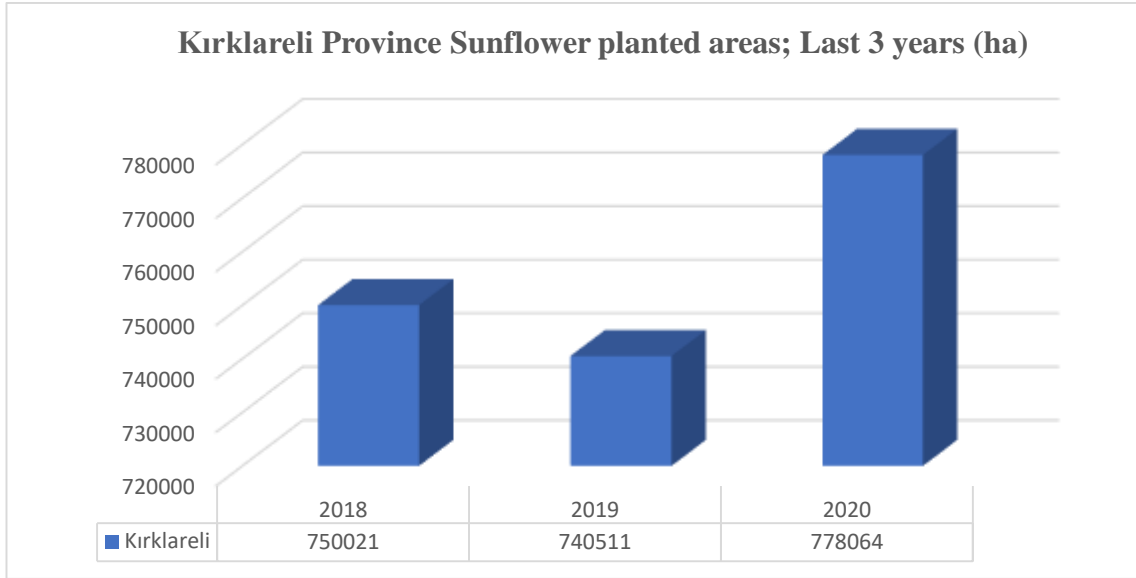


Figure 3. Kırklareli Province Sunflower Plantations Last 3 Years (ha) (Source: TurkStat, 2020)

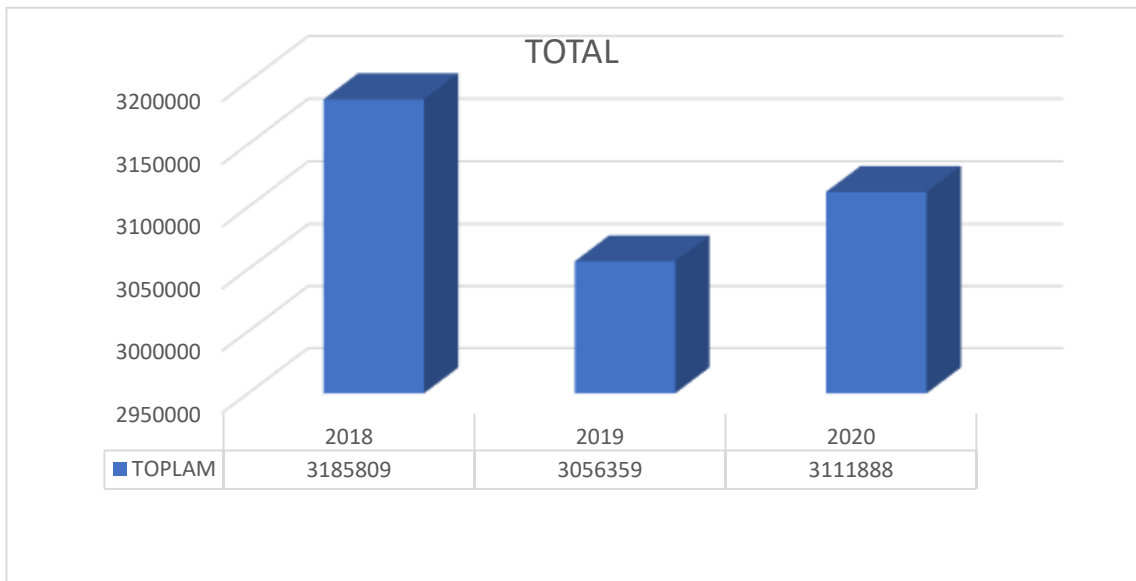


Figure 4. Thrace Region Sunflower Plantations Last 3 Years (ha) (Source: TurkStat, 2020)

**Investigation of Sunflower Production Amounts in the Thrace Region**

When we examine the sunflower production amounts of the Thrace Region in the last three years, it is possible to see an increasing graph. Sunflower, which saw a production figure of 777,807 tons in 2018, has reached 802,798 tons in 2019 and 820,836 tons in 2020 with an increasing graph.

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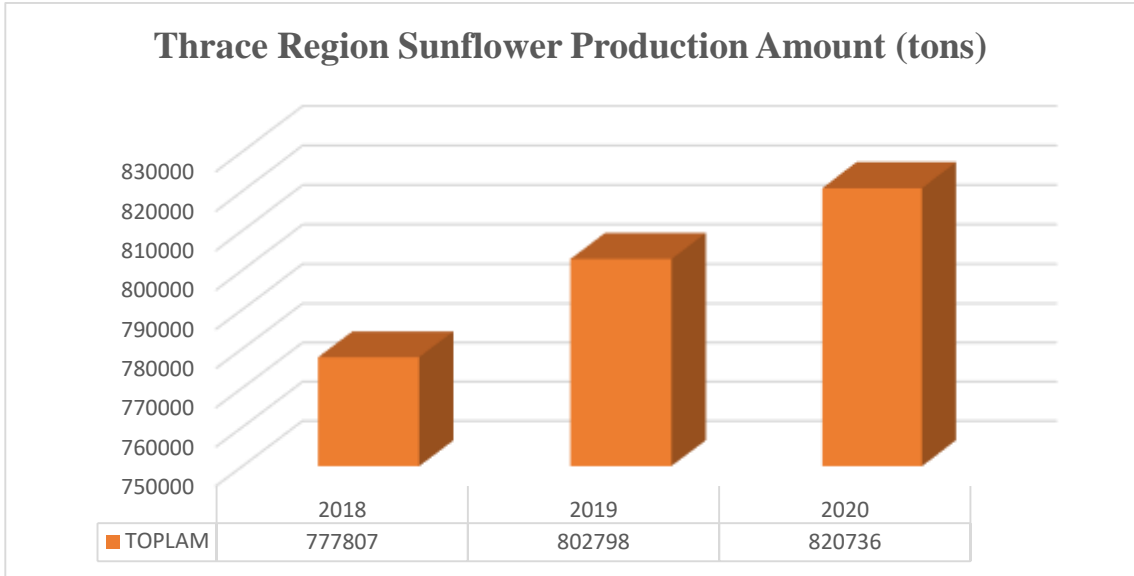


Figure 5. Thrace Region Sunflower Production Amount (tons) (Source: TurkStat, 2020)

**Investigation of Sunflower Yield Averages in the Thrace Region**

If we look at the average yield of sunflower obtained from the decares of the Thrace Region compared to the last three years, it is possible to see an increasing graph. While the producers of the Thrace Region, which is the important sunflower cultivation region of our country, obtained an average yield of 247 kg from the decares in 2018, it is possible to say that this figure reached 266.3 kg / da in 2019. However, the fact that the average yield in 2020 was up to 267.7 kg / da is pleasing for our country with sunflower deficit.

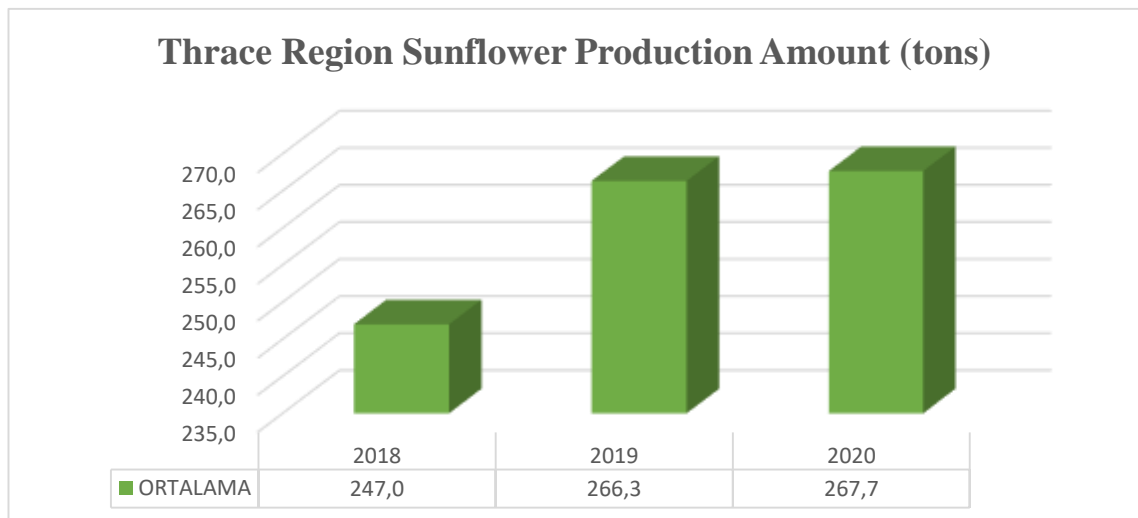


Figure 6. Thrace Region Sunflower Production Amount (tons) (Source: TurkStat, 2020)

When the cultivation areas and production performance of the Thrace Region are examined, it is possible to attribute the increase in the total amount of production to the increase in yield per unit area, especially despite the unbalanced cultivation areas. Especially in 2019 and 2020, the fact that the climatic conditions are suitable for the

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sunflower plant has caused the yield scale and accordingly the production amount to increase.

## **MATERIAL AND METHOD**

The main material of the study consists of primary data obtained from questionnaires carried out at the producer level. In addition, statistical data and literature studies related to the field were also used.

Within the scope of the research, a total of 38 producers engaged in sunflower production within the scope of the Thrace Region area were interviewed. The data collection method was carried out face-to-face and online survey application.

In the questionnaire form used in the study, questions related to the demographic characteristics of the producers, production and product decision selection, production problems, producer satisfaction and evaluation were included. Open-closed-ended and multiple choice question formats were used in the questionnaire form and a total of 32 questions were included. In the analysis and evaluation of the data, simple descriptive statistics were used.

## **RESULTS AND DISCUSSION**

### **General Profile of the Producers Participating in the Study**

#### **Gender, Marital Status, Education Status**

When we look at the producers who participated in our survey study, it is possible to say that men are the majority in terms of gender distribution. 91.7% of the participants were male and 8.3% were female.

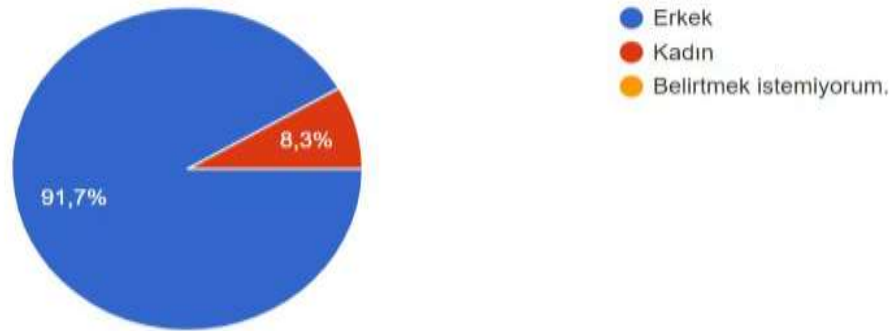


Figure 7. Gender Distribution of Producers Participating in the Study

When the producers who participated in our survey are examined, it is possible to see that 70.8% are married and 25% are single.

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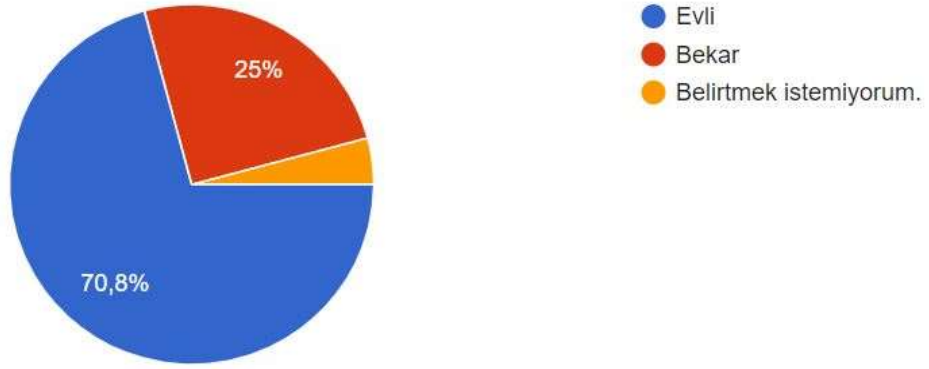


Figure 8. Marital Status Distributions of the Producers Participating in the Study

When the educational status of the producers participating in our survey is examined, we see that 50% of the producers participating in the survey are undergraduate graduates. However, it is seen that the other largest audience consists of producers who have completed their high school education with 20.8%. It is seen that 16.7% of them are Associate Degree graduates. As we can clearly deduce from this table, we can clearly communicate that our survey was attended by more conscious producers who had received a certain level of education, whose educational status was at the medium and high level.

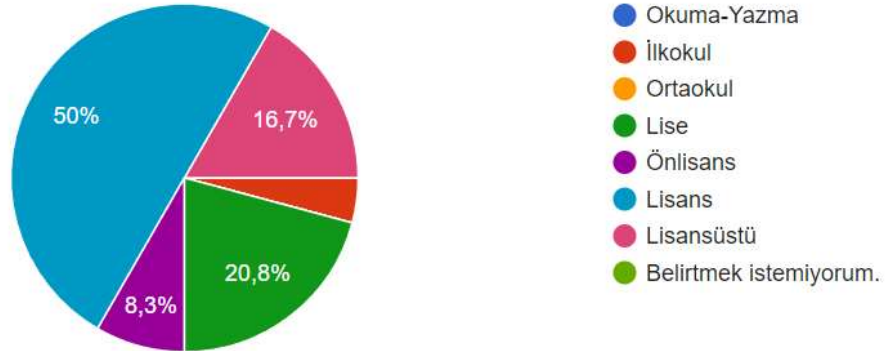


Figure 9. Educational Status Distributions of the Producers Participating in the Study

### **Approach to Problems in the Production Process**

With the questions we asked our farmers who produce sunflowers in the Thrace Region, we put forward approaches to the problems they experienced in the production process. To the question of what is the biggest problem in agricultural production that we have directed to our producers, our producers have pointed out high input costs with a large majority. 91.7% of the producers emphasized that the biggest problem in production is

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the high input costs. While the second place was taken by the Disease and Pest answer with 4.2%, marketing problems were pointed out with 4.2%.

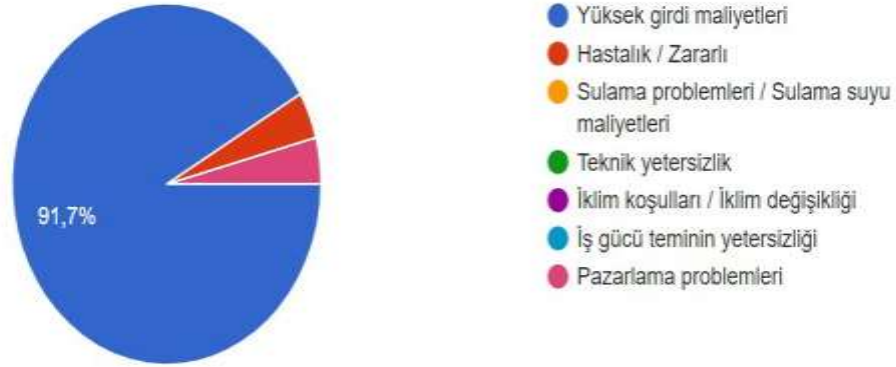


Figure 10. Problems Seen by the Producers Participating in the Study in Agricultural Production

In order to evaluate the difficulties experienced by our producers in sunflower production and their expectations in the face of the problems, we included in our research what is the first responsible or problem element that comes to mind when they experience low yield in sunflower production.

As a result of our investigations, the climate seems to be the first factor that producers hold responsible for the yield losses they experience with a rate of 62.5%. The fact that our producers' concerns and opinions on climate are in this direction is perhaps extremely important in terms of nature sensitivity. On the other hand, it is striking to say that our producers see the second biggest element as the seed variety. Here it is possible to infer that producers have high expectations from seed varieties.

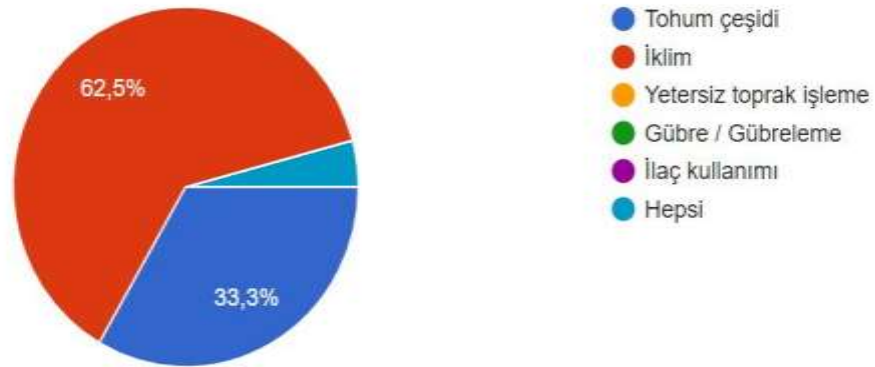


Figure 11. The Perspective of the Producers Participating in the Study on the Productivity Decreases They Experienced

### Factors Determining Sunflower Production Preference

Our producers who produce sunflowers in the Thrace Region are affected by some resources and situations before deciding on the product they will grow. Here, too, when deciding which species the producers will grow, the largest proportion of the producers



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and 50% of the producers see it as completely the adaptation of the area to be produced to the plant. Secondly, when our producers make production decisions, it is seen that they look at how profitable the product they will grow will provide them. This rate is not to be underestimated and is around 41.7%. One of the striking results of the study will be that when producers make production decisions, they are not affected by other producers at all.



Figure 12. Factors Affecting the Production Decision of the Producers Participating in the Study

In our research, our manufacturers are aware of the problems experienced during production. or it is seen that there are sales channels with the highest rate as the unit and place consulted in the pre-production decision stage. 41.7% of the producers consult the sales channels, dealers or cooperatives first when they need to make decisions in the technical sense or in the problems experienced. Secondly, it is seen that producers are seriously affected by other producers. It was seen that 25% of the producers were determined to be the producers in their environment as consults or samplers. We see that another element is the Agricultural Consultants. When we look at this issue and sum it up, it is seen that the vast majority of manufacturers are inclined to receive support from expert professionals.

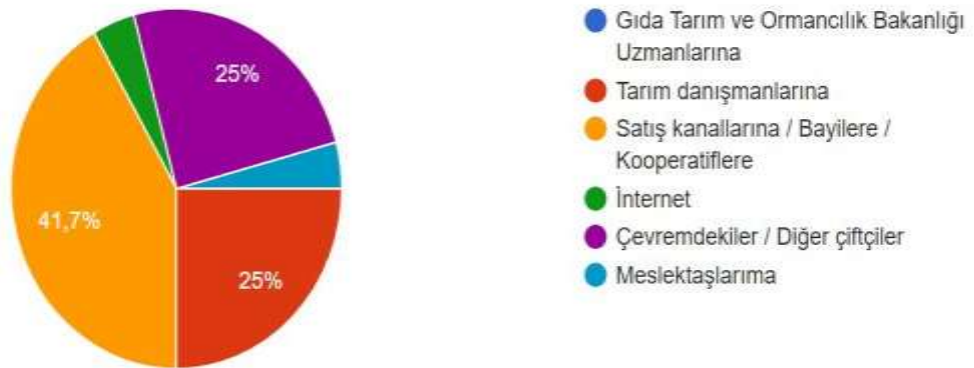


Figure 13. Areas Consulted by the Producers Participating in the Study in Production Problems

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**Factors in Sunflower Seed Selection**

It is useful to consider separately the issue of seed, which is the most important element of production and which producers especially focus on in our study. We will examine the areas where our producers are affected when making their seed preference and the processes in the decision stages.

When we look at the price evaluation when choosing the sunflower seeds of the sunflower producers of the Thrace Region, 54.2% of our producers emphasized that the price is important for them. In addition, our generator rate, which states that price is very important, is at the level of 16.7%. Our producer rate, which does not care about the price policy in sunflower seed preference, is 16.7%.

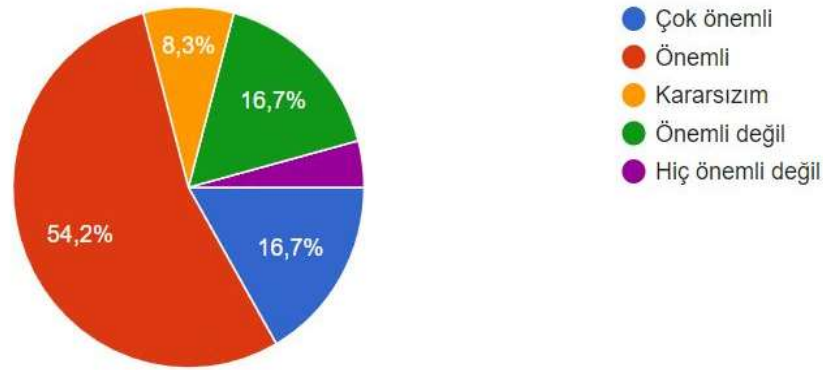


Figure 14. The Importance of Price in the Seed Preference of the Producers Participating in the Study

Producers who produce sunflowers in the Thrace Region are absolutely guided by sales channels and play a decisive role especially in seed preference. When choosing sunflower seeds, our producers consider the guidance of dealers or all other sales channels to be important by 50% and very important by 8.3%. The rate of producers who do not care about the guidance of sales channels remains at the level of only 4%.

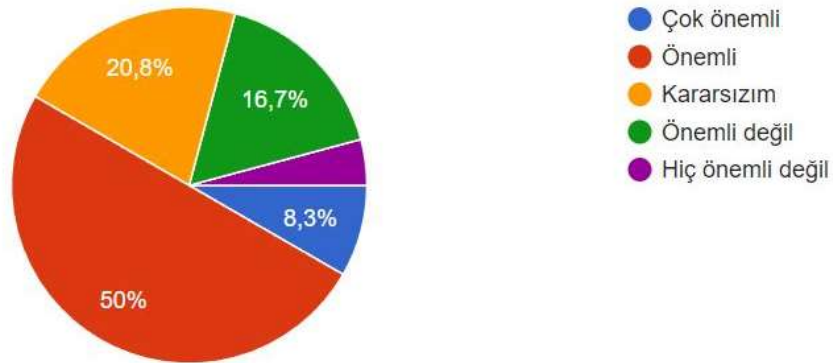


Figure 15. Attitude of the Producers Participating in the Study Towards the Sales Channel Proposal in Seed Preference

### **Sunflower Producer's Perspective on Agricultural Policies**

Sunflower species is one of the products that have great importance for our country and should be supported as an externally dependent country, especially in terms of oil deficit. Currently, when our country is examined in terms of agricultural policies, certain species and plants; fertilizer, diesel and additional additional supports are given.

In our study, we examined the satisfaction of our producers with the support provided to them in terms of agricultural policy in sunflower agriculture.

To put forward the perspective of the sunflower producers of the Thrace Region on sunflower agricultural supports, 75% of the producers stated that they were not satisfied with the agricultural supports available in the current situation and that the agricultural supports were not sufficient. On the other hand, 20.8% of the producers stated that they were satisfied with the existing sunflower production supports.

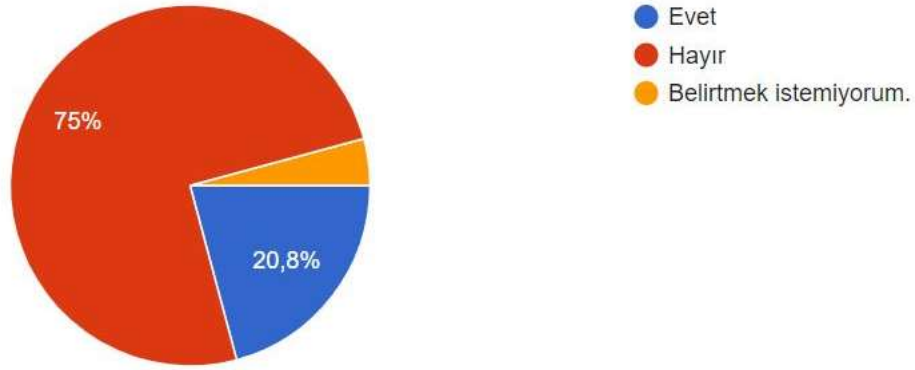


Figure 16. Perspective of the Producers Participating in the Study on Sunflower Production Supports

In addition to the answers given by the producers during the field studies we carry out, it is necessary to evaluate the comments they give to agricultural policy studies. During the face-to-face survey studies conducted in Tekirdağ, Edirne and Kırklareli provinces, it was seen that the producers were highly dissatisfied with the diesel and fertilizer support program currently available in our country. As a result of the study, it is shown that 75% of the producers are not satisfied with the support of the current agricultural policies on the sunflower producer.

Accordingly, in our study, we investigated how manufacturers determine their payment methods as a result of insufficient supports. Here, too, we went to analyze what our producers prefer as a payment method.

It has been observed that 75% of the sunflower producers of the Thrace Region make futures purchases to pay their agricultural inputs and apply to the long-term open account payment method, which we call credit. While 16.7% of the producers apply for installment by credit card, it is seen that only 8.3% of the producers can receive their agricultural inputs in advance.

In this analysis, it is observed that the existing sunflower agricultural supports do not allow the producers to receive their agricultural inputs in advance and the producers to make the biggest expense items more economical with cash purchases.

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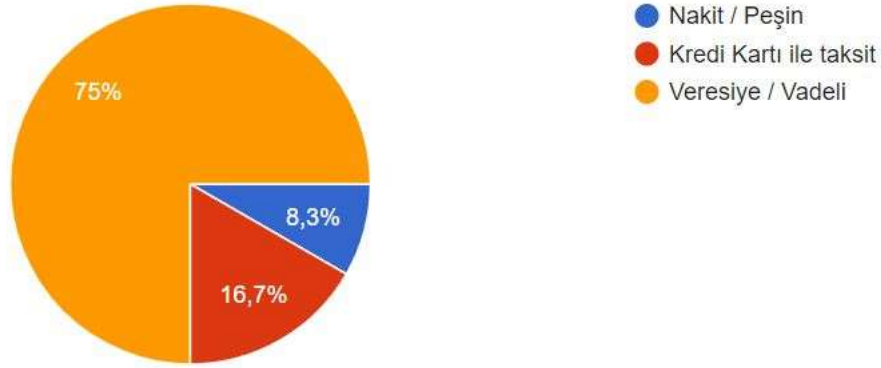


Figure 17. Methods of Purchasing Agricultural Inputs of Producers Participating in the Study

### CONCLUSION AND RECOMMENDATIONS

It is obvious that our country is a serious importer of sunflower construction. However, in our Thrace Region, which is the largest region where sunflower production is made, it is seen that a total of 3,111,888 sunflower production is made in 2020. The average yield of 267.7 kg / da is obtained against this area. In the light of this area and yield result, 820.836 tons of sunflower products were produced in the Thrace Region.

When the habits and attitudes of our producers are examined, it is seen that the producers are mainly in close relations with sales channels. Our study shows that with this habit, manufacturers also receive serious support from sales channels in planning their commercial activities. In fact, this is a contentious development. It is clearly established that when deciding on the agricultural actions of producers, they consult expert knowledge, demand guidance at a certain rate.

On the other hand, the fact that the sunflower producers of the Thrace Region see the first reason for their yield losses as climate is a source of satisfaction. The fact that producers are aware of yield losses as a result of changing climate, increasing temperatures and droughts continues to hope that it will make producers more sensitive to climate and nature.

The Thrace Region is quite difficult for sunflower producers from the high cost of agricultural inputs. The results of our study clearly show that producers draw attention to input costs as the biggest factor that forces agricultural production. However, the result of caring about price parity in sunflower seed preference supports this idea.

It is observed that the producers of the Thrace Region are not satisfied with their support for sunflower agricultural policy. The vast majority of producers find their agricultural support insufficient. As a result of this situation, we see that a large part of shopping habits are realized as maturity, long-term borrowing.

Agricultural support for sunflower and sunflower oil, which have a high strategic importance for our country, should be increased. Incentives and encouraging support should be provided to our producers for the sustainable production of such a strategic product. The supports to be realized should support the inputs of sunflower producers in real terms and direct production.

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**EFFECT OF GENE DOSE ON BROOMRAPE RESISTANCE IN SUNFLOWER**

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**ABSTRACT**

Increasing the genetic resistance of sunflower to broomrape using various approaches attracts breeders from different seed companies. In our work, we studied the effect of the homo- and heterozygous state of the Or7 gene on the degree of resistance to broomrape race G in 12 sunflower breeding genotypes. The resistance was assessed under the conditions of a climatic chamber in the phase of 3 pairs of true leaves in a box with peat soil for growing plants with the addition of 0.2 g of broomrape seeds per 1 kg of soil. Five homozygous susceptible genotypes (or7or7) showed the degree of damage from 14 to 38 broomrape nodules per plant. For two resistant homozygous genotypes (Or7Or7), the infection rate was 2 nodules per plant. Five heterozygous genotypes (Or7or7) were characterized by a wide variation in broomrape damage values from 2 to 29. Therefore, depending on the combination of crossing, the Or7 gene in the heterozygote was dominant, intermediate, and recessive. The data obtained indicate the presence of other mechanisms of resistance to broomrape race G, additional to the Or7 gene.

**Key words:** heterozygote, homozygote, genotype, seed, race, dominant, recessive

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**GENETIC DIVERSITY ANALYSIS OF BROOMRAPE (OROBANCHE CUMANA)  
POPULATIONS IN SUNFLOWER GROWING AREAS IN EUROPE**

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**ABSTRACT**

*Orobanche cumana* or broomrape is an obligate root parasite of sunflower (*Helianthus annuus*) that strongly impacts yield in southern and eastern Europe. The host-parasite system of *O. cumana* and sunflower is characterized by a typical gene-for-gene interaction. The extensive use of sunflower varieties carrying monogenic resistance genes enhanced the selection pressure on the parasite, leading to the emergence of new races. The two most recent races of *Orobanche* that were officially described are referred to as race F and G. This work reviews the results of monitoring broomrape populations in 8 different European countries during the past 10 years. Seeds of *O. cumana* collected in sunflower fields were tested for their virulence on a differential set of sunflower varieties carrying different resistance genes. Race F is still the most predominant in most regions, but in east European countries a wider diversity of races and an increased incidence of the more aggressive race G was observed. The genetic diversity of the isolates was studied using a set of 180 SNPs that allowed to classify them according to their geographic origin and showing higher levels of heterozygosity in eastern Europe populations. These results will be corroborated by more recent GbS data that were obtained for a subset of the collection. All in all, this study provides an overview of the pathogenicity profiles and the molecular diversity of *O. cumana* populations across the major sunflower markets in Europe.

**Key words:** Sunflower, *Helianthus annuus*, Broomrape, *Orobanche cumana*, genetic diversity

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**DNA MARKER FOR MARKER-ASSISTED SELECTION FOR SUNFLOWER  
RESISTANCE TO RACE G OF BROOMRAPE**

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**ABSTRACT**

For marker-assisted selection of sunflower for resistance to race G of broomrape, 2 new SCAR markers have been developed to identification the HaOr7 gene. The marker RORS1 is for the presence/absence of the HaOr7 gene with the length of the specific PCR product  $\approx 168$  bp and the SORS9 marker is for the presence/absence of the wild-type allele with the length  $\approx 217$  bp. Due to the possibility of multiplex PCR, these markers form a codominant marker system. This marker system was tested on 69 sunflower genotypes with different resistance to broomrape race G: 18 resistant and 34 susceptible lines of VNIIMK breeding, 3 resistant and 10 susceptible hybrids of VNIIMK breeding, 2 differential lines to race F of broomrape – LC1093 and P96 and 2 resistant commercial hybrids SI Chester (Syngenta, Switzerland) and P64LC108 (Pioneer Hi-Bred International, Inc., USA). All resistant sunflower lines to race G of broomrape showed the presence of only one specific PCR product of  $\approx 168$  bp in length (marker RORS1), characterizing them as homozygotes by the HaOr7 gene. All susceptible lines, including two differential lines to race F as well as susceptible sunflower hybrids, showed the presence of a specific PCR product of only the SORS9 marker, a wild-type homozygote. Resistant hybrids, including SY Chester and P64LC108, were characterized by the presence of both fragments of the RORS1 and SORS9 markers, making them heterozygous. The marker was used to select sunflower plants from segregating populations at the Laboratory of genetics of VNIIMK. Phenotypic analysis, confirmed that all plants identified by the marker as susceptible were affected by broomrape, while resistant homo- and heterozygotes were not affected or were affected insignificantly – 1–3 tubercles per plant. Thus, the marker we developed is a good tool in sunflower breeding for resistance to race G of broomrape.

**Key words:** DNA marker, sunflower, *Helianthus annuus*, broomrape, *Orobanche cumana*, marker-assisted selection



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**WILD *HELIANTHUS* SPECIES AS A VALUABLE BREEDING SOURCE FOR  
BROOMRAPE RESISTANCE OF CULTIVATED SUNFLOWER (*HELIANTHUS  
ANNUUS* L.)**

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**ABSTRACT**

Sunflower is an important industrial crop. Together with the expansion of the production areas, the challenges in growing sunflower are becoming tougher. Undoubtedly one of the most important problems in sunflower cultivation is the parasitic plant broomrape (*Orobanche cumana*). The first use of wild *Helianthus* species for introduction of broomrape resistance dates from the first half of 20th century when Russian academician Zhdanov developed resistant sunflower genotypes from interspecific crosses with *H. tuberosus*, *H. mollis* and *H. maximiliani*. Taking into account the ability of broomrape to evolve into more aggressive races and thus overcome the resistance genes, scientists are forced to constantly search for new sources of resistance. As a result, sources with different levels of resistance are found in *H. tuberosus*, *H. mollis*, *H. maximiliani*, *H. nuttallii*, *H. debilis*, *H. neglectus*, *H. niveus*, *H. argophyllus*, *H. divaricatus*, *H. bolanderi*, *H. petiolaris*, *H. praecox*, *H. deserticola* and *H. grosseserratus*. Breeding for genetic resistance is even more challenging due to the existence of not only large broomrape interpopulation divergence, but also intrapopulation divergence. One of the areas that has not received enough attention is the anatomical characterization of the root of wild sunflower species and sunflower in general. It is known that pre-haustorial resistance to broomrape is determined by the development of physical barriers in host root cell walls, which prevents linkage of broomrape to the host. A detailed anatomical characterization of root in wild *Helianthus* species can give valuable information about differences between the species and more knowledge about the nature of resistance of certain *Helianthus* species to broomrape.

**Key words:** Wild sunflower, broomrape, resistance

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**IN THE RACE WITH THE BROOMRAPE - IS THERE A WINNER?**

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**ABSTRACT**

Broomrape (*Orobanche* spp.) is a widespread, herbaceous, parasitic plant that has been known to attack various host plants, including sunflower (*Helianthus annuus* L.). It is native to regions in Europe and Asia, and it co-evolved with various host plants and with the expansion of agriculture and the movement of crops around the world, broomrape was unintentionally transported to new areas. Newer studies report about sunflower broomrape detection in African countries such as Tunisia and Morocco. After the discovery of broomrape infestations on sunflower, various control and management strategies have been developed to combat its spread which includes crop rotation, use of resistant sunflower genotypes, herbicide use and other cultural practices aimed at reducing the prevalence of broomrape. Since its appearance on sunflower in the beginning of the 20th Century and development of resistant sunflower varieties, broomrape control was mostly focused on using resistant genotypes with monogenic inheritance. With the expansion of resistant sunflower genotypes in the production, existential pressure on broomrape led to the emergence of more virulent physiological races. Broomrape races are designated with letters, from A to H, and until the middle of 1990s broomrape race E was predominant in the most countries where broomrape was present and was successfully controlled by resistant gene Or5. However, studies from the last several years indicate dissimilarities between broomrape populations with the same letter (above race E) reported in different countries. Newer molecular studies are focused on detecting quantitative trait loci (QTLs) which may act at different development stages of broomrape, providing accumulative resistance mechanisms in order to ensure more durable protection. Despite efforts to control broomrape, it remains a persistent problem in sunflower cultivation in certain regions. New strains of broomrape may emerge, making control measures an ongoing challenge for breeders and farmers. Research and development efforts are ongoing to find more effective and sustainable ways to manage this parasitic plant and protect sunflower crop.

**Key words:** sunflower, broomrape races, quantitative trait loci

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**STUDY THE RESPONSE OF DIFFERENT INTERSPECIFIC SUNFLOWER FORMS  
TO PEG-MEDIATED WATER STRESS**

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**ABSTRACT**

Response of five sunflower genotypes with different origin to drought stress at germination and seedling stage was investigated using polyethylene glycol (PEG 6000) as drought simulator under laboratory conditions. Normal treatment and two levels of osmotic stress were monitored. Germination percentage, root length, shoot length, root to shoot length ratio, and depression were determined for the studied genotypes, represented by three replications to evaluate the response of sunflower variety, cultivated hybrid, two interspecific hybrids and an accession of *H. argophyllus* under normal and simulated drought stress treatments. Plant height for all studied hybrids decreased with increasing water stress. Studied interspecific hybrids showed similar responses at osmotic potentials of both -0.6 MPa and -1.62 MPa. They performed better and were classified as drought tolerant. The cultivated sunflower hybrid Baikal showed medium tolerance and variety Favorit – sensitive one. The variation among studied genotypes was found to be a reliable indicator to screen the drought tolerant ones at primary growth stage.

**Key words:** Interspecific hybrids, osmotic stress, PEG 6000

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**CLIMATE-RESPONSIVE APPROACHES FOR BUILDING DURABLE  
RESISTANCE OF SUNFLOWER TO BROOMRAPE IN EVOLVING  
ENVIRONMENTAL CONDITIONS**

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**ABSTRACT**

The presence of various biotypes of broomrape (*Orobanche cumana* Wallr.), each differing in their virulence towards sunflower (*Helianthus annuus* L.), has been widely documented. Highly virulent races of broomrape (F, G and H) have been observed in numerous sunflower cultivation regions. It is important to note that biotypes of *O. cumana* belonging to the same race can exhibit varying levels of virulence; for instance, race F from Spain may differ significantly from race F in Romania. In light of these variations, conducting systematic multi-environmental testing on available germplasm becomes crucial for identifying stable genetic sources of resistance. The IFVCNS has organized multi-environmental testing to evaluate its breeding material, identifying several resistance genes. Some resistance sources are governed by major genes, while others follow recessive inheritance or exhibit quantitative trait loci (QTL) resistance. Managing broomrape in sunflower production poses challenges due to the parasite's ability to adapt and overcome existing resistance mechanisms. Climate change is considered as a potential factor behind the rapid changes in the racial composition of the parasite. Although resistance to *O. cumana* is frequently breached, utilizing multiple resistance sources is crucial in combating the emergence of new races. While resistance based on a single gene can be easily transferred to elite breeding material and prove effective in the short term, achieving durable resistance necessitates the combination of different resistance genes from diverse sources, including both quantitative and qualitative modes of resistance. Consequently, the incorporation of multiple resistance genes into a single genotype has demonstrated improved resistance durability. Moreover, it is essential to ensure that these genes do not have adverse effects on other desired traits. By capitalizing on the strengths of durable resistance approach, the breeding team at IFVCNS has successfully developed sunflower hybrids with enhanced broomrape resistance and increased sustainability. These advances contribute to sunflower production systems' long-term success and stability, ultimately mitigating the challenges posed by broomrape and changing climatic conditions.

**Key words:** sunflower, broomrape races, genes, resistant hybrids, durability

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**RACES OF BROOMRAPE PRESENT IN SOUTH-EASTERN ROMANIA**

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**ABSTRACT**

In countries around the Black Sea, such as Romania, Bulgaria, Turkey, Ukraine, Russia is present the most virulent races of broomrape (*Orobanche cumana* Wallr). In south-east of Romania, in location Braila, in years 2022 and 2023 we identify races A, B, C, D, E, F, G, H, I or more. In every year we tested differential set with sunflower genotypes for identified races of broomrape present in this area. In flowering time of sunflower genotypes, we make notation about attack degree of broomrape and we observe a higher virulence in year 2022 then in year 2023. An additional differential genotype, old Romanian variety Neagra de Cluj (accession PI 650368), was resistant at broomrape race HRO or IRO in both years. Neagra de Cluj, can be included in international set of differential for races G, H and I of parasite *Orobanche Cumana* and is provided by North Central Regional Plant Introduction Station (NCRPIS), part of United States National Plant Germplasm System (NPGS).

**Key words:** sunflower, broomrape, races

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**NEW APPROACHES FOR ACHIEVING DURABLE RESISTANCE TO  
BROOMRAPE IN SUNFLOWER**

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**ABSTRACT**

Sunflower broomrape (*Orobanche cumana* Wallr.) is a holoparasitic plant that causes significant yield losses to sunflower crops. Hence, the development of broomrape-resistant hybrids is one of the prime breeding objectives. Using conventional plant breeding methods, resistance genes have been identified which led to the development of a number of resistant hybrids, adapted to different growing regions worldwide. However, while there are many studies on genetic of resistance to broomrape in sunflower, the molecular tools that are available for research on *O. cumana* are very scarce. Recent advances in sunflower genomics paved the way for application of modern breeding tools in broomrape breeding and find durable solutions for limiting broomrape spread and virulence. Here we present an overview of those new tools, such as phenotyping, -omics, and genome editing techniques, which need to be introduced into the sunflower breeding programs in order to achieve durable resistance to this parasitic plant.

**Key words:** sunflower, broomrape, new breeding tools, durable resistance

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**A PRELIMINARY STUDY ON THE IDENTIFICATION OF DIFFERENT  
SUNFLOWER VARIETIES WITH THE LEVEL OF RESISTANCE TO RACE G  
MINOR SPECIES AN**

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**ABSTRACT**

*Orobanche cumana* has become a bottleneck factor restricting the healthy development of the sunflower industry in China. Planting sunflower varieties resistant to broomrape is the simplest, feasible, and cost-effective control measure at present. It is important to clarify the mechanism of sunflower resistance for breeding resistant varieties to broomrape. In this study, we used the petri dish filter paper system to evaluate and identify the resistance level of 32 sunflower varieties. We selected two sunflower varieties (JK103 and LD5009) with significant difference in resistance and sensitivity level. After artificial inoculation of the G race, the differences in the number of parasitic nodulation on roots, callose deposition in cell wall, hydrogen peroxide accumulation, ROS scavenging enzyme activity and transcription expression of resistance genes between resistant and susceptible varieties were compared at germination stage, nodulation stage and shoot meristem stage. The results showed as follows: (1) The average number of parasitism tubercle in the roots of JK103 was 3.2, significantly lower than that of LD5009, which was 16.2; The callose mass deposited in the root cell wall of JK103 was significantly higher than that of LD5009; The content of hydrogen peroxide and the activities of different ROS scavenging enzymes in the roots of JK103 and LD5009 showed an initial trend of increase and then later decreased. The magnitude of the changes of the above indicators in the roots of JK103 was significantly higher than that of LD5009 at the same time point; The quantitative transcription level results of the resistance-related genes showed that, except XTH6, the relative expression content of all tested resistance-related genes, such as Ha-PR1, LOX, CAT, etc. in resistant variety JK103 was significantly higher than the susceptible variety LD5009. The above results suggested that sunflower against the infection of broomrape via structural and physiological resistance mechanism, meanwhile, the signal molecules, such as H<sub>2</sub>O<sub>2</sub>, JA and SA were also involved in the establishment of sunflower resistance upon the infection of broomrape.

**Key words:** *Orobanche cumana*; resistance gene; hydrogen peroxide content; antioxidant enzyme activity

**Fund Project:** National Special Oil Industry Technology System (CARS-14)

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**APPLICATION OF SSR MARKERS TO REVEAL THE GENETIC DIVERSITY OF  
SUNFLOWER BROOMRAPE IN CHINA**

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**ABSTRACT**

Broomrape (*Orobanche cumana* Wallr.) is a kind of parasitic seed plant seriously affecting the sunflower industry in China. To clarify the genetic relationship of sunflower broomrape in China, SSR markers were used to determine the population genetic diversity of 93 broomrape samples which were collected from different provinces of China. Results showed 14 SSR primers were screened out from 50 SSR primers, based on their highly polymorphism among tested samples. A total of 108 out of 112 bands were identified as polymorphic bands. The percentage of polymorphic bands was 96.43%. Both Shannon index and Nei's diversity index were raised with these ample population size increasing. Among tested samples, broomrape collected from Inner Mongolia and Xinjiang provinces showed most highly polymorphism. Their Shannon indices were 0.4380 and 0.4967 respectively. Genetic clustering results showed that the samples from 6 different provinces could be divided into 2 clades. Inner Mongolia, Yunnan, Xinjiang, Hebei and Gansu samples were clustered into one clade, and Shanxi were clustered into another separated clade. Samples collected from Gansu and Shanxi province showed the closest relationship, while the same samples from Gansu showed the furthest relationship with samples collected from Xinjiang region.

**Key words:** Sunflower, *Orobanche cumana* ; SSR markers ; genetic diversity

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**DEVELOPMENT CRISPR/CAS9-MEDIATED RESISTANCE IN SUNFLOWER  
AGAINST O. CUMANA**

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**ABSTRACT**

Sesquiterpene lactones (STL) are a group of natural compounds found in various plant species, including sunflowers (*Helianthus* spp.), and they have been studied for their potential role in allelopathy and defense against pests and pathogens. Allelopathy refers to the ability of certain plants to release chemicals that affect the growth and development of neighboring plants and organisms. In the context of sunflower broomrape (*Orobanche cumana*) and sunflowers, STLs have been of particular interest due to their potential role in inhibiting the growth of the broomrape parasite. Some STLs have been shown to possess allelopathic properties, which means they can influence the germination and growth of other plants, potentially including parasitic plants like sunflower broomrape. Research has suggested that certain STLs found in sunflowers may exhibit inhibitory effects on the germination and growth of sunflower broomrape seeds. These compounds could potentially be released from the sunflower roots and into the soil, creating a hostile environment for the parasite. However, the effectiveness of sesquiterpene lactones in controlling sunflower broomrape is still an active area of research, and their practical application as a management strategy requires further investigation. In recent years, secretion of Sesquiterpene Lactones (STLs) from sunflower roots has been found to trigger the germination of broomrape seeds. The genes encoding the enzymes (HaGAS, HaGAO, HaG8H, HaCOS) functional in STL biosynthesis in sunflower have been well characterized. CRISPR-Cas9 is a powerful genetic editing tool that has been used to modify specific genes in various organisms, including plants, for a range of purposes, including crop improvement and pest resistance. In the light of all these information, genes of the enzymes that catalyze the production STLS was aimed to knockout with CRISPR/Cas9 technique in the study. It has been hypothesized that mutant sunflower lines developed in this way will have full resistance to broomrape. The sequences of four genes (HaGAS, HaGAO, HaG8H, HaCOS) encoding the enzymes functional in STL biosynthesis were retrieved from the database and processed with CRISPR-P 2.0 software to find out the best guide RNAs (gRNAs) that can target exon parts of the genes. By this way, four best gRNAs (one gRNA for each gene) were selected for simultaneous targeting of the first exon of the genes. All gRNAs were then transferred into a Cas9 containing agrobacterium plasmid (pHSE401) by using golden gate cloning. gRNA/Cas9 containing vectors were then inserted into agrobacterium rhizogenes and positive colonies were verified with colony PCR. The seed, cotyledon and hypocotyl explant of the sunflower genotype (NS3) was then treated with *A. rhizogenes* to insert of the gRNA/Cas9 into explants and root formation. Rooted mutants' explants were then put into broomrape seed containing tissue culture media. The results indicated that 79% of the mutant roots have high resistance to broomrape. After DNA isolation, the target genes were amplified with PCR and sequenced to see the CRISPR-mediated mutation in the genes. among the broomrape resistant rooted explants 83% of them were recorded to carry mutation in the gene of interest. This is the first study developing broomrape resistant sunflower genotypes by using

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CRISPR genome editing system. Optimization of CRISPR mediated gene transfer and regeneration protocol will fasten and made important contribution to sunflower breeding. Genome editing-based strategies used to enhance crop resistance to parasitic weeds and its prospective applications will be discussed in the congress.

**Key words:** CRISPR, Sunflower, Sesquiterpene Lactones, broomrape, *O. cumana*, resistance

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**INFLUENCE OF BROOMRAPE ON SOME ANATOMICAL AND  
PHYSIOLOGICAL TRAITS IN SUNFLOWER**

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**ABSTRACT**

Parasitic species of *Orobanche* are totally depend on their host for all nutritional requirements, drawing nutrients from host plants via a specialized structure named haustorium. *Orobanche cumana* is a specific parasite of sunflower (*Helianthus annuus* L.) that can cause reductions in crop yield and different yield associated traits, such as head diameters, weight of 1000 achenes, number and weight of seeds per head etc. Here, we evaluate the influence of broomrape on some anatomical and physiological traits in host. Plant height, leaf area, content of pigments (chlorophyll a and b, carotenoids etc.), as well as crop yield of some sunflower hybrids susceptible to the parasite were analysed in two infested and non-infested fields during 2021 agricultural season. In different fields the number of broomrape attachments per host plant varied between 0,6 and 5,1 depending on sunflower genotypes. The most affected was the hybrid noted HT3, which shown the lowest value of yield (1,6 t/ha comparative to 3,4 t/ha in control). The yield of infested plants was significantly diminished (by 34-54%, depending on sunflower hybrids) comparative to non-infested controls. In addition to yield losses, broomrape significantly influences sunflower leaf area. So, an increase in leaf area and leaf area index (by 16-59%) in *O. cumana* infested sunflower was found in comparison to non-infected plants, the results being in concordance with those reported by other researchers. Although changes in chlorophyll content also were reported in some plants attacked by broomrapes, in this study, chlorophyll a, chlorophyll b, chlorophyll a + b content and Ca/Cb did not show any significant differences between the infested and non-infested hybrids. No significant correlations were found between the number of *O. cumana* attachments and the degree of damage to the host.

**Keywords:** sunflower, broomrape, *Orobanche cumana*, yield, leaf area

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THE STIGO PROJECT: DECIPHERING THE MOLECULAR DIALOG OF *O.*  
*CUMANA* SEEDS GERMINATION

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**ABSTRACT**

*Orobanche cumana* is an obligate non-photosynthetic parasitic plant that attaches to the roots of the sunflower (*Helianthus annuus*) for acquiring water and nutrients for its development. Broomrapes (up to 100/sunflower plant) are then new sink for sunflower and lead to yield losses. To date, resistant sunflower varieties are the most effective way to control *O. cumana*. However, it is still necessary to understand the underlying resistance mechanisms in sunflower. While the vast majority of plants germinate thanks to internal hormonal signals and environmental cues, broomrape seeds do not germinate spontaneously and must perceive a biochemical signal produced by the host roots that induces the germination. Sunflower is the only cultivated host species for *Orobanche cumana*. To date, only three major resistance genes have been mapped in sunflower and none of them is involved in the induction of *O. cumana* seed germination. However, a better understanding of this key stage in the interaction between the sunflower and *O. cumana* will lead to new control methods. The LGS1 gene in sorghum is involved in modulating the production of molecules inducing *Striga* seeds germination, enhancing the resistance to *S. hermonthica*, an obligate photosynthetic root parasitic plant. So far, two types of allelopathic signals have been identified, the strigolactones (SL) and sesquiterpenes (eg. dehydrocostus lactone, DHL), but a larger panel of germination stimulant (GS) molecules is expected and remains unknown in sunflower. Our objectives are to develop effective and selective methods of control against *O. cumana* by: (i) identifying the allelochemicals exuded by sunflower and responsible of *O. cumana* seed germination and the genes and alleles involved in their biosynthesis (ii) identifying and characterizing the GS receptor(s) of *O. cumana*, and developing specific germination inhibitors based on their activity towards these receptor(s), (iii) developing new control methods against *O. cumana* and new varieties for sunflowers with low stimulant germination activities.

**Key words:** *Orobanche cumana*, seeds, sunflower root exudate, germination stimulant, GS receptors

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**CONTENT AND OIL YIELD OF SUNFLOWER (HELIANTUS ANNUS) - HYBRID  
DEVEDA DEPENDING ON THE MAIN TILLAGE SYSTEM**

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**ABSTRACT**

The research was conducted in a stationary field experiment of the Dobrudzha Agricultural Institute - General Toshevo in the period 2016 to 2022. The impact of seven main soil tillage systems (MSTS) with and without turning the cultivated layer, No-till, as well as the alternative alternation between them in a 4-field crop rotation (beans-wheat-sunflower-corn) on the content and yield of oil. The MSTS are: 1. CP - conventional plowing (24-26 cm); 2. D – disking (10-12 cm) 3. C – cutting (chisel-plough); 4. NT - No-till (direct sowing); 5. Coventional plowing (for spring crops) – No-till (for wheat); 6. Cutting (for spring crops) - Disking (for wheat) and 7. Coventional plowing (for spring crops) - Disking (for wheat). The main objectives of the study were: (i) to investigate the seasonal variability in sunflower: (i) in the Kernel/husk ratio; (ii) the oil content of the whole seed and its components; (iii) the obtained yields of oil per 1 area. Meteorological conditions during the years of study have an extremely strong influence on the proportion of the kernel and that of the husk in the whole seed. The share of the kernel varies from 74.91% (2016) to 80.20% (2018). This inevitably affects the share of the husk, whose share is higher in 2016, 2019 and 2020. The oil content in the kernel is also characterized by a well-defined dynamic - from 61.81% (2021) to 64.46% and 64.47% ( 2017 and 2022). The highest percentage of oil in the husk was found in 2018. In the whole seed, this high level of differentiation in oil content values depending on weather conditions over the years was preserved. The seed produced in 2019 is the highest oil (50.85%), and the least - in 2016 (46.68%). Yields of kernels, husks and their oil content, as well as whole seed, were more strongly affected by weather conditions during the study period. The tillage systems with or no deep turning treatment of the plow layer applied in crop rotation constantly or in combination with shallow tillage or No-till lead to obtaining seed highest oil content and, accordingly, oil yield compared to the others. The strict adherence to crop rotation, regardless of the diversity in the main tillage systems tested and the high level of selection work lead to a lack of observation of the parasite broomrape. An additional contribution to this fact is that the areas around General Toshevo are lightly infected with aggressive races of this parasite.

**Key words:** Main soil tillage systems (MSTS), Sunflower, Kernel/Husk components, Oil content.

**INTRODUCTION**

Sunflower (*Helianthus annuus* L.) is a major oilseed crop in almost the entire world. In recent years, the consumption of sunflower oil has increased significantly. In recent years, the consumption of sunflower oil has significantly increased. Application and combination of proper agrotechnics and the fact that the sunflower has a very good root system it can actively draw water and nutrients from the 2-meter soil layer (Mc Michael and Quisenberry, 1993; Angadi and Entz, 2002; Balalić et al., 2012). This fact is a prerequisite for expanding the area of distribution in drier conditions. According to Miladinović et al. (2019), due to its ability to grow under different agro-ecological conditions and its moderate drought tolerance, sunflower

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may become a preferred oilseed crop in the future, especially in light of global environmental changes. Significantly earlier, Part of the research in Bulgaria on the problems of sunflower in relation to productivity and quality shows that these characteristics are dynamic and dependent not only on the genetic predisposition of the varieties/hybrids, but also on a number of important agrotechnical factors that fit into the different systems of agricultural production - the weather conditions, tillage, nutritional regime, care during the vegetation and others (Tonev and Nikolova, 1997; Tonev, 2006; Nankov, 2012; Koteva, 2014). Undoubtedly, the methods of soil cultivation under the relevant agro-climatic conditions play an essential role in obtaining maximally satisfactory yields and quality characteristics with the necessary respect for the environment (Botta et al, 2006; Celik et al, 2013). Dang et al (2015) and Peixoto et al (2020) no-till (no-till) practice is one of the three main principles of conservation agriculture. This practice has better protection against soil erosion and offers greater efficiency in plant nutrient uptake. Obtaining higher productive and quality characteristics is undoubtedly the ultimate goal of many researchers dealing with the agronomy and selection of this crop (Habib et al, 2007). In this connection our main objectives of the study were: (i) to investigate the seasonal variability in sunflower: (i) in the Kernel/husk ratio; (ii) the oil content of the whole seed and its components; (iii) the obtained yields of oil per 1 area.

## **MATERIAL AND METHOD**

The research was conducted in a stationary field experiment of the Dobrudzha Agricultural Institute - General Toshevo in the period 2016 to 2022. The impact of seven main soil tillage systems (MSTS) with and without turning the cultivated layer, No-till, as well as the alternative alternation between them in a 4-field crop rotation (beans-wheat-sunflower-corn) on the content and yield of oil. The MSTS are: 1. CP - conventional plowing (24-26 cm); 2. D – disking (10-12 cm) 3. C – cutting (chisel-plough); 4. NT - No-till (direct sowing); 5. Conventional plowing (for spring crops) – No-till (for wheat); 6. Cutting (for spring crops) - Disking (for wheat) and 7. Conventional plowing (for spring crops) - Disking (for wheat). The mineral fertilization in the crop rotation was as follows: Common bean – N60P60K60; Wheat – N120P120K60; Sunflower - N60P120K120 and Maize – N120P60K60.

For the indicated research period, an agrotechnical test of the Deveda hybrid was carried out. According to the main breeder of the team that created the hybrid, it is moderately injured. It is a single interline hybrid (Nenova, 2019). The seed oil content is 51.7% and the protein content-27.1%. The hybrid is resistant to downy mildew /race 731 and 730/ and broomrape and middle resistance to phoma, phomopsis, althernaria and sclerotinia. The oil content of the kernel and husk and the whole seeds was determined by the method of Rujkowski (1957).

## **RESULTS AND DISCUSSION**

Many factors influence the value of a number of sunflower indicators distinguishing crop productivity and oil quality. The abiotic factors of the environment, as well as the applied agricultural techniques, are the basis for the full development of the genetic potential when using the agricultural techniques under the specific weather conditions.

The response of the hybrid “Deveda” seed components in terms of their share in the seed formed, oil concentration and oil yield in kernels and hulls varied widely over the study period average (Table 1). The values of the coefficients of variation are the lowest for the index of the share of the kernel in the seed, the concentration of oil in the kernel and the seed - between 3 and 5%. Values below 30% were found for yields of kernels, oil from kernels and whole seeds, while for the index of oil content from husk it was 43.92%, which is an indication of a high degree of dispersion of the data.

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Table 1. Degree of variation in the performance of the sunflower oil content and yields in seeds components according to MSTs by for the period 2016-2022.

Stat. Parameters	N	Minimum	Maximum	Mean	Std. Deviation	CV%
Kernel % in seed	98	73.50	86.40	77.25	2.45	3.17
Kernel Oil %	98	55.50	68.85	63.53	2.60	4.10
Yield Kernels	196	1239.6	5567.4	3456.5	996.5	28.83
Kernel Oil Yield	196	732.6	3507.5	2200.1	656.4	29.83
Husk %	98	13.60	26.35	22.76	2.45	10.76
Husk Oil %	98	3.10	12.10	7.16	2.08	29.06
Yield Husks	196	505.7	2059.9	1256.4	353.1	28.11
Husk Oil Yield	196	18.0	214.0	75.1	33.0	43.92
Seeds Oil %	98	42.90	56.20	49.20	2.46	5.01
Total Oil Yield	196	763.6	3721.4	2275.2	678.3	29.81

On the basis of the statistical analysis, it was also found that the tested factors in the experiment influence to the maximum extent both the oil content of the individual components of the seed and the obtained oil content from them (Table 2).

Table 2. Analysis of variances of sunflower oil content and productivity by seeds component according to main soil tillage systems (MSTs) averaged for the period 2016 – 2022.

Source	df	Content, %			Yields		
		Dependent Variable	F	Sig.	Dependent Variable	F	Sig.
Years (1)	6	Kernel, % in seed	5381.640	0.000	Kernel Oil Yield	257.341	0.000
	6	Husk, % in seed	15346.791	0.000	Husk Oil Yield	498.755	0.000
	6	Kernel Oil, %	6067.919	0.000	Total Oil Yield	254.409	0.000
	6	Husk Oil, %	11279.234	0.000	Yield Kernels	236.901	0.000
	6	Seeds Oil, %	4870.429	0.000	Yield Husks	211.444	0.000
MSTs (2)	6	Kernel, % in seed	1039.319	0.000	Kernel Oil Yield	129.396	0.000
	6	Husk, % in seed	2928.442	0.000	Husk Oil Yield	206.727	0.000
	6	Kernel Oil, %	1397.607	0.000	Total Oil Yield	130.504	0.000
	6	Husk Oil, %	1979.329	0.000	Yield Kernels	128.102	0.000
	6	Seeds Oil, %	1212.097	0.000	Yield Husks	124.837	0.000
1 x 2	36	Kernel, % in seed	607.218	0.000	Kernel Oil Yield	18.848	0.000
	36	Husk, % in seed	1702.090	0.000	Husk Oil Yield	79.220	0.000
	36	Kernel Oil, %	1712.275	0.000	Total Oil Yield	19.378	0.000
	36	Husk Oil, %	964.402	0.000	Yield Kernels	15.862	0.000
	36	Seeds Oil, %	1342.879	0.000	Yield Husks	13.305	0.000

Weather conditions during the years of study have a strong influence on the proportion of kernel and husk in the whole seed. The strength of their influence on the values for the share of the components with the seed is over 50%. For their oil content, it decreases with values for oil concentration in the kernel and the whole seed. The interaction between the two factors has a stronger influence than the independent influence of MSTs, which is determined between 7-10%.

The proportion of kernel in seed by year of study varied from 74.91% (2016) to 80.20% (2018) (Figure 2). These results have an impact on the values of the share of the husk in the seed

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accordingly. The same is the lowest in 2018 - 19.96%, when the kernel occupies the largest share in the seed. Both have the largest share in the seed in 2016 - 25.11%.

On average for the period 2016-2022, the share of the kernel in the seed in the tested MSTs was 77.25%. Only with the constant application of conventional plowing in the conventional plowing (CP-CP) and the alternative rotation CP-No-till, the share of kernels reaches 78.73% and 78.06%, respectively. The oil content of seed components and whole seed varied significantly between years (Figure 3). Kernels have the highest oil content in 2019 (66.38%). Against the background of the long-term experiment (2016-2022), the hybrid “Deveda” stands out with the highest oil content in 2017 (64.46%) - above the average for the experiment (63.53%). The kernels of the obtained product has the lowest oil content in 2021 - 61.81%. By years of study, oil content ranges from 4.50% (2022) to 9.66% (2018). Thus, the differentiation in the oil content of the whole seed is maximally expressed.

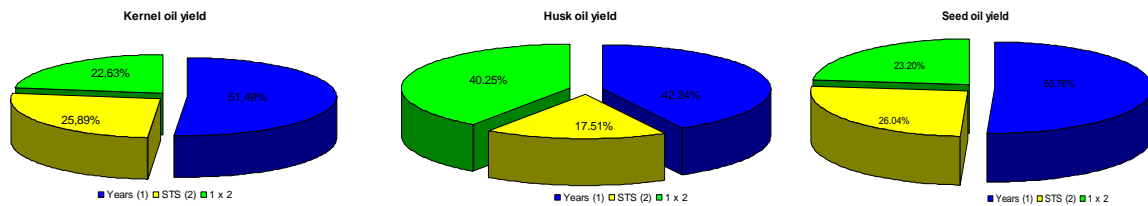


Figure 1. Strength of effect of the factors and their combinations average for the period 2016-2022 on the oil yields of sunflower seed according main STS, %

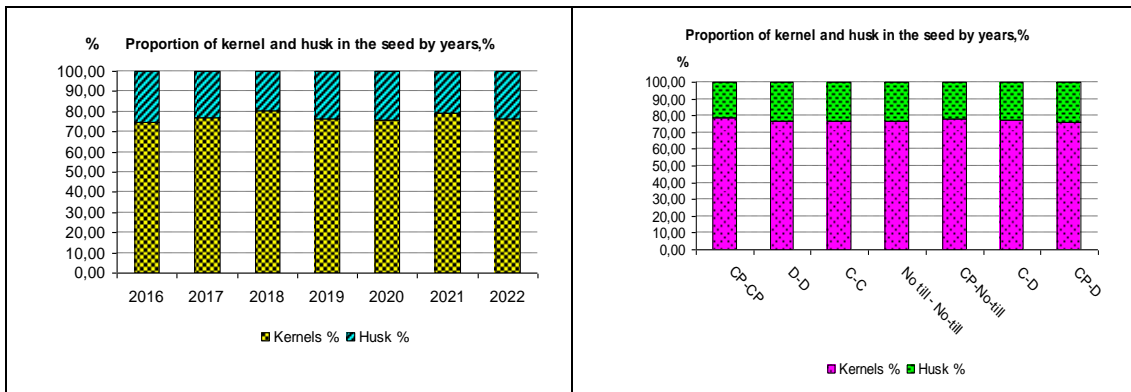


Figure 2. Proportion of seed components in seed according to years and MSTs, %

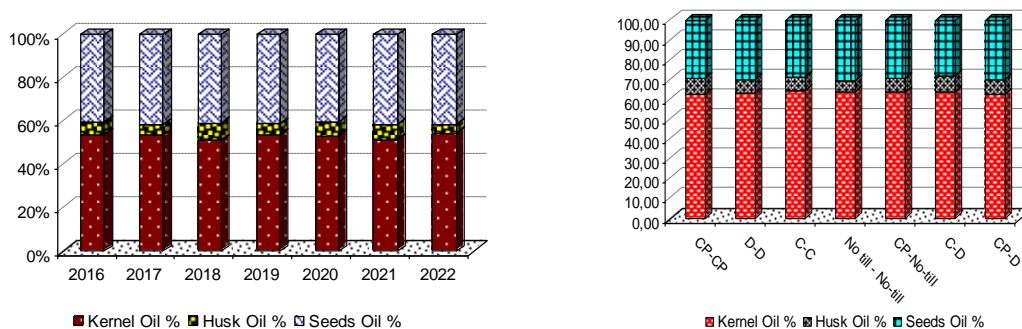


Figure 3. Oil content (%) in sunflower seed and it components, according to years and MSTs

The production of seeds in 2019 and 2018 is distinguished by the highest % oil, 50.85% and 50.45% respectively. The most unfavorable conditions for the processes related to the formation



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of oil in the seed were in 2016 (46.68%) and 2020 (47.48%). In the remaining years of the study, the oil content in the seeds was between 49% and 50%.

The tested tillage systems are also characterized by a well-expressed differentiation in the values of the investigated indices, and to a maximum extent. The percentage of oil in the kernel varied from 62.63% in (CP-D) to 64.83% in the deep non-rotating treatment (C-C).

On average for the period 2016-2022, the C-C systems (49.89%) are distinguished by the largest oil content in the seeds; constant No-till (49.70%); CP-No-till (49.66%); C-D (49.51%) and CP-CP (49.37%).

Discussing the obtained results for oil content from kernels by year we obtain values that distinguish each of the MSTs within the same year (Figure 4). This means that their place is not fixed in a strictly defined order. Almost every year there is a trend towards a lower oil content from kernel with permanent direct seeding. In two of the years (2017 and 2020), the three intermittent deep tillage systems (turned and no-turned tillage) in terms of kernel oil yield approached the constant application of conventional plowing in the crop rotation. In the variant with its 1-year break with (No till-No till) in 2016, 2020 and 2022 an excess over CP-CP was found by 15.48%, 24.96% and 16.56%, respectively.

The influence of MSTs on the values of the obtained oil yields from the husks is expressed in a wide scale variation - from 19.49 kg/ha (2022) with the constant application of direct sowing in the crop rotation (No till-No till) to 156.53 kg/ha (2016) the constant application of conventional plowing in the crop rotation (CP-CP).

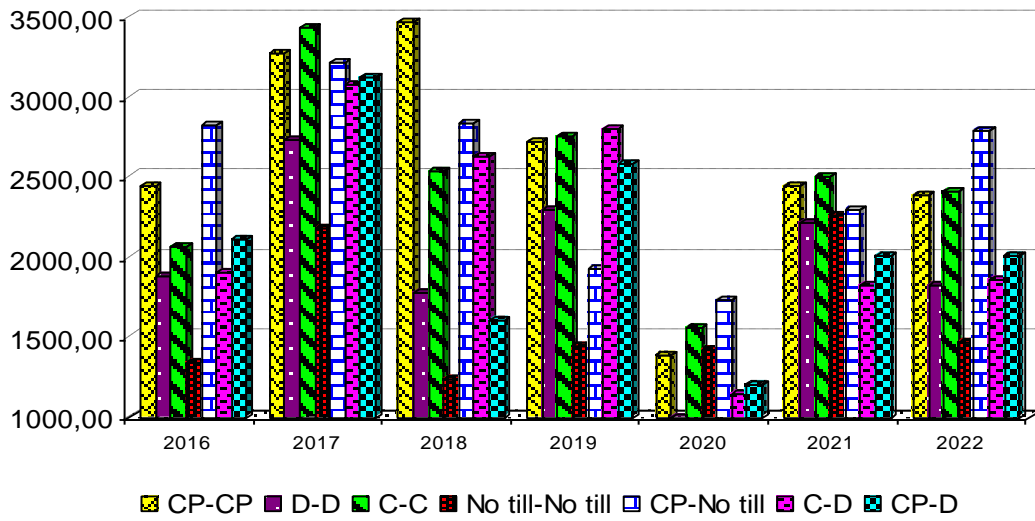
As a result of their share in the seed, the values of oil yields obtained from the husks are significantly lower than those obtained from the kernels. However, they are a valuable raw material in the preparation of animal feed. The indicated trends for the impact of MSTs on kernel oil yield are almost preserved for husk oil yield. Our results corroborates the view of Leon et al (2003) that the oil content and oil yield are complex quantitative traits, determined by genetic and environmental factors, along with interaction between them.

Final, whole seed oil yields as a composite measure also vary significantly by MSTs. The differentiation between the tillage options tested was enhanced in years with unfavorable weather conditions. An example of this is 2020, when seed oil yield varies from 874.13 kg/ha (D-D) to 1804.45 kg/ha (CP-No till). In the year with the most favorable conditions for developing the productive potential of the hybrid "Deveda" (2017), the variation was from 2222.48 kg/ha (No till - No till) to 3526.72 kg/ha (C-C) and 3388.61 kg/ha (CP- CP).

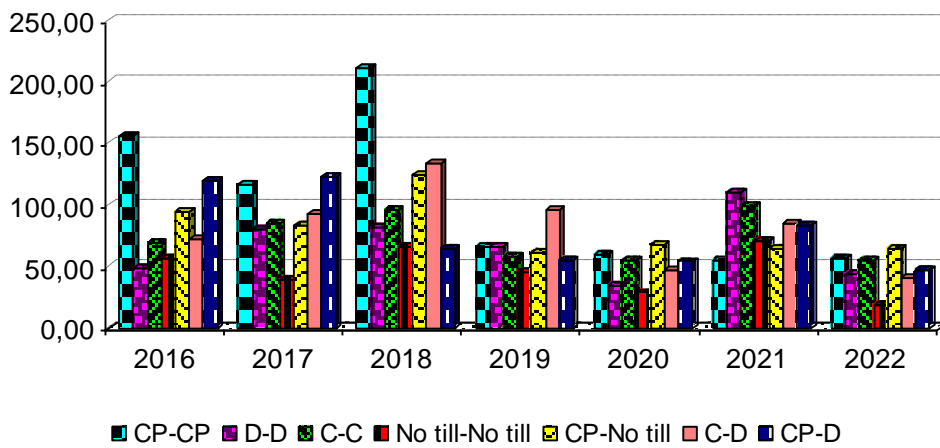
The factor with the greatest influence on the values of the tested indicators is meteorological dynamics in the amount of precipitation and temperature during the growing season. In our experiment, the interaction between these two main meteorological elements is the key factor determining the magnitude of oil yields (Table 3). The final oil yields formed are practically a complex of yields from the components of the seed and their oil content. On average for the long-term study period, the obtained oil yields from the seed ranged from 3095.85 kg/ha (2017) to 1376.62 kg/ha (2020) with an average yield in the experiment for the period 2016-2022 of 2275.15 kg/ha. This means an increase in oil yield by 36.07% on average for the tested MSTs under favorable conditions for culture development and, accordingly, with a combination of different types of stress, a drop to 60.51% of the average for this period of time.

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**Oil yields from the kernels**



**Oil yields from the husks**



**Oil yields from whole seeds**

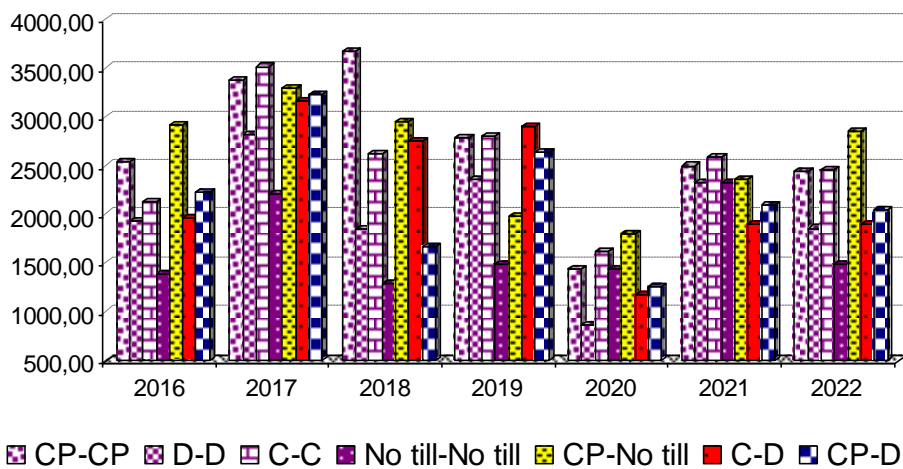


Figure 4. Final yields of sunflower seed oil and their components, kg/ha

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Table 3. Yields of kernels and husks and yields of oil in them and whole seed depending on weather conditions in the years studied, kg/ha

Years	Kernel Oil Yield	Husk Oil Yield	Total Oil Yield	Yield Kernels	Yield Husks
2016	2079.5 b	80.7 d	2160.2 b	3329.4 b	1249.9 c
2017	3006.4 e	89.5 e	3095.9 e	4675.4 d	1669.0 e
2018	2297.4 cd	111.6 f	2409.1 d	3639.5 c	1342.0 d
2019	2361.9 d	64.7 c	2426.6 d	3556.8 c	1194.9 b
2020	1326.5 a	50.1 b	1376.6 a	2122.4 a	795.9 a
2021	2222.2 c	81.7 d	2303.9 c	3600.5 c	1378.3 d
2022	2106.7 b	47.1 a	2153.8 b	3271.6 b	1164.9 b

A number of studies have been carried out in DAI-General Toshevo in relation to sunflower culture and the behavior and application of various agrotechnical practices on its productivity and quality, as well as the economic efficiency of its production in the country (Klochkov and Nankov, 1987; Nankov and Tonev, 1994; Nankov, 1996; Nankova et al, 1999; Nankov et al, 2002; Nenov et al, 2004). In this respect, Nankov's studies (1982a,b, Nankov and Dimitrov, 1985) related to the influence of autumn-winter moisture reserves in sunflower varieties and hybrids deserve special attention.

The MSTs have a strong influence on the amount of yields obtained from the components of the seed and their oil yields (Table 4). The highest yields for the 7-year study period were obtained with the constant application of traditional plowing in the crop rotation. The total oil yield of 2685.68 kg/ha exceeds the average of all tested variants with MSTs by 18.04%. For the individual components of the seed, this increase is respectively for the husks by 25.92% and for the kernels - by 17.78%.

Table 4. Yields of kernels and husks and yields of oil in them and whole seed depending on the MSTs, kg/ha

MSTs	Kernel Oil Yield	Husk Oil Yield	Total Oil Yield	Yield Kernels	Yield Husks
CP-CP	2591.2 e	94.5 e	2685.7 e	4116.1 f	1525.1 f
D-D	1938.3 b	67.9 b	2006.2 b	3057.2 b	1118.7 b
C-C	2466.3 d	74.8 c	2541.0 d	3817.7 d	1351.4 d
No till - No-till	1621.0 a	47.1 a	1668.1 a	2547.1 a	925.9 a
CP-No-till	2517.0 de	80.7 d	2597.7 de	3956.6 e	1439.6 e
C-D	2175.5 c	81.5 d	2257.1 c	3390.6 c	1215.4 c
CP-D	2091.4 c	78.8 d	2170.3 c	3310.3 c	1218.9 c

Three of the tested MSTs in seed oil yield exceeded the experimental average yield (2275.15 kg/ha). These are the two deep (24-26 cm) and constantly applied soil treatments - conventional plowing (CP-CP) with turning the cultivated layer and deep loosening without turning it. The oil yield increase was 18.04% and 11.69%, respectively. Of the alternatively alternating treatments, only CP-No till stands out with an excess of 14.08%. The amount of seed oil yields obtained shows that over this long-term period of time, characterized by diversity in the combination of meteorological factors, none of the MSTs tested outperformed the continuous application of traditional/conventional plowing in the crop rotation. Significant correlations were found between the individual components of the seed, their productivity, oil content and

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the obtained oil yields from the whole seed. There were a number of negative correlations between the proportion of kernel in the seed with that of the husks and with the percentage of oil in the kernel (Table 5). The interrelationship between the proportion of the husk with the concentration of oil in it and the whole seed is also negative. The most strongly expressed positive correlation between the oil in the nut and that in the whole seed - 0.699\*\*. Kaya et al, (2007) through regression analysis results found a different relationship between seed yield of hybrids and oil content. The authors indicate that up to an oil content of 40%-45% the correlation with yield is negative.

Table 5. Pearson Correlation between sunflower seed components content and their oil concentration, 2016-2022

Indices	Kernel, %	Husk ,%	Kernel Oil, %	Husk Oil, %	Seeds Oil, %
Kernel %	1				
Husk, %	-0.995(**)	1			
Kernel Oil,%	-0.203(*)	0.221(*)	1		
Husk Oil, %	0.445(**)	-0.439(**)	-0.314(**)	1	
Seeds Oil, %	0.485(**)	-0.468(**)	0.699(**)	0.034	1

High levels of correlations were found between the oil yields of the seed components and their relative share in the seed (Table 6). The R-values between oil extraction of the kernel with the indicated indices is in the order of over 0.900, while in oil extraction in the husk the level of R is above 0.600. The correlation between the yield of oil in the kernel and the yield of oil in the whole seed stands out with the highest correlation value - 0.999.

Table 6. Pearson Correlation between sunflower seed components yields and their oil yields, 2016-2022

Indices	Kernel Oil Yield	Husk Oil Yield	Total Oil Yield	Yield Kernels	Yield Husks
Kernel Oil Yield	1				
Husk Oil Yield	0.649(**)	1			
Total Oil Yield	0.999(**)	0.677(**)	1		
Yield Kernels	0.993(**)	0.669(**)	0.994(**)	1	
Yield Husks	0.944(**)	0.680(**)	0.946(**)	0.976(**)	1

\* Correlation is significant at the 0.05 level (2-tailed).

\*\* Correlation is significant at the 0.01 level (2-tailed).

## CONCLUSIONS

Meteorological conditions during the years of study have an extremely strong influence on the proportion of the kernel and that of the husk in the whole seed. The share of the kernel varies from 74.91% (2016) to 80.20% (2018). This inevitably affects the share of the husk, whose share is higher in 2016, 2019 and 2020. The oil content in the kernel is also characterized by a well-defined dynamic - from 61.81% (2021) to 64.46% and 64.47% (2017 and 2022).

The highest percentage of oil in the husk was found in 2018. In the whole seed, this high level of differentiation in oil content values depending on weather conditions over the years was preserved. The seed produced in 2019 is the richest in oil (50.85%), and the least - in 2016 (46.68%). Yields of kernels, husks and their oil yields, as well as whole seed, were more strongly affected by weather conditions during the study period compared to the effect of the

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MSTS. With the highest yields of the components of the seed, oil from the kernels and the whole seed, 2017 is noted, and unfavorable - in 2020.

The tillage systems with or no deep turning treatment of the plow layer applied in crop rotation constantly or in combination with shallow tillage or No-till lead to obtaining seed richer in oil content and, accordingly, oil yield compared to the others. Close to this systems are the results obtained when traditional plowing is interrupted with No-till when sowing wheat in the crop rotation. The independent permanent application of deep cutting (chisel-plough) oil yield is less with 144.7 kg/ha compared to traditional plowing, while in the application of CP-No-till system this difference is only 88 kg/ha.

Shallow tillage alone and in combination, as well as long-term self-application of No-till lead to an increase in the share of husks and a lower yield of oil compared to the deep main tillage. The lowest yields of oil in the seed were obtained with the constant application of No-till - 1668.1 kg/ha, i.e. with 1017.6 kg/ha less compared to the constant application of traditional plowing.

The reliability of the obtained results is of the maximum degree of expression. The influence of meteorology as a factor is more pronounced than that of main STS. It has approximately the same values for kernel, %/husk, % in the seed, as well as for the percentage of oil in the kernel and the whole seed. However, it was found to have a much stronger influence on oil content in the husk compared to the kernel and whole seed.

The strict adherence to crop rotation, regardless of the diversity in the main tillage systems tested and the high level of selection work lead to a lack of observation of the broomrape parasite (*Orobanche* spp.). An additional contribution to this fact is that the areas around DZI-General Toshevo are lightly infected with aggressive races of this parasite.

The proportion of kernel in the seed is strongly negatively correlated (-0.995\*\*) with that of the husk. The proportion of kernel in the seed was also in a well-pronounced positive correlation with the percentage of oil in the seed (+0.485\*\*) and the oil content in the husk (+0.445\*\*). There is also a well-expressed correlation between the oil content of the kernel and that of the seed (+0.699\*\*). The correlation dependences between oil yields from the individual components of the seed are positive and with a very high level of statistical reliability. The highest correlation value was found between oil yields in kernel and seeds - 0.999.

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**TRANSCRIPTOME ANALYSIS AND GENE MINING OF BROOMRAPE IN  
SUNFLOWER-BROOMRAPE PATHOSYSTEM**

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**ABSTRACT**

*Orobanche cumana* (sunflower broomrape), a weedy root parasite, mainly attack sunflower and entirely attract water and nutrition from the host. Broomrape causes severe yield losses of sunflower worldwide, but it is difficult to control by traditional practices because of its complex life cycle. In order to elucidate the molecular mechanism of interaction between sunflower and broomrape, some genes about infestation are being uncovered in broomrape by our RNA-seq analysis. In this study, two sunflower varieties (resistant/sensitive sandaomei strain) were selected and inoculated with broomrape respectively. During the interaction between sunflower and broomrape, broomrape nodule was sampled and transcriptome sequencing was performed. We finally deployed the Deseq2 assay to screen differentially expressed genes (DEGs) from the broomrape that inoculating in sunflower resistant cultivars and sensitive cultivars respectively. A total of 868 DEGs were obtained, including 437 up-regulated genes and 431 down-regulated genes. The GO enrichment analysis showed that DEGs were mainly enriched in the sucrose/starch metabolic process, cell wall and protein serine/threonine kinase activity. The process of sugar metabolism can affect the osmotic pressure of sunflower broomrape, causing the variation of water content and nutrition in the broomrape derived from host. The enzymes that catalyze sucrose into reducing sugars are located at the cellulose synthesising tissues, mainly in the cell wall. The study of the genes about sucrose/starch metabolic process can provide the detail that how to the enzyme promote the broomrape infesting sunflower, and provide insight into exploiting effective measures to control the weedy parasite.

**Key words:** Broomrape, Sunflower, Transcriptome analysis, Gene mining, Pathosystem



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**MECHANISM OF ‘JINMIAO TARGET’ IN INHIBITING OROBANCHE CUMANA  
PARASITISM OF SUNFLOWER**

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**ABSTRACT**

In this study, two different sunflower varieties (LD5009 and JK103) were treated with ‘Jinmiao target’ by root irrigation. The root samples were collected at 0, 24, 48 and 72 h after irrigation for histochemical analysis. H<sub>2</sub>O<sub>2</sub> content, ROS scavenging enzyme activity and resistance related gene expression in roots were measured. The growth index and tubercle number of sunflower were measured 20 days after irrigation. The aim of this study was to elucidate the mechanism of ‘Jinmiao target’ in inhibiting *Orobanche cumana* parasitism of sunflower. The results showed that: (1) compared with the control (water application), the number of tubercles in LD5009Jinmiao target decreased by 95.5 and the parasitism rate decreased by 98.20%, the fresh mass and dry mass of tubercles decreased by 94.60% and 81.63%, the height and stem diameter of sunflower increased by 2.09 cm and 0.52 mm respectively, the growth rates were 14.92% and 15.29% respectively. The number of tubercles in the JK103Jinmiao target reduced by 37.5 compared with the control, the parasitism rate decreased by 98.04%, the fresh mass and dry mass of tubercles decreased by 97.06% and 82.69%, the height and stem diameter of sunflower increased by 2.07 cm and 0.39 mm respectively, the growth rates were 12.26% and 9.70% respectively. (2) After irrigating with ‘Jinmiao target’ inducer, the corpus callosum deposition in the roots of both sunflower cultivars increased. However, the JK103 showed the most significant increase after 48 h. The content of H<sub>2</sub>O<sub>2</sub> after 24 h reached the maximum in JK103 and LD5009 varieties, which were 3.53 and 2.68 μmol·g<sup>-1</sup>, respectively. Compared to the control, the most significant increase of H<sub>2</sub>O<sub>2</sub> content was recorded in LD5009, an increase of 208.05%. (3) The activities of four ROS scavenging enzymes in two varieties showed an initial trend of increasing and then decreasing, all of them reached the maximum value after 48 h of treatment. Compared with the control, the activities of SOD, POD, CAT, PPO in JK103Jinmiao target treatment increased by 69.77 U·g<sup>-1</sup>, 5.44 U·g<sup>-1</sup>·min<sup>-1</sup>, 1.88 U·g<sup>-1</sup>·min<sup>-1</sup> and 527 U·g<sup>-1</sup>·min<sup>-1</sup>, respectively. However, the activities of the above four ROS scavenging enzymes were increased by 25.91 U·g<sup>-1</sup>, 13.16 U·g<sup>-1</sup>·min<sup>-1</sup>, 0.50 U·g<sup>-1</sup>·min<sup>-1</sup> and 313 U·g<sup>-1</sup>·min<sup>-1</sup> in LD5009Jinmiao target treatment. (4) Transcriptional analysis of related resistance genes indicated that the two varieties were induced in different degrees after treatment. However, induction degree of LD5009Jinmiao target was the most obvious, especially CAT, Mn-SOD and XTH6. The relative expression levels were more than 50 times higher than the control. The results showed that the ‘Jinmiao target’ inducer had significant inhibitory effect on the parasitism of sunflower, the effect was better before parasitizing sunflower (before the formation of tubercles). ‘Jinmiao target’ inducer could promoted callose deposition in sunflower root cells and resisted the infection of sunflower root by *O. cumana* at the structural level, it also induced the increase of ROS scavenging enzyme activity and the expression of CAT, PAL, Mn-SOD and XTH6 genes in sunflower roots, so that sunflower increased

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resistance to *O. cumana* parasitism. However, the degree of induction varies from cultivar to cultivar.

**Key words:** Sunflower; *Orobanche cumana*; Jinmiao target; induced resistance

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**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 al., 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.

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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 Or7 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

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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.

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.

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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 (LC109 3)	A-F (R 96)	A-G (Tunka)	A-G (RG)		
<b>Rostov region</b>								
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
<b>Voronezh region</b>								
Kalachevsky	41	20	22	9	9	0	G	E
Pavlovsky	36	11	10	5	2	0.7	E	G, H
Novousmanskyy	31	30	21	16	4	2	F+ G	H
Kashirsky	33	13	6	0	1	0.3	E	F, G, H
<b>Samara region</b>								
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
<b>Orenburg region</b>								
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
<b>Stavropol region</b>								
Kochubeevsky	38	29	14	9	4	0	E	G
Sovetsky	56	39	23	7	11	1	E+ F+ G	H
<b>Krasnodar region</b>								
Primorsko-Akhtarsky	32	23	22	3	6	05	G	E
Krylovskoy	34	33	30	7	3	0.8	G	H
Vyselkovskyy	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

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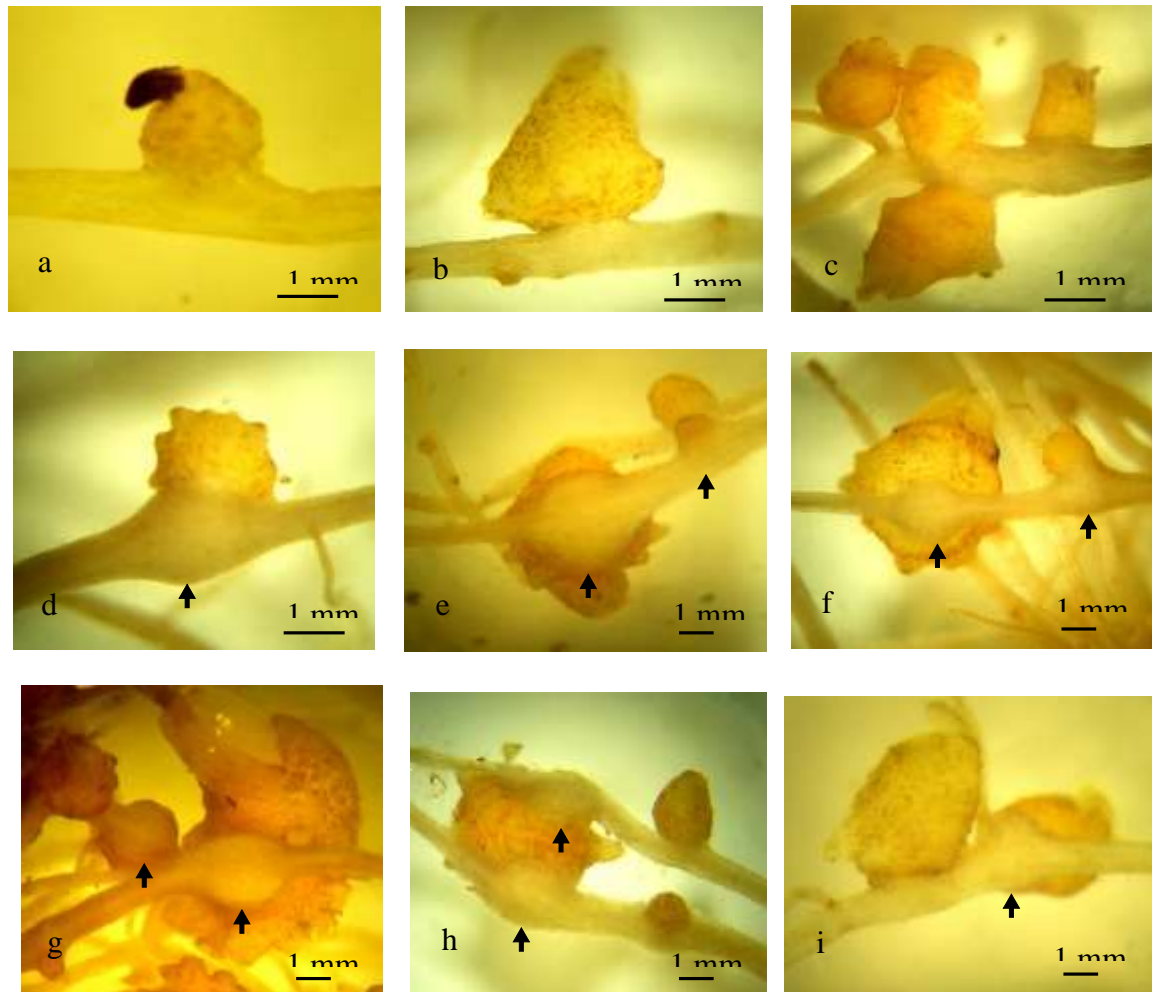


Fig. 1 a-i. The area of connection of the broomrape tubercle with the sunflower root: a, b, c - absence of root thickening under the tubercles; d, e, f, g - presence of thickenings (indicated by arrows); h, i - tubercles next to each other on the same root without thickenings under them and with thickenings (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.

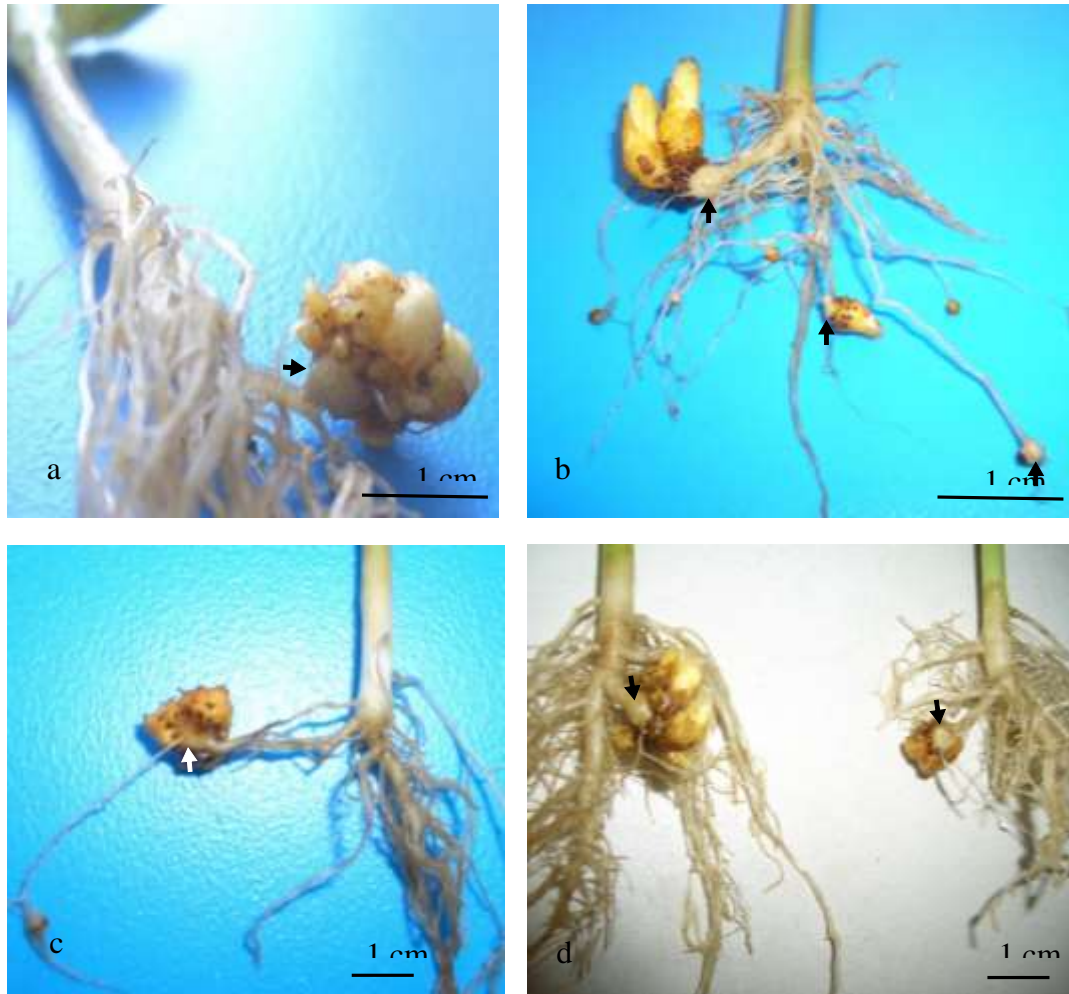


Fig. 2 a-d. Multiple shoots developing from *Orobanche cumana* tuber with thickening of the sunflower root underneath (a, c, d); b - thickening of the root under broomrape shoots and tubercles that are just beginning to develop.

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 *Orobancha cumana* on sunflower: a - mature bush; b - flowering bushes of some individuals (indicated by arrows) on the same host plant.

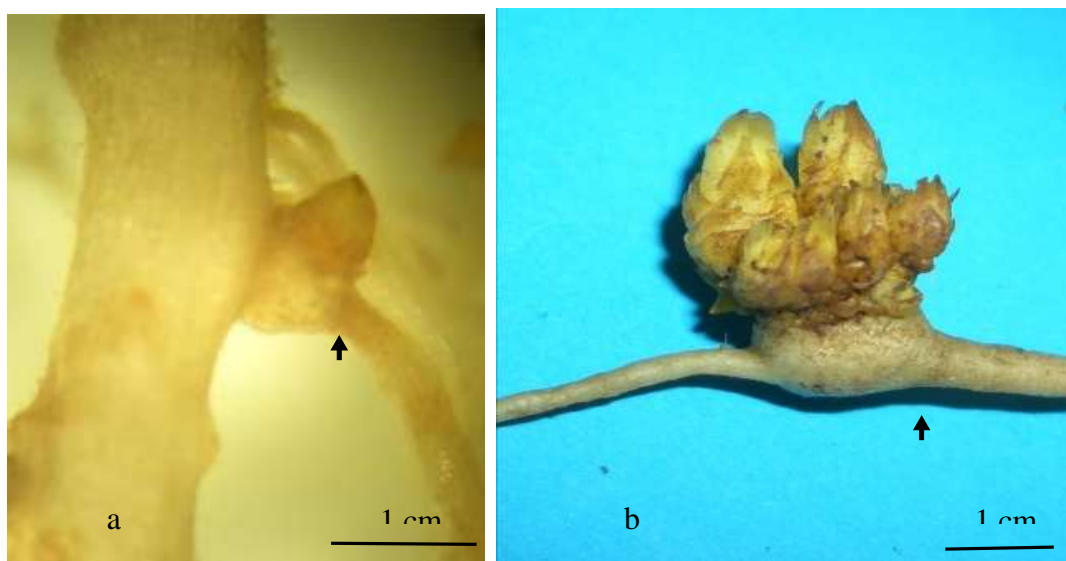


Fig. 4 a, b. Thickenings (indicated by arrows) of the haustorial area in the sunflower root, hiding the broomrape tubercle: a – complete absorption of the tubercle, the broomrape shoot that has started to develop emerges directly from the thickening in the sinus of the additional root; b - partial absorption 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

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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 anatomico-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 anatomico-histochemical study of the described thickenings of the haustorial area in the sunflower root under the tubercle of the parasite.

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**ADVANCING BIOCONTROL STRATEGIES FOR BROOMRAPE MANAGEMENT**

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**ABSTRACT**

Broomrapes are invasive, troublesome, and plant-parasitic weeds considered highly challenging to manage in the Mediterranean, including Türkiye. Their biological capabilities favor broomrapes to establish a parasitic relationship with their hosts and enormous seed bank and distribution. Therefore, conventional weed management strategies are inefficient to prevent, suppress, and control broomrape infestations. Broomrape management strategies should be purposefully designed, such as reducing or even inhibiting the seed bank, the ability to detect host plants, germination rates, and the capability to penetrate the host vascular system. Biological control of broomrapes has focused on insect herbivores, but the majority are not found to be broomrape-specific predators except *Phytomyza orobanchia*, which is reported to be a broomrape-specific biocontrol agent. However, the efficacy of *P. orobanchia* in reducing broomrape populations is quite limited by cultural practices. On the other hand, some bacteria, such as *Pseudomonas aeruginosa*, *P. fluorescens*, *Bacillus atrophaeus*, and *B. subtilis*, are reported to target the growth of broomrape radicles. Moreover, *Fusarium oxysporum* f. sp. *orthoceras*, *F. arthrosporioides*, *F. solani*, *Macrophomina phaseolina*, *Alternaria alternata*, and *Rhizoctonia solani* were isolated from the diseased inflorescences of Egyptian broomrape and were found to be pathogenic to the broomrape. *Azospirillum brasilense* could even inhibit the broomrape radicle growth. Moreover, mycorrhizal fungi populations such as *Rhizobium leguminosarum* or *Azospirillum brasilense* may mislead some broomrape seeds to find crop roots. Nevertheless, none of these biocontrol agents might disperse uniformly across the desired agricultural land, nor their broadcast application is easy. The scientific gap among these management strategies essentially requires further research.

**Key words:** Broomrape, Management strategies, Biological control

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**MONITORING OF OROBANCHE CUMANA WALLR RACES IN SUNFLOWER  
FIELDS OF NORTH EAST GREECE**

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**ABSTRACT**

Weeds and especially the holoparasite *Orobanche cumana* (broomrape) are a serious problem in sunflower crop in Greece and worldwide, while the use of herbicide-resistant hybrids (HRC) dominates as a production system offering a valuable solution to the problem. The objectives of this work were, a) to assess the spatio-temporal spread of broomrape and the most important weeds in the main sunflower cultivation zone in Evros ( NE Greece) and b) to evaluate the effectiveness of the new sunflower production system through the technologies Clearfield®, Clearfield® Plus, and ExpressSun®. Surveys were conducted in August 2022 in 71 fields in the aforementioned crop zone in order to, a) record the degree of broomrape infestation and the emergence of new noxious weeds, b) determine the effectiveness of the three applied technologies, and c) derive information on the farming practices followed by the farmers based on a questionnaire. The results were compared with those of surveys carried out in 2012 and 2015 where the abundance of broomrape and major weeds had been recorded in the same crop zone. The weed flora was recorded using sampling frames of 1m<sup>2</sup>, in a W pattern routes inside the fields and the population density of each species was estimated using the visual estimation according to Braun Blanquet methodology. The Abundance Index of broomrape and other important weeds was calculated and the data were saved in a geodatabase along with the data of farming practices and the use of applied herbicides. The spatial distribution of broomrape and important weeds in sunflower crop was mapped based on GIS as well as the prediction of their potential emergence in the surveyed zone. According to the records during 2022 survey, the species *Chenopodium album*, *O. cumana*, *Convolvulus arvensis*, *Echinochloa crus-galli*, *Xanthium strumarium* and *Cannabis sativa* were recorded in higher abundance. The applied technologies Clearfield® Plus, and ExpressSun® proved to be effective in broomrape control, while special attention needs to be paid to the use by the producers of the recommended hybrids by each technology and the correct application of herbicides. Importantly, a significant change in the weed flora was detected during the three reporting years of the surveys.

**Key words:** Broomrape, weeds, sunflower, spatio temporal dispersal, GIS

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**DETERMINING THE YIELD PERFORMANCES AND THE RESISTANCE TO  
BROOMRAPE AND DOWNY MILDEW OF IMI TYPE SUNFLOWER  
(HELIANTHUS ANNUUS L.) HYBRIDS IN DIFFERENT LOCATIONS**

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**ABSTRACT**

Sunflower (*Helianthus annuus* L.) is the most grown oil plant due to its suitability to agricultural mechanisation and it is the most preferred vegetable oil for consumers in Turkey. One of the main challenging factors in sunflower cultivation is broomrape (*Orobancha cumana*) and downy mildew (*Plasmopara halstedii*). Additionally, some weeds such as *Xanthium strumarium* L. and *Cirsium arvense* also cause a problem. Sunflower hybrids which are resistant to imazamox (IMI) herbicides, play an important role to tackle broomrape and the other weeds. In this study, the yield performances of the hybrids, which are resistant to IMI herbicides and are developed within the scope of TARI's National Sunflower Project, have been investigated in different locations (Vakıflar, Ahmetbey and Edirne) in 2022. The resistance of the varieties to broomrape and downy mildew were evaluated under the natural conditions and also through the artificial inoculation. The experimental design was a Randomized Complete Block Design with four replicates. The four rows plots were 7,50-m long with the 70 x 30 cm plant spacing. 4 commercial hybrids, which are widely cultivated in Turkey, took place as checking varieties. Weed control was with IMI herbicide (Imazamox (40 g/l) with 1.25 l / ha dose after 6-8 leaf stage. Statistical analysis was performed with JMP statistical program. As a result, some varieties of the experiments have shown high resistance to broomrape and downy mildew under the field conditions. The results of artificial inoculation tests display that there were a number of varieties determined as highly resistant to broomrape and downy mildew. Some of the selected varieties are promising and are ready to be nominated for the registration in Turkey.

**Keywords:** Sunflower, broomrape, downy mildew, breeding for resistance, IMI herbicides, hybrid breeding

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**UPDATE ON SUNFLOWER BROOMRAPE SITUATION IN SPAIN**

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**ABSTRACT**

Sunflower broomrape (*Orobanche cumana* Wallr.) is an allochthonous parasitic plant species in Spain, where it only occurs in sunflower crops. It was introduced in the 1950s in central Spain and in the 1970s in southern Spain. Recent studies suggested that they were two separate introductions, probably from different geographic areas. Both gene pools evolved from race E to race F in the 1990s, presumably by point mutations since no increase in genetic diversity was detected. The racial situation of the parasite in both race-F gene pools, named Guadalquivir Valley (FGV) and Cuenca (FCU), was stable until recently, when more virulent populations were detected in both. Our studies suggested that the increased virulence in the GV was caused by the genetic recombination of avirulence alleles of both gene pools FGV and FCU, resulting in a new race named GGV that parasitizes race-F resistant hybrids carrying Or7 allele but not DEB2 line, with Ordeb2 gene that provides resistance to race-G populations from eastern Europe. In central Spain, a recent study has revealed that populations from the GV (FGV and/or GGV) have been introduced in the area at a large scale, with genetic recombination between CU and GV populations that will probably result in an expansion of the GGV race in this area. Additionally, we have detected a new race in CU characterized by parasitization on DEB2 but not on hybrids carrying Or7. This new race, named FCU+, did not result from the introgression of any external avirulence gene since no increased intrapopulation diversity was detected. Accordingly, it is hypothesized that a point mutation caused it. The increasing complexity of the sunflower broomrape situation in Spain urges control measures aimed at curtailing its expansion into new regions, together with the identification of novel sources of resistance to ensure the sustainability of this crop, currently essential for Spanish agriculture.

**Keywords:** Sunflower broomrape, parasite, wild species, resistance

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**BROOMRAPE RESISTANCE FROM WILD SPECIES**

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**ABSTRACT**

Sustaining sunflower production necessitates the comprehensive management of pests and diseases, primarily focusing on bolstering the crop's genetic resistance. Sunflower broomrape (*Orobanche cumana* Wallr.) poses a significant challenge in this regard, as its rapid evolution of virulence hinders traditional breeding efforts for resistance. Presently, all known resistance genes have succumbed to more virulent parasite strains, prompting renewed endeavors to discover novel sources and mechanisms of resistance. Wild species of *Helianthus* currently emerge as the primary reservoirs of untapped resistance genes. In this presentation, we will explore the strategies being developed by research groups worldwide to identify and characterize fresh resistance genes against sunflower broomrape and to incorporate them into cultivated sunflower varieties.

**Keywords:** Sunflower broomrape, parasite, wild species, resistance



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**RECENT DEVELOPMENTS IN BROOMRAPE IN SUNFLOWER IN THE WORLD**

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**ABSTRACT**

Orobanche which is a angiosperm parasite one of the most restraining factor in especially Eastern European sunflower-producing countries which has more than half of world sunflower production. There is big challenge between sunflower breeders and broomrape; whenever breeders develop resistant cultivars against this pathogen, broomrape responds by evolving another virulent race overcoming the resistance every 20 years. In the last over 50 years, several research groups have made relevant contributions on sunflower breeding for resistance and dominant genes for resistance to races A, B, C, D, E, and F have been found and incorporated into cultivated sunflower genotypes mostly utilizing classical breeding. The recent studies also indicated that new broomrape populations have been determined also in some countries called G and H races. However, there is still the existing doubt in the description of races and identification of resistance genes in different countries especially recent races so it needs for international cooperation using common differentials and molecular tools. Therefore, sunflower breeders and geneticists have focused on to solve that uncertainty recently and they have achieved significant results in the use of molecular markers for identifying broomrape races. Consequently, in addition to classical breeding methods, marker-assisted selection and some molecular screening tools should be used even more in the future research to obtain broomrape resistance. As well as developing broomrape resistance genes, some research should be performed on also altering the plant anatomy of plant organs, biochemical parameters (mechanical barriers, induced germination, hormones, etc) aspects of the parasite side such as breeding system and genetics of virulence to understand the dynamics of broomrape populations and race evolution. On the other hand, Clearfield system is also an alternative and efficient control method with using of imidazolinone (IMI) herbicide plus resistant hybrids. The combining both herbicide with genetic resistance could supply more horizontal and durable resistance and successful advances for broomrape control in the future.

**Key words:** Sunflower, broomrape, races, distribution, resistance, breeding,

**INTRODUCTION**

This holoparasitic plant is considered a very important biotic pest because it spreads over many areas in the Old World and causes yield reductions of up to almost 100% and restricts sunflower production (Figure 1.4). The reason why orobanş is considered so harmful is; Because its seed sizes are quite small and it can produce thousands of seeds from its flowers, it can spread quickly and easily to very long distances by factors such as wind, insects, water, etc., and its seeds can germinate again and remain dormant in the soil for years to continue its lineage (Barut, 2017). For this reason, sunflower is seen as a major threat by producers and researchers; Although blight-resistant sunflower varieties have been developed, due to climate changes in nature, they break this resistance by creating new physiological races (Aksoy and Pekcan, 2014). In addition to reducing grain yield in sunflower, orobanche negatively affects many

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important characters such as oil and protein ratio, thousand seed weight, yield per plant, plant height, quality and table size. Sunflower cultivation in our country started in the Balkans after the First World War, and then the cultivation areas spread throughout the country. The problem of bitch in sunflower production in our country was first seen in 1956, and in 1981, new races of bitch emerged in the Thrace region. Between 1956 and 1982, the yield in sunflower producing areas in Turkey decreased by approximately 50% due to blight (Bulbul et al., 1991). It is known that in recent years, in our country and Eastern European countries, the bitch parasite has started to become a problem again by creating new races. Thus, it has been reported that the borer parasite can renew itself and break the resistance mechanism against resistant varieties approximately once every twenty years (Kaya, 2003; Labrousse et al., 2004).

Orobanche populations are divided into races according to their degree of virulence. So far, eight races have been reported, denoted by letters A to H (Kaya, 2014). Although it can be estimated that the bitch parasite currently found in our country is F, G and H races, it is thought that the first races seen may be D and E races (Kaya et al., 2004; Kaya et al., 2012; Molinero-Ruiz and Domínguez, 2014). The evaluation of the germplasm of the *Helianthus* species to create resistance against different races of bitch has shown that it is the main source of resistance genes that provide resistance to new virulent races of bitch in wild sunflowers. In addition, very valuable resistance sources have been observed in cultivated sunflower germplasms. Genetic studies carried out by (Vranceanu et al., 1981) in Romania revealed that five bumblebee races were observed, and the dominant genes containing bitch resistance against these known races were determined and Or 1, Or 2, Or 3, Or 4 and Or were identified to facilitate the detection of bumblebee races. A “racial discriminant set” was created for 5 genes.

When resistance was developed to the old races of Orobanche, it was not seen as a significant problem in sunflower agriculture until the F race, which was a very virulent race in Spain and could not be controlled by Or resistance genes, emerged in 1995. The spread of the F race occurred very quickly to many countries in Europe (Alonso et al. ., 1996; Domínguez, 1999). In studies conducted to control race F, the presence of genes containing resistance to race F was detected in the germplasm of cultivated sunflower and wild sunflower populations (Fernández-Martínez et al., 2000; Rodríguez-Ojeda et al., 2001; Sukno et al., 1999). For more than twenty years, a new race, the G race, has emerged, killing the F race-resistant varieties controlled by the Or genes (Molinero-Ruiz et al., 2015). Recently, Türkiye (Kaya et al., 2009a); Romania (Pacureanu-Joita et al., 2009); In Russia (Antonova, 2014), a more aggressive new race, race H, emerged in sunflower fields. In addition, recent studies have shown that the race seen in the Şahinköyü region in the Thrace region exhibits a different infestation pattern than other races, and it has been suggested that this race may be race I (Yonet et al., 2018). Table 1.3 shows the beagle breeds seen in different countries of the world.

The rapid change of *O. cumana* populations, leading to the emergence of new virulent strains, requires continuous research to develop new sources of resistance to orobansha. The results of evaluating sunflower germplasm for resistance against different races have shown that wild *Helianthus* species are the main source of resistance genes providing resistance to new virulent races, but cultivated germplasms are also considered valuable sources of resistance. Although it has been determined that most of the sources of resistance to orobansha are controlled by major genes (vertical resistance), it has been emphasized that they also occur by quantitative (horizontal) loci (Cvejić et al., 2020). Dominant genes specific to Orobanche breeds are generally accepted by seed companies as ideal sources for single crosses, as it is sufficient for the resistance gene to be present in only one parent. In addition, combining vertical and horizontal gene resources of the same genotype and obtaining more permanent resistance with the gene pyramid is extremely important for successful and long-term resistance.

The first sunflower variety resistant to race A of the orobanche parasite was the variety developed by Plachek in 1918 in the western regions of the former Soviet Union (Škorić and

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Pacureanu, 2010). Changes in the race composition of the orobanchi have led to the emergence of the new race B in other sunflower cultivation areas, in the now independent countries of Moldova and Ukraine (Duca, 2014; Miladinovic et al., 2012). A new race C was observed in Moldova in the 1960s (Alonso et al., 1998) and a different race C was observed in Bulgaria in the 1970s (Škorić and Pacureanu, 2010). from the Agricultural Research and Development Institute in Fundulea, Romania (Vranceanu et al., 1981); By taking advantage of the gene pool created by the integration of *Helianthus tuberosus* into cultivated sunflowers, they determined the existence of five hornbill races. By making the first comprehensive race classification of this parasite, they identified and cataloged the *O. cumana* races from race A to race E and the dominant genes that provide resistance against them. A set of sunflower differential lines carrying five dominant resistance genes named Or1, Or2, Or3, Or4 and Or5, respectively, using the dominant genes for resistance to race A, races A + B, races A + B + C and races A + B + C + D. Five hornbill races have been described, from to E. Until the mid-1990s, races D and E were dominant, and hybrids resistant to race E, controlled by the Or5 resistance gene, were successfully developed and sunflower production increased worldwide (Vranceanu et al., 1981). In the mid-1990s, resistance was developed with the discovery of resistance genes in the F race wild sunflower germplasm and cultivated species, which were observed in some regions of Spain and broke all Or resistance genes (Alonso et al., 1996; Domínguez, 1999; Fernández-Martínez et al., 2000; Louarn et al., 2016; Rodríguez-Ojeda et al., 2001; Sukno et al., 1999). In recent years, strains that overcome Or5 resistance with increasing virulence have also been observed in Romania, Turkey, Bulgaria, Kazakhstan, Ukraine and Russia (Antonova, 2014; Fernández-Martínez et al., 2015; Martín-Sanz et al., 2016; Molinero-Ruiz et al. , 2006). Although all of them were initially called the F race, it was understood that the parasite seen in other regions was not the same race. The new G race, which eliminated F race resistant varieties, first appeared in Romania in 2005 (Pacureanu-Joita et al., 2009); In Molinero-Ruiz et al., 2015 and subsequent studies, it was determined that it was one of the more lethal races (race H) (Rîșnoveanu et al., 2016). According to the results in Moldova, it was emphasized that less aggressive races up to race E dominated in the central parts, showing the unequal spatial distribution of races, and it was observed that more than 60% of the bitch samples in the southern and northern parts of the country were more lethal races, races G and H ( Duca, 2014; Gisca et al., 2013). Again, the new H race was observed in Russia (Antonova, 2014), northeastern Ukraine (Maklik et al., 2018) and Turkey (Kaya et al., 2009b). In addition, in recent studies conducted in our country, it was seen that the race seen in the Şahinköyü region in the Thrace region exhibited a different infestation pattern than other races, and this race was called race I (Yonet et al., 2018). In the last 30 years, more virulent orobanchi strains have emerged, probably as a result of the widespread expansion of sunflower acreage, host selective pressure, and a rapid evolution in interaction complexity (Kaya, 2014).

Sunflower broomrape (*Orobanche cumana* Wallr.) which is the holoparasitic reduces sunflower (*Helianthus annuus* L.) yield until %100 infecting the sunflower roots. In addition to Russia, Ukraine, Romania, Bulgaria, Turkey, and Spain as the main sunflower producers in the world, broomrape is also present in Serbia, Hungary, Moldova, Greece, Tunisia, Israel, Iran, Kazakhstan, China, Mongolia, and Australia, and possibly in a few other countries as well (Dedic et al., 2009; Molinero-Ruiz et al., 2009; Burlov and Burlov, 2011; Pacureanu-Joita et al., 2012; Amri et al., 2012, Ma et al., 2012; Kaya et al, 2012b; Antonova et al, 2012a, b; 2013; Cantamutto, 2012; Miladinović et al. 2012b; Eizenberg et al 2012, Gisca et al 2013; Marinkovic et al. 2014). Due to having light and producing many seed in one plant, it is dispersing easily with spreading of sunflower growing area. Therefore, broomrape needs to be controlled by using available strategies, and breeding for resistance proved to be the most reliable way to control the parasite (Skoric, 2012; Fernandez- Martinez et al, 2012; Kaya et al, 2013).

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One of the best options for controlling broomrape uses resistant and tolerant cultivars in the sunflower production. The rapid changes in the race composition of broomrape requires search for new resistant sources persistingly. Therefore, it need to identify sunflower lines and other genetic material firstly for resistance to highly virulent races of broomrape, which is of great importance for current breeding programs, as source of resistance for gene transferring and sunflower hybrid production. After the determination of genes controlling resistance and their action, inbred lines could be used for transferring resistance in inbred lines with good agronomic traits using currently in the breeding programs, or could be used directly for developing commercial hybrids (Skoric et al., 2010; Skoric and Pacureanu- Joita, 2010, Fernandez- Martinez et al, 2010; 2011; Skoric, 2012; Cvejic et al, 2012; Kaya et al. 2012a).

**RECENT STIUATION OF THE DISTRIBUTION OF NEW BROOMRAPE RACES IN SUNFLOWER AREAS**

Broomrape races on sunflower are designated by the letters of Latin alphabet: A, B, C, D, E are old races and F, G and H are the last and the most virulent ones. They were found in the course of the last decade at first in Romania Spain and in Turkey (Fernandez-Escobar et al., 2009; Pacureanu-Joita et al., 2012; Fernandez-Martinez et al., 2012; Kaya et al., 2012a). To identify the recent races of broomrape; Romanian differentials are using generally by sunflower researchers, resistant to races D, E, F, G; the inbred line P-1380 (differential for E race), the inbred line LC 1093 (the differential for F race), the Spanish line P 96, resistant to race F under control of two recessive genes or6 or7 and two Russian sunflower lines: VK 623 and VT 62, resistant to new broomrape races respectively in Russia (Antonova et al., 2009, 2010, 2011, 2012a b; 2013; Pacureanu-Joita et al., 2010, 2012; Gisca et al 2013). The recent development in broomrape races in last four years in sunflower production areas by countries were given below.

Broomrape populations which overcomed the resistance gene Or5 (race F) were identified in Spain in the mid 90's and F race has infested widespread in the main sunflower growing areas of the country for 20 years. On the other hand G race populations were also observed especially in Southern Spain (Andulacuea) and Central Spain (Cuenca) which were main sunflower planting areas and exhibited similar aggressiveness with Turkey populations (Molinero-Ruiz et al., 2009, 2014; Fernandez- Martinez et al, 2009, 2010; 2012;Velasco et al. 2012; Akhtouch et al 2013).

In Romania, more than 60% of the sunflower cultivated area is infested with broomrape. There are three important areas, as the presence of the broomrape races and infestation degree, situated in Braila, Constanta and Tulcea locations. The three more spread broomrape populations in the largest area cultivated with sunflower, are very different regarding the virulence and dissemination of the parasite The race G was definitely found in Tulcea and Constanta counties in Romania and latest surveys showed possible appearance of even more virulent race (Pacureanu et al., 2009, 2012).

During the last years in the Russian Federation, the biotypes of broomrape have appeared and propagated especially after free exchange of sunflower sowing material between the countries, then overcomed rapidly other sunflower production areas (Antonova et al., 2009; 2013). The Rostov region was covered by the broomrape mostly and had the most virulent races affecting highly of all studied sunflower genotypes. While the broomrape populations in Volgograd region were less virulent and mixed with E and F races, the race F existed commonly in Stavropol region. In Krasnodar region, the mix of races C, D, E prevailed but in the northern districts bordering on Rostov region, the biotypes of a parasite affecting differential resistant to F and G races were observed. Thus, high virulent broomrape races overcoming action of known Or5, Or6, Or7, or6o7 sunflower resistance genes have spread in the South of the Russian

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Federation (Goncharov, 2009; Gorbachenko et al., 2011; Antonova et al., 2010, 2011, 2012a b; 2013).

The new broomrape races have developed in last years in Republic of Moldova. The broomrape population were observed more virulent than the race E, but not as aggressive as the F race is in Balti area, in the North part of the Republic of Moldova. A high infestation with virulent F race was found also in the Central and South areas of the Republic of Moldova (Duca et al., 2010 and 2013; Glijin, 2012; Gisca et al., 2013).

New F race infestation have spread recently almost all part of in Trakya region which is European part of Turkey and covers more than 50% of sunflower areas in Turkey (Semerci et al, 2010) . Moreover, new races have spread to other sunflower areas recently such as Cukurova, Middle Anatolia and Black Sea regions that were immune areas 4-5 years ago. On the other hand, there was no race determination study in Turkey so it could not be indicated that which new races as G or H existed other than F (Evci et al., 2011; Kaya et al 2009, 2012a, b).

Burlov and Burlov (2011) performed a combined research in artificial conditions also with comparing Turkish and Russian broomrape races with Ukrainian ones. They indicated that there were more virulent G and H races of the parasite in populations of broomrape collected in four regions in Ukraine. They observed that new F and race was not found in the Russian Rostov population and the new virulent races F and G and, possibly, race H as well (especially the Turkish population of the parasite) was observed in Odessa, Donetsk and Turkish populations of broomrape includes.

In Bulgaria, Shindrova and Penchev (2012) observed that E and G races were most widely distributed in Bulgaria but E race population gradually decreased but it was still predominant in the region of northeastern Bulgaria. They mentioned also that race F was rare and random occurrence, G race populations were in only the central north Bulgarian regions nowadays and its infestation could increase gradually in southeastern Bulgaria and in other regions as well in the upcoming years.

Broomrape in sunflower fields in Serbia has observed with varying intensity almost every year. Broomrape population in Serbia, race E was dominant but some broomrapes also have noticed in several regions in Vojvodina (North part of Serbia) on some resistance sunflower hybrids against races A-E indicating possibly changes in broomrape population. Monitoring of the broomrape population in Serbia has been performing regularly to observe in race composition changes spreading or not of more virulent races from neighboring countries (Dedic et al., 2009; Maširević and Medić-Pap, 2009; Hladni et al., 2009, 2010, 2012; Masirevic 2012a b; Cvejic et al, 2012; Miladinović et al. 2012a; Marinkovic et al. 2014).

Pacureanu et al. (2012) evaluated fourteen populations of broomrape collected from Bulgaria, China, Moldova, Romania, Russia, Serbia, Turkey, Spain and Ukraine in the artificial infestation conditions to determine in differentiation of broomrape races utilizing RAPD markers. They observed that the most aggressive populations were in Moldova, Romania, Russia and Turkey there were two or three different populations of broomrape in each country. They also mentioned that the broomrape populations in Russia are very similar to Romanian ones, except from Rostov's, this being almost similar with the most virulent Turkish population. They also observed that from Chinese population has not attacked to P-1380 line (race E) and Serbian one has not attacked to LC 1093 (race F), as well as Ukrainian population, Bulgarian and Spain populations attacked in lower rate to LC 1093. Their RAPD markers analyze results indicated that the ten studied broomrape populations divided in three groups and the genetic distance was not correlated with the geographic area.

On the other hand, Mladinovic et al. (2012a) also indicated that climatic parameters such as the altitude, latitude, longitude, mean hottest month temperature, mean coolest month temperature, average rainfall of 10 habitats and as well as soil parameters such as soil texture, total N, humus and calcareous content and pH had no effect on *Orobanche* attack intensity. However,

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Cantamutto et al. (2012) indicated broomrape seed dormancy was this most important parameter even than temperature and day length (latitude dependant) and winter temperature could adjust the germination time of the weed.

However, Molinero-Ruiz et al., (2014) indicated that homogeneous groups of highly virulent broomrape populations were related to geographical origin in Europe. They also found that some molecular groups related to only to one pathogenic group (i.e. race F from Southern Spain), while others include different pathogenic groups from the same geographical origin (i.e. races F and G from Turkey).

## **BROOMRAPE PARASITE BIOLOGY AND CONTROL METHODS**

On the other hand, to understand sunflower resistance mechanism, the studies should be focus on parasite side such as the reproductive biology of parasite, the genetic structure of the populations, inheritance mode of traits related to parasitism, host relationships, germination factors and environmental factors affecting development of parasite, etc... Recent studies on seed production of *Orobancha* revealed that broomrape was self-compatible and tolerated seed production under isolation, both under self-fertilization and controlled hybridization (Ephrath et al., 2010; Plakhine and Joel, 2010; Cantamutto et al., 2012; Dongo et al., 2012; Gevezova et al., 2012; Rodríguez-Ojeda et al., 2012; Spring and Raupp, 2012; Eizenberg et al., 2012, 2013; Duca et al., 2010, 2013; Duca and Glijin, 2013; Seiler, 2011, 2012, Dimitrijević et al., 2013; Habimana et al., 2014). However, the bag type used for isolation clearly played important role on determining seed production and micro-perforated transparent plastic bags were the best for not differing in germination and infectivity capacity (Rodríguez-Ojeda et al., 2010). On the other hand, the inheritance of unpigmented mutant lacking anthocyanin was studied by Rodríguez-Ojeda et al., (2011) and they mentioned that the unpigmented trait was controlled by recessive alleles at a single locus, with the F1 hybrid being phenotypically distinguishable from both pigmented and unpigmented parents.

Clearfield System with Imidazolinone (IMI) herbicides controls efficiently both major weeds and also broomrape, this system presents practical solutions to farmers so it has increased market share rapidly in recent years especially in Eastern European countries. The resistant IMI genes transferred from wild types to cultivated types, the trait is controlled by one gene *Imr1*, also known as *Ahas1-1*, exhibiting partial dominance, also affected by a second modifier *Imr2* gene. Therefore, to obtain IMI herbicide resistant hybrids both inbred lines should be IMI resistant. On the other hand, a new IMI-resistant trait called CLHA-plus was developed by mutagenesis and selection controlled by partially dominant allele *Ahas1-3*. Lines and hybrids carrying the CLPlus mutation have better tolerance to IMI herbicides then it need only one parent of a Clearfield hybrid allowing hybrid development rapidly (Demirci and Kaya, 2009; Fernandez- Martinez et al, 2009, 2010; 2012; Sala et al., 2012, Evci et al., 2011, 2012, Kaya et al., 2012, 2013). New sources of resistance to IMI herbicides have been recently identified in populations of wild *H. annuus* and *H. argophylus* (Christov et al., 2010).

Another approach to control broomrape is utilizing from germinations stimulants to decrease broomrape population in the soil when sunflower did not plant. Honiges et al. (2009) indicated that the most widely known such broomrape germination stimulants are strigol, electrol, orobanchol, and GP 24 synthetic stimulant. In addition to Dehydrocostus lactone (Joel et al. 2012), Raupp and Spring (2013) also mentioned that germination stimulant from sunflower root exudates as dehydrocostus lactone, a sesquiterpene lactone, dehydrocostus lactone, costunolide, tomentosin, and 8-epixanthatin were purified and identified spectroscopically and that compounds induced germination of *O. cumana* at nano-to micromolar concentrations. On the other hand, Zhang et al. (2013) indicated that soybeans could induce sunflower broomrape germination and could be used potentially as a trap crop for sunflower broomrape. Likewise,

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Ma et al (2012) observed similar effect on Chinese herb and in another studies (2013) in maize production.

**CLASSICAL AND MOLECULAR BREEDING STUDIES ON GENETIC RESISTANCE AND FINDING NEW GENETIC RESISTANT SOURCES AGAINST BROOMRAPE**

Since broomrape is an extremely variable parasite and the breakdown of resistance is a so common and frequent occasion, resistance breeding is continuous process and multiple sources of resistance are needed. Sources of resistance to broomrape races found in the early sunflower breeding programs in the Former Soviet Union originated from land races of cultivated sunflower, later from some chemical or radiation mutations but genetic resistance was also introduced into susceptible sunflower from wild species, mainly *H. tuberosus*. Recent studies on evaluation of sunflower germplasm for resistance to broomrape races proved that wild *Helianthus* species constitute the major reservoir of genes conferring resistance to new virulence races (Evcı et al., 2009; Pacureanu-Joita et al., 2010; Terzic et al., 2010; Škorić and Pacureanu-Joita, 2010, 2011; Petcu, and Păcureanu, 2011; Skoric, 2012; Fernandez-Martinez et al., 2010, 2012; Kaya et al., 2012a, 2014).

Progressions of old broomrape races (A, B, C, D, E) are controlled by five single dominant genes Or1 to Or5, respectively. A new race F that overcomes the gene Or5 was identified in Spain, Romania, Turkey. Furthermore, more virulent race G that affects cultivars resistant to race F, was identified (Skoric et al., 2010; Pacureanu-Joita et al., 2011; Fernandez-Martinez et al., 2012). Wild *Helianthus* species remain the main source of resistance to new virulent races of pathogen. The resistance to races E, F, G have been found in certain wild species of the genus *Helianthus* and incorporated into cultivated sunflower genotypes by interspecific hybridization. The crosses with the wild sunflower species *H. maximiliani*, *H. grosseserratus*., and *H. divaricatus* with cultivated sunflower and developed populations contributed greatly to develop sunflower varieties through interspecific hybridization and also recently *H. tuberosus* was used as the donor of Or5 and Or6 genes (Skoric et al., 2010).

Sunflower researchers mostly focused on wild species recently to find new resistance genes and develop new resources against broomrape. Velasco et al. (2011) found resistance to race G in a wild *Helianthus debilis* subsp. *tardiflorus* and the F1 plants from the cross with cultivated sunflower were resistant indicating dominance of resistance gene(s). Seiler and Marek (2011) indicated that while especially perennial wild species exhibited higher resistance, annual species demonstrated lower resistance to Race F. However, although there are some difficulties on crossing perennial species with cultivated sunflower, some novel techniques such embryo culture and chromosome doubling of the F1s, amphiploids, etc.. could facilitate the transfer of broomrape-resistant genes from the wild perennial species. Consequently, some amphiploids were obtained from perennial wild species *H. grosseserratus*, *H. maximiliani*, and *H. divaricatus* resistant to Race F releasing of four germplasm populations resistant to Race F named BR1 through BR4. Resistance to Race F appears to be controlled by dominant-recessive epistasis, complicating the breeding by requiring the genes to be incorporated into both parental lines of a resistant hybrid derived from interspecific amphiploids of *H. annuus* and of two wild perennials, *H. divaricatus* and *H. grosseserratus* controlled by a single dominant gene. However, recent studies indicated that the resistance of the sunflower germplasm derived from *H. Grosseserratus* proved to be digenic, the second gene being influenced by environmental factors (Seiler and Jan, 2010; Seiler, 2012, Skoric, 2012; Skoric and Pacureanu-Joita, 2011; Fernández et al. 2012).

Cvejic et al (2012), found that new source of resistance to race G and other new races in population from interspecific hybridization with *H. divaricatus* and *H. tuberosus* and then

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developed later inbred lines resistant to race G. They also indicated that different sunflower germplasm sources could be used as reservoir of genes conferring resistance to new virulent races of broomrape in the future. The latest research conducted in Romania under field and greenhouse conditions showed that sources of resistance to the newest population of *Orobanche* that were found in Romania, Turkey and Spain are present in the lines LC-009 and AO-548 (Rodríguez-Ojeda et al., 2013).

The differences in sources of the resistance have led to development of lines that are resistant to the same race of broomrape but vary in their genetic constitution (Imerovski et al., 2011; 2013). Until today, different authors reported different modes of inheritance of resistance to race F; controlled by single dominant gene, Or6 or two recessive genes or two partially dominant genes. Or7, whose expression was influenced by the environment. Changes in the race composition of broomrape in Romania have been reviewed by Pacureanu-Joita et al. (2008). Preliminary results of resistance to race G indicate that it is controlled by dominant alleles at a single locus (Velasco et al., 2011). Studies by Antonova et al. (2009) and Goncharov (2009) both discuss the dynamic change of broomrape races in Russia. It is known that *Orobanche* races change frequently in Ukraine and Moldova too, and that, although no public reports have been made yet, there are at least seven races of the pathogen in the two countries. New races F and G of broomrape have spread in many parts of Bulgaria and that races appeared one after another and have complicated the sunflower breeding process. Christov et al. (2009) have achieved outstanding results via interspecific hybridization in identifying broomrape resistant genes in wild *Helianthus* species incorporating them into cultivated sunflower and developing elite sunflower lines. Christov (2013) indicated that some genetic materials originating from wild sunflower species such as *H. tuberosus*, *H. pauciflorus*, *H. eggertii*, *H. x laetiflorus*, *H. decapetalus*, *H. hirsutus*, *H. divaricatus*, *H. giganteus*, *H. maximiliani*, *H. nuttallii* ssp. *rydbergii*, *H. salicifolius*, *H. smithii*, *H. annuus* (wild), *H. argophyllus*, *H. debilis*, *H. petiolaris* and *H. praecox*) and also five species from genera *Calendula*, *Carduus*, *Grindelia*, *Inula* and *Tithonia* of family *Compositae* to obtain resistance to broomrape. He also indicated that some developed inbred lines were demonstrated fully resistant to new races. Encheva et al. (2013) mentioned that also some inbred lines originated from wild *H. salicifolius* showed 100% resistance to *Orobanche* against artificial infection both in field and laboratory conditions developed by interspecific hybridization. Additionally, Encheva and Shindrova (2012) indicated that AAL and DIA accessions of wild *H. annuus* and *H. petiolaris* accessions were completely resistant to broomrape and *H. petiolaris* could have a great potential for broomrape resistance for cultivated sunflower.

To improve the durability of broomrape resistance, different breeding strategies should be applied like gene pyramiding or combining vertical and horizontal resistance mechanisms using more genes from different sources. That mechanism could be obtained by a resistant hybrid from the combination of lines carrying two different resistant mechanisms consisting one of failing of *Orobanche* seed germination and other one supplying necrosis of the parasite structures at early development stage (Fernandez-Martinez et al., 2012; Skoric, 2012).

### **Molecular markers**

Due to the influence of environmental factors, inadequate amount of broomrape seeds in the soil, hard process to collect broomrape seeds, its seed germination problems, etc., broomrape tests do not produce reliable results always. In order to attain their breeding goals and identify sources of broomrape resistance certainly, sunflower breeders choose the appropriate inoculation method and molecular marker technique (MAS). Since the most reliable and most easily applicable method of screening breeding materials for broomrape resistance is the use of molecular markers. QTL, RFLP, RAPD, TRAOP, and SSR markers have so far been used for



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this purpose (Imerovski et al., 2013; Perez-Vich et al., 2013). Molecular research for the purposes of race characterization and mapping is developing rapidly. For instance, Pacureanu et al. (2009) found that the resistance to the race F could be controlled with either two recessive genes or one dominant gene, in inbred lines depending on the origin of the inbred lines. These results were proved that by Imerovski et al. (2011) at the molecular level, and indicating that the marker is specific to the material on which it has been developed.

The utilizing from RFLP markers for the characterization of broomrape races, a linkage group containing the Or5 gene were integrated with the GIE Cartisol RFLP map. Besides working on the development of markers for Or5, current research is aimed to the mapping of a new gene that provides resistance to broomrape races higher than F (Cvejić et al. 2012). By comparing the molecular profile of resistant and susceptible genotype, a polymorphism was observed on LG3 of SSR map. Accordingly, it can be assumed that the new gene is located on this linkage group (Imerovski et al. 2012). Further tests on the mapping population will determine the exact position of the gene, and enable the development of specific molecular marker that will accelerate the introduction of resistance to new races of broomrape into the commercial sunflower lines.

Pineda-Martos, et al. (2014) studied genetic diversity of 50 broomrape populations in Spain utilizing 15 microsatellite markers and observed the existence of two distant gene pools, one in Cuenca province and other in the Guadalquivir Valley. They indicated that also both inter- and intrapopulation variability were extremely low within each gene pool and but genetic recombination between distant gene pools was an important mechanism for creating new variation and also having an effect on race evolution. In another study, Pineda-Martos, et al. (2014) used 4200 simple sequence repeat (SSR) markers to identify and characterize broomrape and 217 SSR primer pairs were used for validation. From them, 87 SSR primers produced reproducible, high quality amplicons of the expected size that were polymorphic among 18 broomrape populations from different locations. They also indicated that *O. cumana* SSR markers were highly transferable to the closely related species *Orobanche cernua* and utilizing that SSR markers could be possible to classify of *Orobanche* spp. samples into species (*O. cumana* and *O. cernua*), geographical origin and host properly.

## **CONCLUSIONS AND FUTURE PROSPECTS**

Sunflower breeders recently have big challenge recently both developing high yielding cultivars and also adaptable to marginal environments due to sunflower production being pushed into lower-fertility soils. In addition, that abiotic stress conditions, some biotic factors such as broomrape, downy mildew, and other diseases also limit and threaten sunflower production recently in increasingly year-by-year. The limited genetic variability and missing resistant genes for broomrape in cultivated sunflower provided especially from wild species. Recent studies in especially MAS proved that novel molecular techniques could be so valuable in sunflower breeding programs for resistance gene identification, accelerating and facilitating broomrape resistance breeding.

The virulence of broomrape populations varies differently in the world and the most aggressive parasitic races are in the Black Sea having more than 50% of sunflower planting areas with disseminating rapidly. However sunflower breeders and geneticists found resistant genes from some wild *Helianthus* genus and then incorporated them into sunflower inbred lines to develop *Orobanche*-resistant hybrids. However, rapid changes in the race composition of broomrape need urgently to set up an international project on both investigating and screening of all wild sunflower species natural and artificial conditions against new races of broomrape and molecular screening too. There is also an urgent need with collaboration among public institutions and private companies to establish a proper set of differential lines for the new races

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appeared in Russia, Romania, Ukraine, Turkey, Bulgaria, etc.. These efforts will help and lead to launch universal methods be established for both screening for resistance to broomrape in field and greenhouse conditions and molecular methods more accurately.

On the other hand, combining IMI or SU herbicide resistance or both of them together with broomrape resistance will supply choose to sunflower farmers with controlling both broomrape and major broad leaf weeds together. Similarly, some studies focused on broomrape parasite biology, germination stitumulants, To speed up the progress of sunflower breeding for resistance to Orobanche, there should be a greater level of collaboration between the breeders from public institutions and private companies.

#### **BULGARIA**

The samples collected from the region of central southern Bulgaria were determined as race G+ of the parasite broomrape (origin Stara Zagora). The samples collected in the region of Northern Bulgaria, race G was found (origin Lozitsa). The results, obtained in the last 10 years, showed the high potential for genetic variability of the parasite and the dynamics of change in its racial composition by area. Among the most affected by the parasite are the regions of central southern Bulgaria and the central and northeastern regions of northern Bulgaria, where established races are more aggressive than race E. Outbreaks and separate micro-districts with races F and G of the parasite were identified at numerous locations during the monitoring. In the last 7 years, the studied fields related to race E represent an increasingly smaller percentage of the total number of studied fields in Bulgaria, both for the current year 2022/2023 and for the entire monitoring period. This shows that we have a mass distribution of the G race in the affected areas of the country, and also the presence of many micro-districts heavily affected by the more aggressive populations (G+ or H).

The results obtained in some areas in the last two years provoke interest in the ongoing changes in the populations of the parasite and the possibility of the appearance of a new race in the near future.

#### **MOLDOVA**

The last 20 years sunflower has consistent planting areas, more then 360 000 ha, exceeding (2.5 times) the admissible limits of this crop in crops rotation. Significant expansion of sunflower area lead to irrational exploitation of the land increasing frequency and aggressiveness of various pathogen... and especially of broomrape *O. cumana* Wallr.

For this purpose, we collected seeds of 43 populations from Republic of Moldova, Ucraina, Spain and Romania, as well as a collection of 37 populations from Moldova, Serbia, Bulgaria, China, Romania and Turkey. For the discrimination between broomrape races a large set of differential was used. In the populations collected from 27 administrative districts of the Republic of Moldova was established the presence of E, F, G, H races in 2014 and the appearance of more aggressive populations, possible a more virulent race, which was called H + in 2019. The comparative analysis of the results from 2014 with that of 2019 confirmed for some localities the presence of the same broomrape races, while in majority of them – it was revealed the appearance of a more evolved race. In 5 localities a strain of broomrape, which affected the differentiators for the H race was found. Making a retrospective analysis of the appearance of the races on the territory of the Republic of Moldova, it was confirmed the idea already presented by other researchers about the faster evolution of broomrape races and the emergence of a new races every 5-10 years. The most efficient set of differentials was used in subsequent research including broomrape populations from different countries. Thus, the population belonging to Spain was identified as race G, the population from southern Ukraine was more virulent and it was referred to the race H. 2 populations from Tulcea and Brăila highlighted the presence of the most evolved broomrape race in southeastern part of Romania,

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confirming the already existing data. The populations from Serbia did not infect the differentials for upper races. 5 populations from Turkey, Bulgaria and China were attributed to the race G and other 6 populations - to the race H. The population of Chesan, Turkey, infected also the differentials for the H race, thus finding the emergence of a more evolved race, which can be considered as a race H +. Thus, except Serbia, it was revealed the presence of high virulent races G and H in the majority of countries. For the first time it was identified race H in China. Among the populations collected in the Black Sea region, including Ukraine, Turkey, Romania and Moldova, race H predominates, and in some cases, a new strain (H+) were found. As a result of these studies we concluded that according to virulence the races identified in different country are the same.

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**MOLECULAR CHARACTERIZATION OF SOME WILD SUNFLOWER SPECIES  
(HELIANTHUS SPP.) AND INTERSPECIFIC HYBRIDS BASED ON BROOMRAPE  
RESISTANCE**

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**ABSTRACT**

Sunflower knows a significant source of oil globally and are the most favored oil and the most produced oil crop in Turkey. Broomrape parasite (*Orobanche cumana* Wallr.) is one of the biggest limited factor for sunflower production in Turkey which causes yield loss up to 100% in sunflower. The parasite creates new races by constantly renewing itself, then researchers are continuously searching for new resistance genes. The use of wild gene sources, which are the gene sources of new races of broomrape, together with marker assisted selection (MAS) is one of the most effective and efficient methods for the development of new hybrid sunflowers. The wild sunflower species, subspecies used in the study were obtained from the North Central Regional Station in Ames, IA, USA. Resistant and sensitive control varieties and hybrid varieties were obtained from Trakya Agricultural Research Institute, Edirne Turkey. Tolerance tests were performed on 65 genotypes consisting of wild species and subspecies and 33 genotypes, which are hybrid varieties. Afterwards, the presence of desired resistance genes was tried to be determined by molecular markers. As a result of the study with the ORS665 marker, the 281bp bands were amplified and compared with the morphological observation results. Within the scope of the disease test, it was observed that 67 individuals were resistant and 44 of them had 281bp fragments; these 44 individuals were predicted to contain the Or4 endurance gene. In the study, the RGC172 marker, the 550bp and 620bp bands were amplified, and 68 individuals were observed to be resistant in the test, and 45 of them gave conclusive results in terms of the 550bp allele, so it is thought that the RGC172-550bp alleles of the Or5 resistance gene of these 45 individuals are related. The 620bp DNA fragment of the RGC172 marker was observed in 44 individuals and these 44 individuals are thought to contain the Or5 resistance gene. In the study, the RGC181 marker, the 550bp and 600bp bands were amplified and compared with the morphological observation results. It was observed that 68 individuals were morphologically resistant, and 38 of them were considered to contain the Or5 resistance gene, since they gave conclusive results in terms of both alleles of the RGC181 marker. In the study; with the ORS1036 marker, 240bp bands were amplified and compared with the morphological observation results. The 68 individuals were observed to be resistant, and since 41 of them gave confirmatory results in terms of the 240bp allele, these 41 individuals are thought to contain the Or6 resistance gene. In the study; with the ORS1114 marker, 265bp bands were amplified and compared with the morphological observation results. The 67 individuals were observed to be resistant, and since 52 of them gave conclusive results in terms of the 265bp allele, it was predicted that these 67 individuals contained the Or6 resistance gene.

**Keywords:** Sunflower, Wild sunflower, MAS, *Orobanche cumana* Wallr., Resistance, Molecular marker

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## **INTRODUCTION**

Sunflower broomrape (*Orobanche cumana* Wallr.) is a parasitic plant that does not have the ability to photosynthesize because it does not contain chlorophyll, and attaches to the host plant root to provide growth requirements such as nutrients, water and minerals (Cvejić et al., 2020; González, 2021). The parasite, known to be diploid, has  $2n = 38$  chromosomes. Additionally, the genome size was determined to be 1.42 Gb by densitometer measurements (Pujadas-Salva and Velasco, 2000; Weiss-Schneeweiss et al., 2006).

Wild sunflowers have perennial and annual species seen wild in the northern regions of Canada, the Western USA and Mexico. They are highly competitive against other wildflowers that grow on their own for the water, sunlight and nutrients in the soil necessary for their growth and development. When Europeans came to settle in that region, they started planting wild sunflowers for their oil and appearance; They increased the popularity of sunflower oil and it started to be cultivated for this purpose (Schilling 2006; Stebbins et al., 2013; Miklic et al., 2004).

The first report containing broomrape resistance was obtained from the varieties "Progress" and "Novinka", developed using the "Group Immunity" breeding approach with germplasm derived from the perennial wild *H. tuberosus* species (Pustovoit and Gubin, 1974). Many researchers (Fernández-Martínez et al., 2010; Fernández-Martínez et al., 2000; Škorić ve Pacureanu, 2010; Terzić et al., 2010) evaluated sunflower germplasm for resistance to boreal races and found that wild *Helianthus* species are an important species that provides resistance to new virulence races as forming a gene reservoir. The source of the first resistance gene identified in sunflower, the Or1 gene, was obtained from the *H. tuberosus* wild species, which also contains a wealth of other resistance genes (Parker and Riches, 1993; Vranceanu et al., 1981). In addition, resistance to races E, F and G was also obtained from wild sunflower species. Sukno et al., (1998) emphasized that especially the perennial species *H. resinous*, *H. laevigatus*, *H. giganteus* and *H. pauciflorus* provided resistance to new races in Spain. Hladni et al., (2009) stated that the restorer line derived from *H. deserticola*, an annual wild sunflower species in Serbia, showed resistance against the E and F races of the broomrape. They made significant contributions to the breeding of sunflower varieties through crosses and interspecific hybridizations with wild sunflower species *H. maximiliani*, *H. grosseserratus* and *H. divaricatus* and cultivated sunflowers and developed populations.

In another study, the resistant Or5 and Or6 genes were detected in the *H. tuberosus* species (Škorić and Pacureanu, 2010). Pérez-Vich et al., (2002) examined the transfer of resistance created by the dominant gene against the F race using two perennial species, *H. grosseserratus* and *H. divaricatus*, and the cultivated *H. annuus* species, and Petcu and Joița-Păcureanu, (2012) emphasized that the hybrids obtained with *H. argophyllus* contained resistance to E and F races found in Romania. Christov, (2013) found that 17 wild perennial *Helianthus* species, *H. pauciflorus*, *H. tuberosus*, *H. eggertii*, *H. decapetalus*, *H. hirsutus*, *H. giganteus*, *H. divaricatus*, *H. smithii*, *H. maximiliani*, *H. salicifolius*, *H. laetiflorus*, *H. nuttallii* ssp. *rydbergii* and annual *H. praecox*, *H. annuus* (wild), *H. petiolaris*, *H. debilis* and *H. argophyllus* are resistant to broomrape races A to G in Bulgaria. The perennial diploid species *H. giganteus*, *H. divaricatus*, *H. glaucophyllus*, *H. mollis*, *H. grosseserratus*, *H. smithii* and *H. nuttallii* and their interspecific hybrids have been reported to be resistant to broomrape. Nikolova et al., (1998) have achieved extraordinary results in resistance to broomrape through interspecific hybridizations. Again, resistance to race G, which is considered a more virulent race by overcoming varieties resistant to race F, has been described in wild species (Molinero-Ruiz and Melero-Vara, 2004; Škorić and Pacureanu, 2010). It has been observed recently that there is resistance against race G and other unknown races in the *H. debilis* ssp. species and in the lines obtained as a result of

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hybridization studies with *H. divaricatus* (Cvejic et al., 2012; Velasco et al., 2012). Wild annual species that are resistant to broomrape races are given in Table 1.7 (Seiler, 2015).

Table 1. Resistance to annual wild species and broomrape races

Species	Resistant to Races
<i>H. petiolaris</i>	A-G
<i>H. praecox</i>	A-G
<i>H. exilis</i>	E-F
<i>H. anomalus</i>	E-F
<i>H. deserticola</i>	E-F
<i>H. argophyllus</i>	F
<i>H. debilis ssp. tardiflorus</i>	G

Labrousse et al., (2001) investigated in their study that the relationship between wild sunflower roots and broomrape, *Helianthus debilis ssp. debilis* species produced an impenetrable encapsulation layer that blocked the parasite entering the sunflower plant roots and then died. Yet another interspecific hybrid from the same species was found to show reduced stimulation of broomrape seed germination and rapid necrosis at an early stage of parasite development. Resistance has also occurred in an interspecific hybrid derived from the species *H. argophyllus*, occurring primarily in the fourth stage of parasite development, as necrosis occurs before broomrape flowering. (Encheva et al., 2013) emphasized that some inbred lines from wild *H. salicifolius* showed 100% resistance to artificial infection of the broomrape parasite under field and laboratory conditions developed by interspecific hybridization. In order to increase the durability of broomrape resistance, it is thought that different breeding strategies should be applied, such as combining vertical and horizontal resistance mechanisms, through the gene pyramid or by using more genes from different sources. This mechanism can be achieved by a resistant hybrid from the combination of varieties carrying two different resistance mechanisms, which ensure the failure of the germination of the bitter seed and the necrosis of parasite structures at the early developmental stage (Fernández-Martínez et al., 2012; Škorić et al., 2012). A, B, C, D and E blight resistance genes are present in various cultivated sunflower populations. Since the broomrape has an extremely variable parasitic structure, pole breaking is a constant occurrence. Multiple sources of resistance are needed to control new strains that emerge as resistance breaks. Genes that confer resistance to F, G, H races and other wild sunflower species are identified by race name, and unidentified genes are combined and introduced into hybrid sunflowers through interspecies hybridization. Sunflower germplasm evaluations for resistance to boreal strains have proven that novel virulent boreal strains in sunflower species constitute important reservoirs for the resistance genes discussed. Additionally, reported resistance to blubber, including seven annual species and thirty-two perennial species, provides a broad genetic basis for breeders seeking resistance to existing and emerging strains of the bumblebee parasite. The latest research conducted under field and greenhouse conditions in Romania; The newest population of roobanchi found in Romania, Turkey and Spain showed that sources of resistance are present in lines LC-009 and AO-548 (Rodríguez-Ojeda et al., 2013 ). It has been stated that wild sunflower species contain many valuable genes for cultivated sunflowers, including blight resistance, but especially perennial species have been neglected and have not been used adequately due to difficulties in hybridizing with cultivated sunflower varieties. The main problems are concern for outcross incompatibility, F1 sterility and loss of genetic material from wild species, and the fact that perennial polyploid species involve a time-consuming backcrossing program due to extra

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chromosome elimination. However, in the 1980s, embryo recovery techniques improved and the possibility of effective selection quickly and accurately became a more routine process with interspecific hybridization performed in advanced greenhouses.

With the use of marker-assisted selection, researchers have developed many marker varieties that are still in use. RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction fragment length polymorphism), TRAP (Target Region Amplification Polymorphism), QTL (Quantitative trait locus) and SSR (Simple Sequence Repeats) markers have been used for this purpose so far (Imerovski et al., 2013; Pérez-Vich et al., 2013). Previously, molecular research for race characterization and mapping purposes was conducted with microsatellites (Bilgen et al., 2019). For example, (Pacureanu-Joita et al., 2009) found that resistance to strain F could be controlled by two recessive genes or one dominant gene in inbred lines, depending on the origin of the inbred lines. These results are in accordance with (Imerovski et al., 2011) and show that the marker is specific to the material on which it was developed. RFLP maps were created for the first time in sunflower by (Berry et al., 1995; Leon et al., 1996) and (Gentzbittel et al., 1999; Gentzbittel et al., 1995). Using RFLP markers for the characterization of Orobanche breeds, a linkage group containing the Or5 gene was integrated with the GIE Cartisol RFLP map. In addition to working on the development of markers for Or5, current research aims to map a new gene that confers resistance to higher oroban strains than the F strain (Cvejic et al., 2012). Molecular studies have been carried out aiming to map the genes that confer resistance to races E and F. The Or5 gene, which confers resistance to race E, has been mapped to a telomeric linkage group (LG) 3 in the sunflower genetic map (Lu et al., 2000; Tang et al., 2003). The observed linkage between markers from LG3 and three different hornworm resistance genes indicates that the Or genes are closely related and likely to be in the same LG (gene family). By comparing the molecular profile of the resistant and sensitive genotype, a polymorphism in LG3 of the SSR map was observed. Accordingly, it is assumed that new resistance genes are on this linkage group (Imerovski et al., 2013). Further testing on the mapping population will determine the precise position of the gene and enable the development of specific molecular markers that will accelerate the development of resistance to new sunflower lines and commercial sunflower lines. (Tang et al., 2002) created the first linkage map using SSR markers in sunflower, and subsequently (Yu et al., 2003) increased the density of the map created with SSR markers by using a new recombinant inbred line population. (Eizenberg et al., 2004) confirmed the importance of molecular markers for the study of sunflower orobang. Imerovski et al. (2013) screened twenty NS sunflower genotypes resistant to different hornbeam races (A-F) with SSR markers and found a significant relationship between ORS1036\_240 and ORS1114\_265 alleles of the Or6 gene through statistical analysis. (Rodríguez-Ojeda et al., 2014) examined the genetic diversity of 50 broomrape populations in Spain using 15 microsatellite markers and observed the existence of two distant gene pools, one in the province of Cuenca and the other in the Guadalquivir Valley. They also noted that both intergenic and interpopulation variability are extremely low within each gene pool and that genetic recombination between distant gene pools is an important mechanism for generating new variation and also influencing breed evolution. Another study (Rodríguez-Ojeda et al., 2014) used 4200 simple sequence repeat (SSR) markers to identify and characterize the broomrape, and 217 SSR primer pairs were used for validation. From them, 87 SSR primers produced reproducible, high-quality amplicons of the expected size that were polymorphic among 18 broomrape populations from different locations. They found that the RAPD patterns of DNA extracted from soil-derived broomrape seeds were the same as DNA in vegetative plant material, provided the seeds were not spoiled (Pacureanu, 2014). (Rieseberg et al., 1995) examined the effects of chromosomal structural changes using markers; (Burke et al., 2004) created the SSR/RAPD genetic linkage map, and some researchers (Langar et al., 2003) created other genetic maps using AFLP markers.

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In this thesis study, resistance tests to the broomrape parasite were carried out and the presence of the desired resistance genes was tried to be detected with molecular markers. For this purpose, ORS665, ORS1036, ORS1114, ORS683, RGC172, RGC181 markers, which are known to be associated with bumblebee resistance genes, were used.

## **MATERIAL AND METHOD**

Pathogen material for use in disease testing was provided by Trakya Agricultural Research Institute (TTAE). The material consists of seeds containing the latest races collected from different cultivation areas in the Thrace Region (Figure 1).

### **Broomrape Tests**

The surface of the seeds of the sunflower material to be tested was sterilized by keeping it in 8% NaOCl (sodium hypochlorite) for 15 minutes and rinsed with distilled water. These procedures were carried out in petri dishes in groups of five. The seeds, washed with distilled water, were placed in petri dishes in which filter paper was previously placed and the name and number of the sample were written on it. Ethaphon (artificial plant growth regulator) was added to the Petri dishes at 25 ppm. The seeds were kept at 26 °C for 1-2 days, rinsed with distilled water and placed in ozonated water. As the seeds in the Petri dishes germinated, they were transferred to plastic cups. Disease tests were carried out within the TTAE institute. In order to grow plants in TTAE greenhouses, there is a mixture of soil and broomrape seeds in plastic cups. The plastic cups were filled halfway with a mixture of soil and Broomrape, and soil was added again by placing germinating seeds on top. The plants were watered frequently for 2 days to ensure their growth. Each plant sample that germinated in the Petri dishes was placed in plastic cups as it germinated. Thus, the cultivation of all plant samples did not occur at the same time. Although the seeds that did not germinate and did not grow were sown again, some varieties did not grow because they could not adapt to the ambient temperature and wild species germinated under sensitive conditions. At the end of 40-50 days, plants with a length of 10-25 cm were separated for disease testing. The roots of the plants removed from the plastic cups were examined and the number of orobanche nodules or the presence or absence of orobanche were determined. Each plant sample, which grew sequentially, went through these stages (Figure 2.3) and information was obtained about the sensitivity of the broomrape. In some individuals, no orobanche nodule was found on the root and these individuals were recorded as resistant individuals. In some individuals, one or more nodule formation was observed on the plant root, and even on the soil in some varieties, and these individuals were determined as sensitive individuals and recorded for comparison with the results of molecular studies.

Morphological observation was made as a result of the Broomrape test of the varieties that germinated; Resistant and susceptible varieties were determined. Then, its usability for MAS purposes was tested using molecular markers reported in the literature to be associated with Or resistance genes (Or2-Or6). 241 wild sunflowers and 38 hybrid sunflower varieties consisting of species and subspecies were germinated for disease testing. However, 65 species and subspecies of wild species and 26 hybrid varieties of sunflower were taken into account as plant material because they germinated and were observed, and this material was used in molecular marker studies. Plant material used for resistance and sensitivity to orobansha; It was determined by the Thrace Agricultural Research Institute (TTAE) among the materials used in sunflower breeding programs. In order to determine the resistant gene region and which races of the broomrape it is resistant to, the varieties that are known to contain which resistance gene and to which race they are resistant were selected from among the materials used in sunflower breeding programs by TTAE.

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Figure 1. Collection of disease tested samples



Figure 2. Material used as parasite in the study

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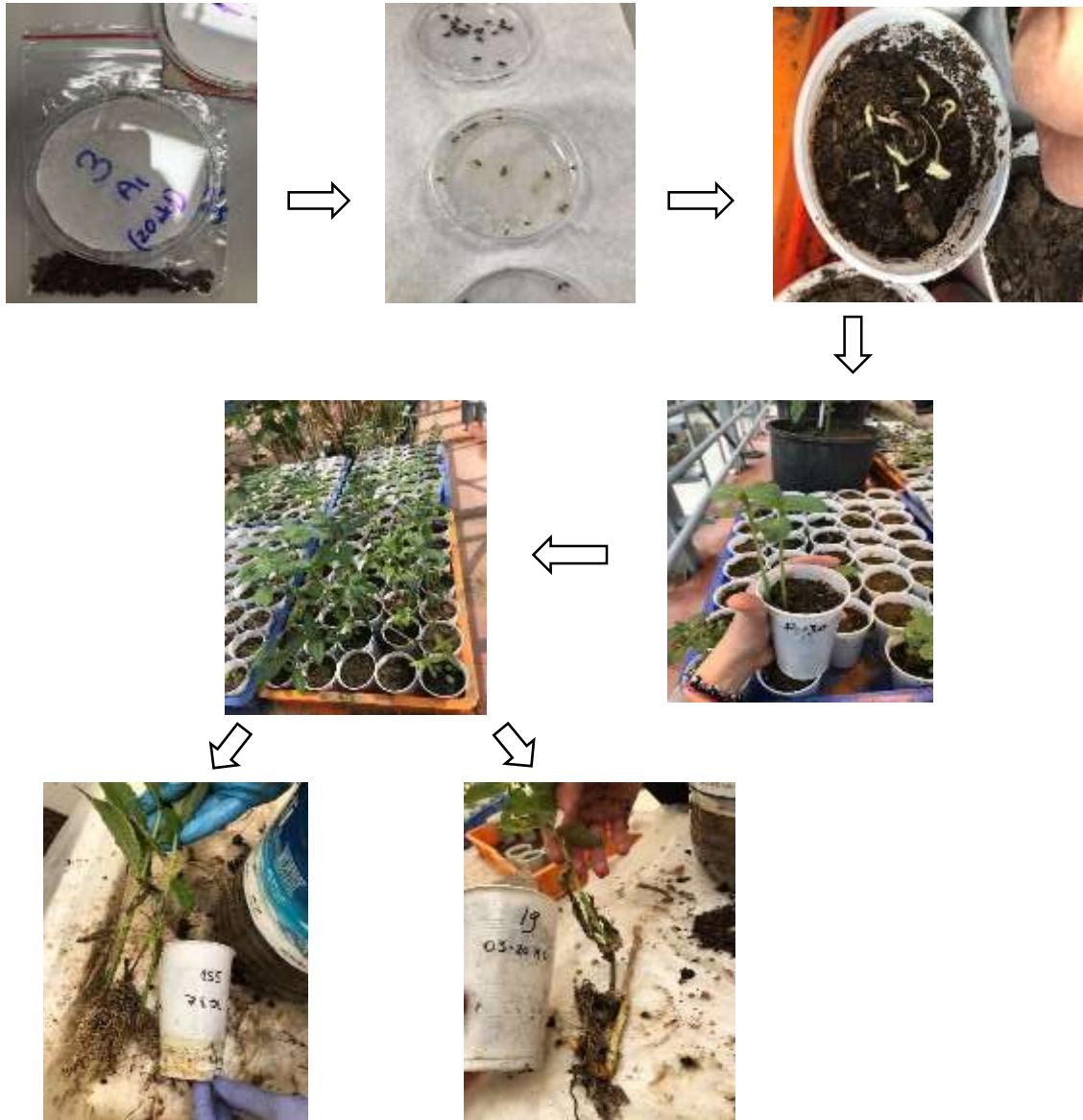


Figure 3. Germination and morphological observation of plant samples

### **Molecular Analyzes**

All molecular studies for this thesis were carried out in the Biotechnology Laboratories of Trakya University, Faculty of Engineering, Department of Genetics and Bioengineering.

#### **DNA Isolation**

The leaves of the materials tested for the disease were placed in 2ml tubes with 150-200mg of plant tissue per sample. After the samples were collected, they were frozen in liquid nitrogen at  $-196^{\circ}\text{C}$  and stored at  $-20^{\circ}\text{C}$  until the DNA isolation phase.

The DNA of the samples taken from the leaves of the plant material was applied to the Norgen Plant/Fungie DNA Isolation Kit (Product#26200) protocol, detailed below.

- 1) 150-200mg leaf samples, stored at  $-20^{\circ}\text{C}$ , were taken into 2ml tubes and frozen in liquid nitrogen, and 10mg PVP and two 3mm metal balls were added to each sample.
- 2) The samples with added metal balls were shredded in a tissue disintegrator device (RETSCH MM400) at a speed of 30 revolutions per second for 90 seconds to ensure that the tissues turned into homogeneous powder.

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- 3) 500µl Lysis and 1µl RNase A were added to the samples.
  - 4) The samples were kept in a 65 °C water bath for 10 minutes by turning them upside down.
  - 5) 100µl Binding Buffer was added to the samples taken from the water bath and the samples were kept on ice for 5 minutes.
  - 6) The samples were centrifuged at 14000rpm for 2 minutes and the supernants were taken.
  - 7) 70% ethanol was added as much as the amount of supernatant taken and vortexed.
  - 8) 650µl of the samples were taken and transferred to gray column tubes and centrifuged at 10000rpm for 1 minute.
  - 9) The remaining liquid at the bottom of the column is discarded and after taking 650µl, the DNA samples remaining in the tube are added to the same gray column tubes. It was centrifuged at 10000rpm for 1 minute.
  - 10) After centrifugation, the part remaining at the bottom of the tube was discarded, 500µl Solution WN was added and centrifuged at 14000rpm for 1 minute.
  - 11) Again, the liquid part accumulated at the bottom of the tube was discarded, 500µl Wash Solution A was added and centrifuged at 14000rpm for 1 minute. This stage was repeated once more.
  - 12) The liquid accumulated at the bottom of the tube was discarded and centrifuged empty at 14000 rpm for 2 minutes without adding any solution.
  - 13) Sample names were written on the elution tubes and the gray columns were placed in these tubes.
  - 14) 70µl Elution Buffer B was added and kept at room temperature for 5 minutes.
  - 15) Centrifuged at 10000rpm for 5 minutes.
  - 16) The samples were placed on ice and concentration measurements were made.
- After quantity and quality determination, DNA main stocks were stored at +4 °C until dilution, and at -20 °C for long-term storage after reconstitution.

#### 2.4.2. DNA Concentration and Quality Determination

OPTIZEN NanoQ Spectrophotometer was used for DNA quantification. The amount of DNA for the samples was recorded in ng/µl, paying attention to the OD260/OD280 (for nucleic acid purity) ratio.

In order to see whether there were DNA breaks and to determine the DNA quality, considering the DNA amounts, approximately 800ng of each sample was loaded onto the gel containing EtBr (30 µl/L) (Ethidium bromide) with a concentration of 0.8% and was run at 120V 80mA for 1 hour. . After the electrophoresis process, the DNA quality of the samples was examined under UV light on a gel imaging device.

#### Marker Studies for Or2, Or4, Or5, Or6, Orab-vl-8 Gene

For the thesis study, the Or5 gene, which provides resistance to the bumblebee races in the region (from A to E), the Or2 gene, which provides resistance to B-C races, the Or4 gene, which shows resistance to D race, and the Or6 gene, which is thought to contain resistance to H race, were targeted, depending on the proximity of the marker to the gene region. attention has been paid. Gene-related markers were identified as a result of literature review. These are RGA181, RGA172 (Radwan et al., 2009), ORS1114, ORS665, ORS1036 (Imerovski et al., 2013), ORS683 (Imerovski et al., 2016). Since the recombination rate varies depending on the gene region of the marker, testing markers close to this distance will produce an effective result (Figure 4).

Relevant marker sizes (Table 3) and Forward and Reverse primers (Table 4) used to detect genes containing resistance to races E and G of sunflower broomrape are given.

Since it is very important to determine appropriate PCR reaction conditions for molecular studies, optimization studies have been carried out to find the most suitable temperature and cycle conditions. Primer PCR contents (Table 2.6) and primer PCR conditions (Table 2.7, Table 2.8, Table 2.9) are given for PCR experiments.



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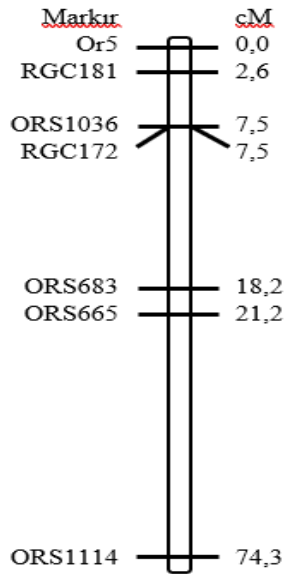


Figure 4. Markers and Their Distances in Genetic Mapping of the Sunflower Or5 Gene Region (Tang et al., 2003)

Table 3 Molecular markers used in the study and the Or genes they are linked to

Broomrape race	Resistant gene	Marker type	Distance to Locus	Associated Marker Size	Reference
E	Or2	ORS1114	-	260bp	Imerovski et al., (2013)
E	Or4	ORS665	-	281bp	Imerovski et al., (2013)
E	Or4	ORS1114	-	264bp	Imerovski et al., (2013)
E	Or5	RGC181	2.6 cM	-	Radwan et al., (2009)
E	Or5	RGC172	7.5 cM	-	Radwan et al., (2009)
E	Or6	ORS1036	-	240bp	Imerovski et al., (2013)
E	Or6	ORS1114	-	265bp	Imerovski et al., (2013)
G	Orab-vl-8	ORS 683	1,5 cM	-	Imerovski et al., (2016)

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Table 4. Molecular markers and primers used in the study for recent resistant to Or genes

Primer name	Forward and Reverse primers 5'- 3'
ORS665	F: GCACATGAGGTATGGATCTCCT R: TGCAAATACAACCTCGGGAAA
ORS1114	F: AGATGGTGGCAGGAGAGTTAAAG R: GCAGAAACAGATCAGGAGGGTAT
ORS1036	F: CCCTTTCACCTTCTATTTTCTATTCA R: CTAAGAGGGGTCGGTATGATTTC
ORS683	F: ATAGAAACCACCACCCATCG R: GGTCCTAATGTTTGGACACTT
RGC181	F: TGGCATTAGATATGAGGTTTGGGA R: TTGGACAAGAAGTCAAGTCAAGG
RGC172	F: TACCATGTGTCTCCCTCTGATGT R: GATGTCCTTGATGTTCCCAATAA

Table 5. The suitable temperature and cycle conditions for PCR

Temperature (°C)	Duration	Cycle
95	3 Minutes	1
95	Seconds	
60	45 Seconds	35
72	3.5 Minutes	
72	10 Minutes	1

Table 6. Primer PCR contents

PCR Content (15 µl)	Final Amount and Concentration
Forward Primer (10 µM)	0,5 µl
Reverse Primer (10 µM)	0,5 µl
gDNA Sample (20 ng/µl)	2 µl
H2O	4,5µl
Master Mix	7,5 µl

Table 7. Primer binding temperatures applied for SSRs (Touchdown)

Primer Name	Cycle	Temperature	Duration	Cycle Number
ORS1114	G. Denaturation	95	5 min	1
ORS1036 ORS665	Denaturation	95	1 min	40 (Each 10 Cycle after, 1°C lower down)
	Binding	62 → 52	1 min	
	Synthesis	72	2 min	
	Final Synthesis	72	10 min	

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Table 8. Primer binding temperatures applied for RGA (Touchdown)

Primer Name	Cycle	Temperature	Duration	Cycle Number
RGA172 RGA181	G. Denaturation	94	3 min	1
	Denaturation	94	30 seconds	35 (Each 10 Cycle after, 1°C lower down)
	Binding	68 → 58	1 min	
	Synthesis	72	30 seconds	
	Final Synthesis	72	15 min	1

Table 9. Primer binding temperatures applied for ORS683

Primer Name	Cycle	Temperature	Duration	Cycle Number
ORS683	G. Denaturation	94	2 min	1
	Denaturation	94	30 seconds	30 (Each 10 Cycle after, 1°C lower down)
	Binding	60	40 seconds	
	Synthesis	70	1 min	
	Final Synthesis	72	2 min	1

The resulting PCR products were visualized by running gel electrophoresis to see whether the PCR study was successful or not and to see how many base pairs of bands formed in the region. In the study, a DNA standard coded Generuler #SM0371(372) that could distinguish 50-1000bp band sizes was used. Comparisons were made based on morphological observation results, taking into account the fragment sizes of the PCR products. Information was obtained about the resistance of wild and hybrid populations to broomrape races. Additionally, an idea was obtained about the usability of markers in selection.

## RESULTS AND DISCUSSION

### Disease Test Results

After the plant varieties planted in the same cup with the pathogen seed germinated in the greenhouse and reached a certain length, the plant roots were examined and the number of nodules and the presence or absence of broomrape were recorded (Table 3.1 and Figure 3.1). Varieties with nodules on plant roots are sensitive individuals; Varieties in which no nodule formation was observed were recorded as resistant individuals. Although the entire collection was germinated and planted, results could not be obtained for all genotypes in the collection because it was difficult for the wild sunflower genotypes to attach to the roots because germination and emergence from the soil were very difficult and took a long time, their development was extremely slow during this period, and some accessions died during or after germination of the seeds. While nodule formation was observed in 29 of 98 different plant materials whose disease tests could be monitored, 69 of them were found to be resistant to blight. Among the wild sunflower species, *H. mollis*, *H. pauciflorus*, *H. winteri*, *H. argophyllus*, *H. debilis* subsp. Some accessions of *cucumerifolius*, *H. petiolaris*, *H. tuberosus*, *H. maximiliani*, *H. agretis*, *H. paradoxus* appear as plants sensitive to orobanche. Hybrid sunflower: SLT\_Ay\_146 x *H. arg14*, SLT\_Ay2\_146 x *H. deb\_581*, Solso *H. deb-581*, SLT\_Ay\_146 x *H. deb\_581*, Solso 30 x *H. arg\_1093*, SLT009 x *H. arg19*, Solso 30 x *H. arg\_1093*, SLT009 x *H. annuus9*, SLT\_Ay\_146 x *H. arg19*, SLT\_Ay\_146 x *H. bol28*, SLT\_Ay-146 x *H. winteri237*, Solso 30 x *H. deb\_581* varieties were recorded as susceptible individuals.

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The remaining accessions were seen as orobanche-resistant individuals. Again, the results were obtained by performing the bittersweet test on the P130 variety [Figure 3.2 (a)], which is known to be resistant to the last races of the bitter and used as genetic material in molecular studies, and the 9661 variety [Figure 3.2 (b)], which is sensitive to the bitter.

Table 10. Cut-off strength states of the accessories as a result of cut-off tests

PI no	Accession Number	Accession or cross name	Broomrape tolerance or nodule number at root
673209	1	<i>H. agretis</i>	8
597890	2	<i>H. annuus</i>	Resistant
468651	3	<i>H. argophyllus</i>	5
490291	4	<i>H. argophyllus</i>	Resistant
649863	5	<i>H. argophyllus</i>	Resistant
649865	6	<i>H. argophyllus</i>	Resistant
664803	7	<i>H. argophyllus</i>	7
468659	8	<i>H. atrorubens</i>	Resistant
673141	9	<i>H. bolanderi</i>	Resistant
673280	10	<i>H. bolanderi</i>	Resistant
649958	11	<i>H. cusickii</i>	Resistant
597908	12	<i>H. debilis subsp. cucumerifolius</i>	Resistant
613753	13	<i>H. debilis subsp. cucumerifolius</i>	2
649870	14	<i>H. debilis subsp. cucumerifolius</i>	Resistant
547169	15	<i>H. decapetalus</i>	Resistant
649971	16	<i>H. decapetalus</i>	Resistant
673143	17	<i>H. divaricatus</i>	Resistant
664641	18	<i>H. eggertii</i>	Resistant
664733	19	<i>H. floridanus</i>	Resistant
547184	20	<i>H. giganteus</i>	Resistant
649984	21	<i>H. giganteus</i>	Resistant
547193	22	<i>H. grosseserratus</i>	Resistant
649994	23	<i>H. grosseserratus</i>	Resistant
649996	24	<i>H. grosseserratus</i>	Resistant
649998	25	<i>H. grosseserratus</i>	Resistant
673183	26	<i>H. heterophyllus</i>	Resistant
547203	27	<i>H. hirsutus</i>	Resistant
547204	28	<i>H. hirsutus</i>	Resistant
653545	29	<i>H. laciniatus</i>	Resistant
586896	64	<i>H. maximiliani</i>	Resistant
613794	65	<i>H. maximiliani</i>	Resistant
468746	30	<i>H. maximiliani</i>	Resistant
468747	31	<i>H. maximiliani</i>	Resistant
586892	32	<i>H. maximiliani</i>	6
586894	33	<i>H. maximiliani</i>	Resistant
613794	34	<i>H. maximiliani</i>	Resistant

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650013	35	<i>H. mollis</i>	1
673147	36	<i>H. mollis</i>	Resistant
592349	37	<i>H. nuttallii</i>	Resistant
650024	38	<i>H. nuttallii</i>	Resistant
531044	39	<i>H. nuttallii subsp. nuttallii</i>	Resistant
531052	40	<i>H. nuttallii subsp. nuttallii</i>	Resistant
586905	41	<i>H. nuttallii subsp. nuttallii</i>	Resistant
597918	42	<i>H. nuttallii subsp. rydbergii</i>	Resistant
664789	43	<i>H. occidentalis subsp. plantagineus</i>	Resistant
673239	44	<i>H. paradoxus</i>	12
673253	45	<i>H. paradoxus</i>	8
503233	46	<i>H. pauciflorus</i>	Resistant
547211	47	<i>H. pauciflorus</i>	Resistant
650026	48	<i>H. pauciflorus</i>	1
673214	49	<i>H. porteri</i>	Resistant
664695	50	<i>H. resinusus</i>	Resistant
435880	51	<i>H. simulans</i>	Resistant
664698	52	<i>H. smithii</i>	Resistant
547223	53	<i>H. strumosus</i>	Resistant
357299	54	<i>H. tuberosus</i>	Resistant
503279	55	<i>H. tuberosus</i>	Resistant
547227	56	<i>H. tuberosus</i>	Resistant
547237	57	<i>H. tuberosus</i>	Resistant
650093	58	<i>H. tuberosus</i>	Resistant
650099	59	<i>H. tuberosus</i>	Resistant
650105	60	<i>H. tuberosus</i>	Resistant
664619	61	<i>H. tuberosus</i>	Resistant
664624	62	<i>H. tuberosus</i>	4
673290	63	<i>H. winteri</i>	1
	68	<i>SLT 009 x H.deb-581</i>	2
	67	<i>SLT_009 x H. arg_1093</i>	Resistant
	69	<i>SLT_009 x H. deb-581</i>	7
	78	<i>SLT_009 x H. deb-581</i>	4
	70	<i>SLT_Ay_146 x H. deb_581</i>	2
	79	<i>SLT_Ay_146 x H.arg14</i>	1
	80	<i>SLT_Ay_146 x H.arg15</i>	Resistant
	81	<i>SLT_Ay_146 x H.arg17</i>	Resistant
	82	<i>SLT_Ay_146 x H.arg19</i>	5
	83	<i>SLT_Ay_146 x H. bol28</i>	5
	84	<i>SLT_Ay_146 x H. bol673141</i>	Resistant
	87	<i>SLT_Ay-146 x H. winteri237</i>	5
	73	<i>SLT_Ay2_146 x H. deb_581</i>	1
	91	<i>SLT009 x H. annuus9</i>	4
	89	<i>SLT009 x H. arg19</i>	3

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90	<i>SLT009 x H.bol.28</i>	Resistant
71	<i>Solso 30 x H. arg_1093</i>	3
72	<i>Solso 30 x H. arg_1093</i>	2
66	<i>Solso 30 x H. deb_581</i>	20
85	<i>Solso x H. arg14</i>	1
86	<i>Solso x H. arg15</i>	1
88	<i>Solso x H. winteri237</i>	Resistant
75	<i>Wild species 2019 France (H. arg)</i>	1
74	<i>Wild species 2019 France (H. deb silvestris)</i>	Resistant
76	<i>Wild species 2019 Turkey (H. arg)</i>	Resistant
77	<i>Wild species 2019 Turkey (H. arg)</i>	1



Figure 5. Control of broomrape resistance of wild sunflowers and hybrids

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Figure 6. (a): Broomrape Resistant P-130 hybrid; (b): Broomrape Sensitive 9661 Inbred line  
Results of Markers Used for Or4

#### ORS665 Marker

It is known that the ORS665 marker is located 21.2 cM away from the Or5 gene region and produces a 281 bp selective band. As a result of the study conducted with the ORS665 marker, 281bp bands were amplified in wild sunflower genotypes and hybrid sunflower varieties with resistant material (P130, LC1002A) and sensitive material (9661) (Figure 3.3) and compared with the morphological observation results. Within the scope of the disease test, 67 individuals were observed to be resistant and 281bp sized fragment was seen in 44 of them (Table 3.2); These 44 individuals were predicted to contain the Or4 resistance gene.

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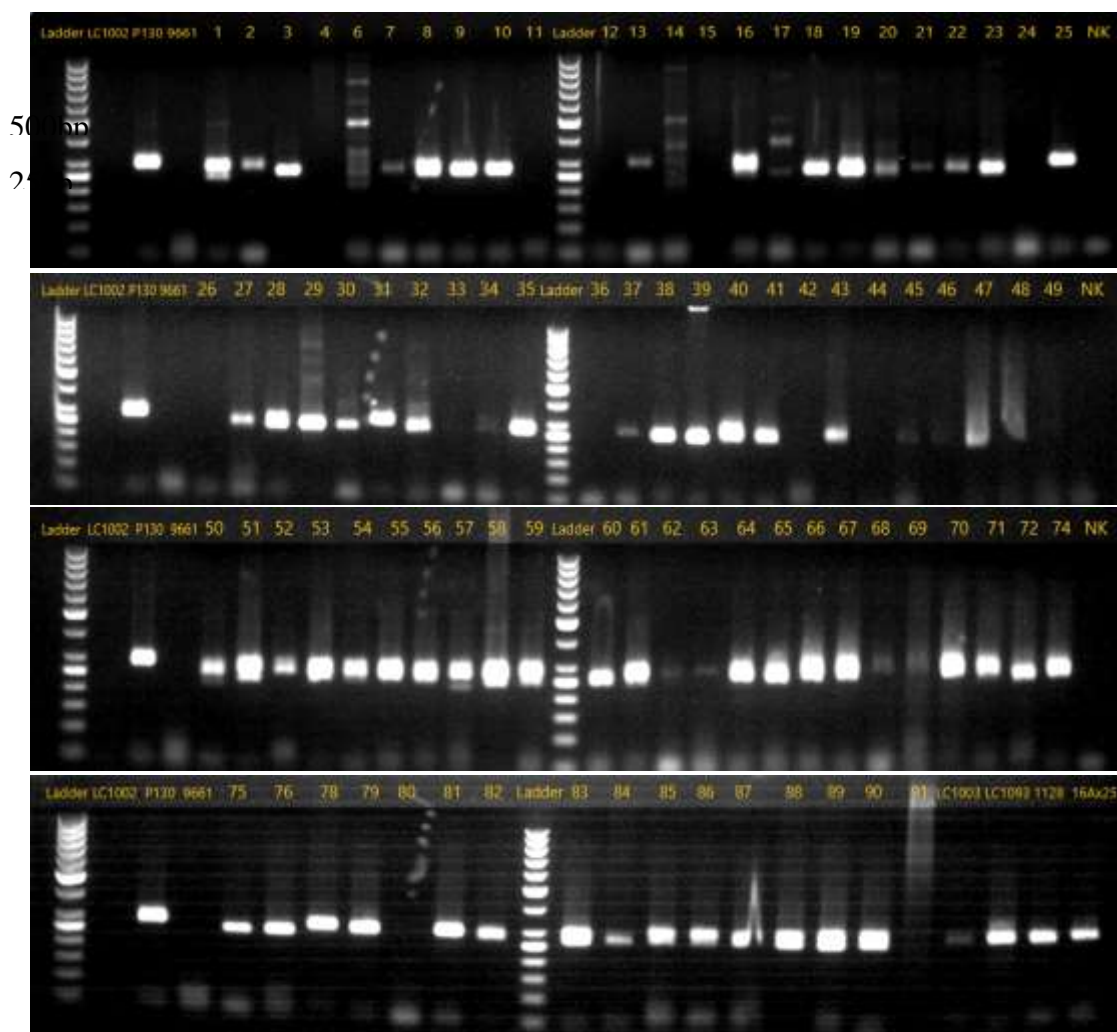


Figure 7. Gel electrophoresis image of the PCR result using the ORS665 marker (P130 = resistant control type, 9661 = sensitive control type, NK = negative control)

#### Results of Markers Used for Or5

##### RGC172 Marker

It is known that the RGC172 marker is located 7.5 cM away from the Or5 gene region and produces selective bands of 550bp and 620bp. As a result of the study conducted with the RGC172 marker, 550bp and 620bp bands were amplified in wild sunflower genotypes and hybrid sunflower varieties with resistant material (P130) and sensitive material (9661) (Figure 3.4) and compared with the morphological observation results. Within the scope of the disease test, 68 individuals were observed to be resistant, and since 45 of them (Table 3.2) gave confirmatory results for the 550bp allele of the RGC172 marker, it is thought that these 45 individuals are related to the RGC172-550bp alleles of the Or5 resistance gene. The 620bp DNA fragment of the RGC172 marker was observed in 44 individuals, and these 44 individuals are thought to contain the Or5 resistance gene.



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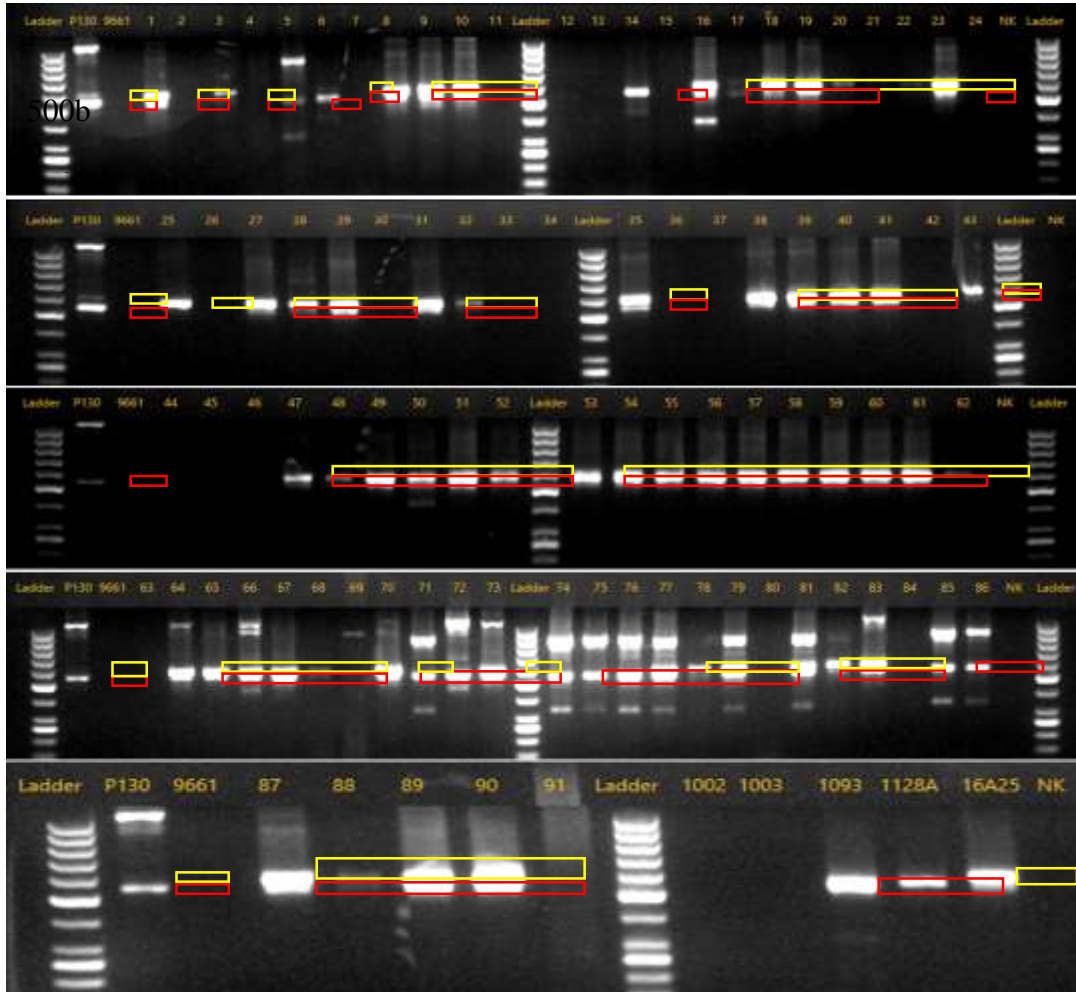


Figure 8. Gel electrophoresis image of the PCR result using the RGC172 marker

#### RGC181 Marker

It is known that the RGC181 marker is located 2.6 cM away from the Or5 gene region and produces selective bands of 550bp and 600bp. As a result of the study conducted with the RGC181 marker, 550bp and 600bp bands were amplified in wild sunflower genotypes and hybrid sunflower varieties with resistant material (P130) and sensitive material (9661) (Figure 3.5) and compared with the morphological observation results. Within the scope of the disease test, 68 individuals were observed to be morphologically resistant, and since 38 of them (Table 3.2) gave confirmatory results for both alleles of the RGC181 marker, it is thought that these 38 individuals contain the Or5 resistance gene.

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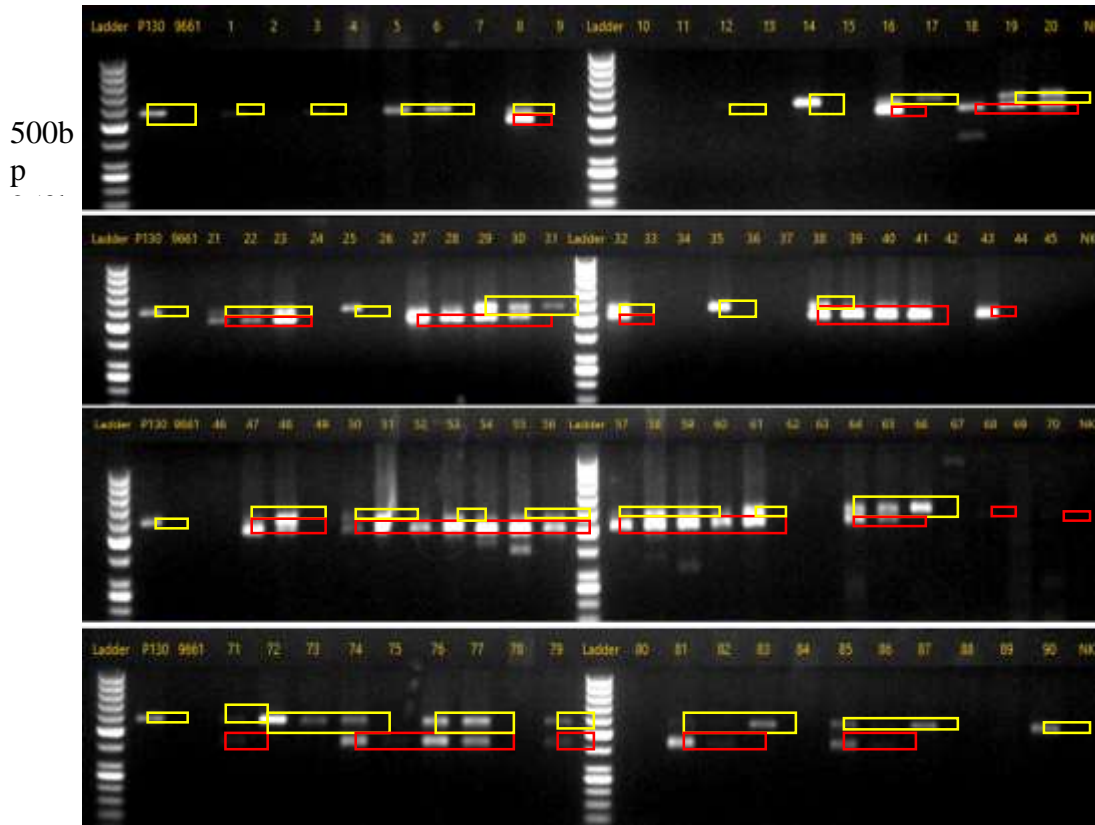


Figure 9. Gel electrophoresis image of the PCR result using the RGC 181 marker (P130 = resistant control type, 9661 = sensitive control type, NK = negative control)

#### Results of Markers Used for Or6

##### ORS1036 Marker

It is known that the ORS1036 marker is located 7.5 cM away from the Or5 gene region and produces a 240bp selective band. As a result of the study conducted with the ORS1036 marker, 240bp bands were amplified in wild sunflower genotypes and hybrid sunflower varieties with resistant material (P130, LC1093A) and sensitive material (9661) (Figure 3.6) and compared with the morphological observation results. Within the scope of the disease test, 68 individuals were observed to be resistant, and since 41 of them (Table 3.2) gave correct results in terms of the 240bp allele, it is thought that these 41 individuals contain the Or6 resistance gene.

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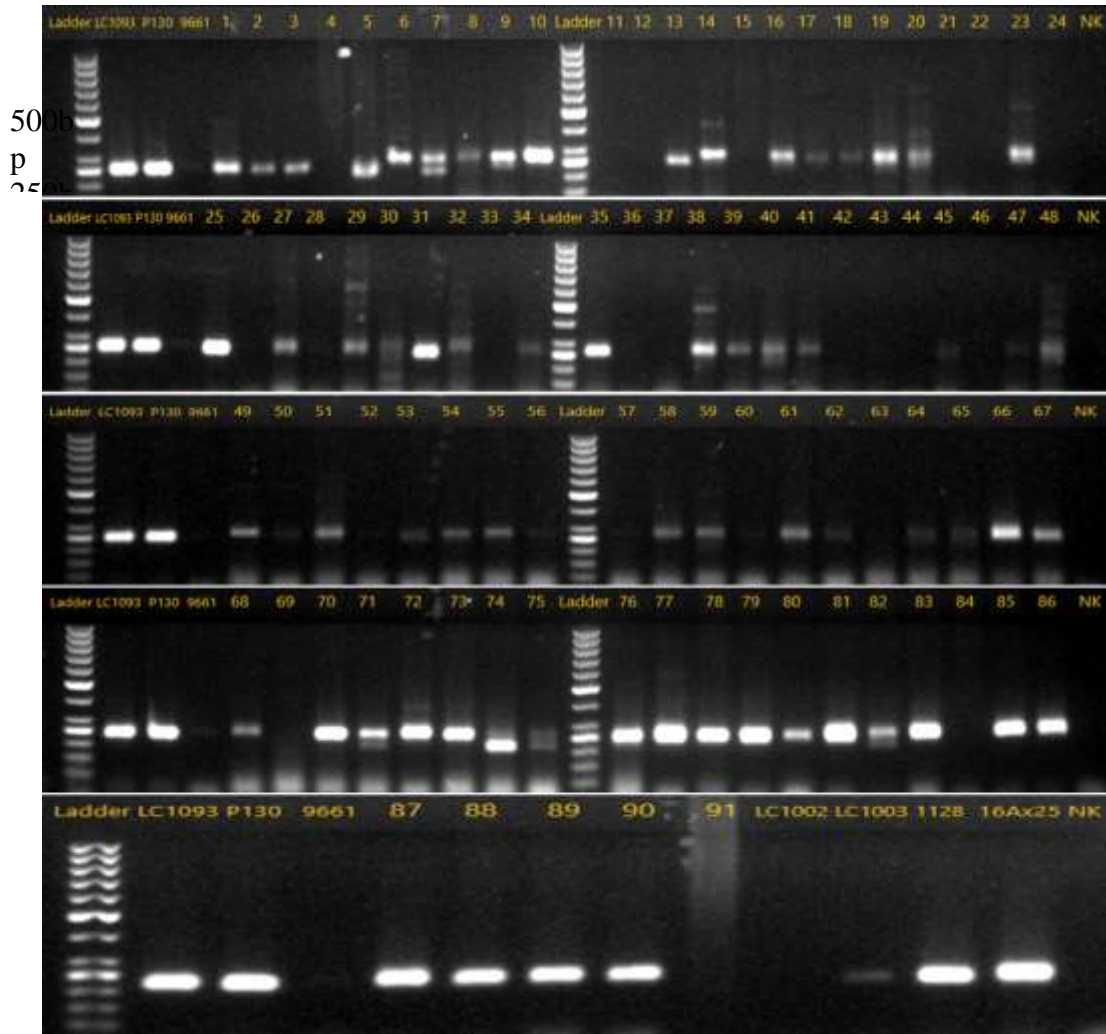


Figure 10. Gel electrophoresis image of PCR result using ORS 1036 marker (P130, LC1093A = resistant control type, 9661 = sensitive control type, NK = negative control)

#### ORS1114 Marker

It is known that the ORS1114 marker is located 74.3 cM away from the Or5 gene region and produces a 265bp selective band. As a result of the study conducted with the ORS1114 marker, 265bp bands were amplified in wild sunflower genotypes and hybrid sunflower varieties with resistant material (P130, 1128A, LC1002A, 16Ax25) and sensitive material (9661) (Figure 3.7) and compared with the morphological observation results. Within the scope of the disease test, 67 individuals were observed to be resistant, and since 52 of them (Table 3.2) gave correct results for the 265bp allele, it was predicted that these 67 individuals contained the Or6 resistance gene.

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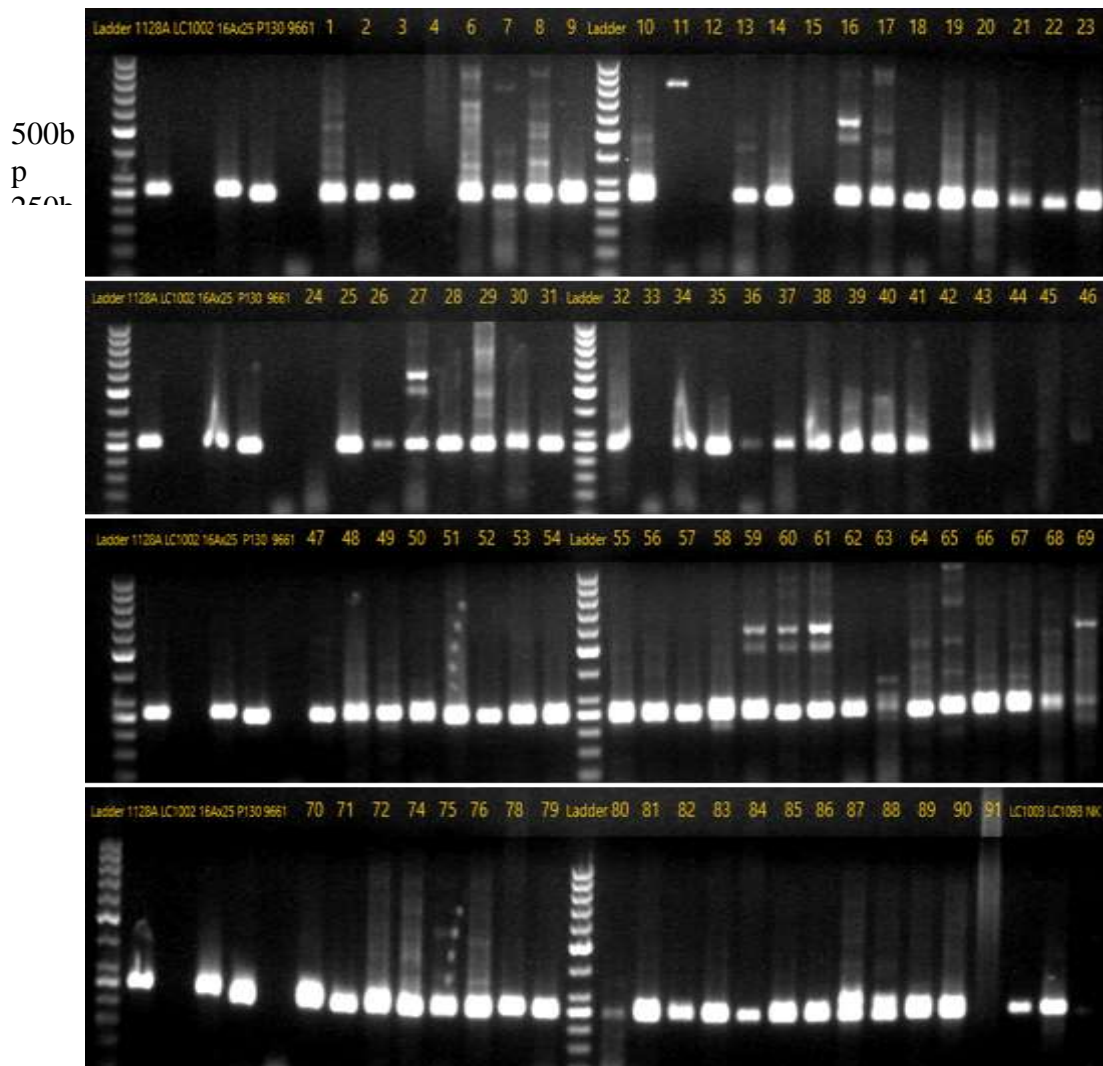


Figure 11. Gel electrophoresis image of the PCR result using the ORS1114 marker (P130 = resistant control type, 9661 = sensitive control type, NK = Negative Control)

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Table 11. Bandage resistance states of accessions and molecular marker tests

Taxonomy	#	BT	RGC172		RGC181		ORS 1036	ORS 1114	ORS 665
			550 bp	620 bp	550 bp	600 bp	240 bp	264 bp	281 bp
<i>H. agretis</i>	1	8	+	+	-	+	+	+	+
<i>H. annuus</i>	2	R	-	-	-	-	+	+	+
<i>H. argophyllus</i>	3	5	+	+	-	+	+	+	-
<i>H. argophyllus</i>	4	R	-	-	-	-	-	-	-
<i>H. argophyllus</i>	5	R	+	-	-	+	+	yok	yok
<i>H. argophyllus</i>	6	R	+	+	-	+	-	-	-
<i>H. argophyllus</i>	7	7	-	-	-	-	+	+	+
<i>H. atrorubens</i>	8	R	+	+	+	+	-	+	+
<i>H. bolanderi</i>	9	R	+	+	-	-	-	+	+
<i>H. bolanderi</i>	10	R	+	+	-	-	-	+	+
<i>H. cusickii</i>	11	R	-	-	-	-	-	-	-
<i>H. debilis subsp. cucumerifolius</i>	12	R	-	-	-	+	-	-	-
<i>H. debilis subsp. cucumerifolius</i>	13	2	-	-	-	-	+	+	+
<i>H. debilis subsp. cucumerifolius</i>	14	R	+	-	-	+	-	+	-
<i>H. decapetalus</i>	15	R	-	-	-	-	-	-	-
<i>H. decapetalus</i>	16	R	+	+	+	+	+	+	+
<i>H. divaricatus</i>	17	R	+	+	-	+	+	+	+
<i>H. eggertii</i>	18	R	+	+	+	-	+	+	+
<i>H. floridanus</i>	19	R	+	+	+	+	+	+	+
<i>H. giganteus</i>	20	R	-	+	+	+	+	+	+
<i>H. giganteus</i>	21	R	-	+	+	+	-	+	+
<i>H. grosseserratus</i>	22	R	-	+	+	+	-	+	+
<i>H. grosseserratus</i>	23	R	+	+	+	+	+	+	+
<i>H. grosseserratus</i>	24	R	-	-	-	-	-	-	-
<i>H. grosseserratus</i>	25	R	-	+	-	+	+	+	-
<i>H. heterophyllus</i>	26	R	-	-	-	-	-	+	-
<i>H. hirsutus</i>	27	R	+	+	+	-	+	+	+
<i>H. hirsutus</i>	28	R	+	+	+	-	-	+	+
<i>H. laciniatus</i>	29	R	+	+	+	+	+	+	+
<i>H. maximiliani</i>	30	R	-	-	+	+	-	+	+
<i>H. maximiliani</i>	31	R	+	+	-	+	+	+	-
<i>H. maximiliani</i>	32	6	+	+	+	+	-	+	+
<i>H. maximiliani</i>	33	R	-	-	-	-	-	-	-
<i>H. maximiliani</i>	34	R	-	-	-	-	-	+	-
<i>H. mollis</i>	35	1	+	+	-	+	+	+	+
<i>H. mollis</i>	36	R	-	-	-	-	-	-	-
<i>H. nuttallii</i>	37	R	-	-	-	-	-	+	+
<i>H. nuttallii</i>	38	R	+	+	+	+	+	+	+
<i>H. nuttallii subsp. nuttallii</i>	39	R	+	+	+	-	+	+	+

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<i>H. nuttallii</i> subsp. <i>nuttallii</i>	40	R	+	+	+	-	+	+	+
<i>H. nuttallii</i> subsp. <i>nuttallii</i>	41	R	+	+	+	-	+	+	+
<i>H. nuttallii</i> subsp. <i>rydbergii</i>	42	R	-	-	-	-	-	-	-
<i>H. occidentalis</i> subsp. <i>plantagineus</i>	43	R	+	+	+	-	-	+	+
<i>H. paradoxus</i>	44	12	-	-	-	-	-	-	-
<i>H. paradoxus</i>	45	8	-	-	-	-	-	-	-
<i>H. pauciflorus</i>	46	R	-	-	-	-	-	-	-
<i>H. pauciflorus</i>	47	R	+	+	+	+	+	+	+
<i>H. pauciflorus</i>	48	1	+	+	+	+	+	+	-
<i>H. porteri</i>	49	R	+	+	-	-	-	+	-
<i>H. resinosus</i>	50	R	+	+	+	+	+	+	-
<i>H. simulans</i>	51	R	+	+	+	+	+	+	+
<i>H. smithii</i>	52	R	+	+	+	-	-	+	+
<i>H. strumosus</i>	53	R	+	+	+	+	+	+	+
<i>H. tuberosus</i>	54	R	+	+	+	-	+	+	+
<i>H. tuberosus</i>	55	R	+	+	+	+	+	+	+
<i>H. tuberosus</i>	56	R	+	+	+	+	+	+	+
<i>H. tuberosus</i>	57	R	+	+	+	+	+	+	+
<i>H. tuberosus</i>	58	R	+	+	+	+	+	+	+
<i>H. tuberosus</i>	59	R	+	+	+	+	+	+	+
<i>H. tuberosus</i>	60	R	+	+	+	-	+	+	-
<i>H. tuberosus</i>	61	R	+	+	+	+	+	+	+
<i>H. tuberosus</i>	62	4	-	+	-	-	+	+	-
<i>H. winteri</i>	63	1	-	-	-	-	-	-	-
<i>H. maximiliani</i>	64	R	+	+	+	+	+	+	+
<i>H. maximiliani</i>	65	R	+	+	+	+	+	+	+
<i>Solso 30 x H. Deb_581</i>	66	20	+	+	-	+	+	+	+
<i>SLT_009 x H. Arg_1093</i>	67	R	+	+	-	-	+	+	+
<i>SLT_009 x H.deb-581(Fransa)</i>	68	2	+	+	+	-	+	+	+
<i>SLT_009 x H. deb-581(Türkiye)</i>	69	7	-	-	-	-	-	-	-
<i>SLT_Ay_146 x H. Deb581</i>	70	2	+	+	+	-	+	+	+
<i>Solso 30 x H. Arg_1093</i>	71	3	+	-	+	+	+	+	-
<i>Solso 30 x H. Arg_1093</i>	72	2	+	-	-	+	+	+	+
<i>SLT_Ay2_146 x H. Deb_581</i>	73	1	+	+	-	+	+	yok	yok
<i>Wild species 2019 France (H. deb) silvestris</i>	74	R	+	-	+	+	-	-	-
<i>Wild species 2019 France (H. Arg)</i>	75	1	+	-	+	-	+	-	-
<i>Wild species 2019 Turkey (H. Arg)</i>	76	R	+	-	+	+	+	-	-
<i>Wild species 2019 Turkey (H. Arg)</i>	77	1	+	+	+	+	+	yok	yok
<i>SLT_009 x H. deb-581</i>	78	4	+	+	-	-	+	+	+
<i>SLT_Ay_146 x H.arg14</i>	79	1	+	+	+	+	+	+	+
<i>SLT_Ay_146 x H.arg15</i>	80	R	-	-	-	-	+	-	-
<i>SLT_Ay_146 x H.arg17</i>	81	R	+	+	+	+	+	+	+
<i>SLT_Ay_146 x H.arg19</i>	82	5	+	+	+	+	+	+	+
<i>SLT_Ay_146 x H.bol28</i>	83	5	+	+	-	+	+	+	+

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<i>SLT_Ay_146 x H.bol673141</i>	84	R	-	-	-	-	-	-	-
<i>Solso x H.arg14</i>	85	1	+	-	+	+	+	+	+
<i>Solso x H.arg15</i>	86	1	+	-	+	+	+	+	+
<i>SLT_Ay-146 x H.winteri237</i>	87	5	+	+	-	+	+	+	+
<i>Solso x H.winteri237</i>	88	R	+	+	-	-	+	+	+
<i>SLT009 x H.arg19</i>	89	3	+	+	-	-	+	+	+
<i>SLT009 x H.bol.28</i>	90	R	+	+	-	+	+	+	+
<i>SLT009 x H.annuus9</i>	91	4	-	-	-	-	-	-	-
LC 1002		R	-	-	-	-	-	-	-
LC 1003		R	-	-	+	+	+	+	+
LC 1093		R	+	-	+	+	+	+	+
1128A		R	+	-	-	+	+	+	+
16AX25		R	-	+	+	+	+	+	+
P130		R	+	+	-	+	+	+	+
9661		S	-	-	-	-	-	-	-

#### Results of Marker Used for Orab-vl-8

##### ORS683 Marker

It is known that the ORS683 marker is 1.5cM away from the Orab-vl-8 gene region. As a result of the study conducted with this marker, no discrimination was observed in wild sunflower genotypes, hybrid varieties and individuals known to be resistant or sensitive to blight.

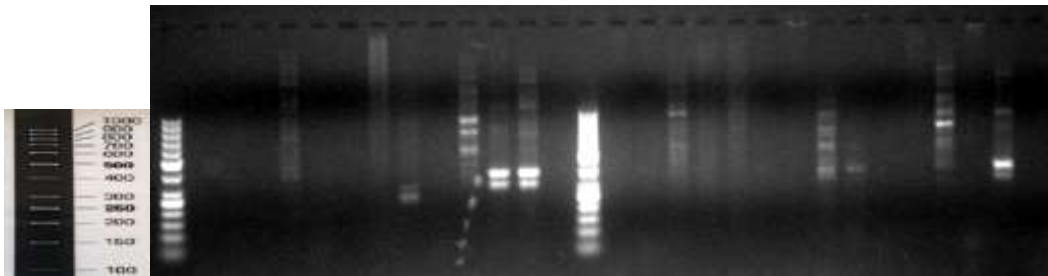


Figure 12. Gel electrophoresis image of PCR result using ORS683 marker

## CONCLUSIONS

When it comes to oilseed crops in our country, the first plant that comes to mind is sunflower, most of which is grown in the Thrace region. However, since it does not meet the needs of the consumer society due to efficiency losses and environmental reasons in production, this production gap is tried to be eliminated by importing. The most important biotic factor affecting the quality of sunflower and causing yield loss reaching 100% is seen as the borer parasite. This parasitic plant breaks the resistance mechanism created against it by creating new races in our country, the Balkans and Europe. Orobanche seeds spread quickly because they are numerous and small. One of the most important goals of sunflower breeding programs is; ensuring sustainable and effective broomstick resistance (Fernandez-Martinez et al., 2010; Kaya et al., 2012b). At the beginning of the 20th century, interspecific hybrids were created using classical breeding methods and began to be used for certain purposes (Satziperov, 1916). Later studies showed that the new sunflower forms resulting from the hybridization of wild sunflower species

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with cultivated sunflowers were resistant to orobans and different diseases (Pustovoit, 1960; Škorić, 1985). Wild sunflowers are of great importance at this point. It is known that wild sunflower species have a gene source that is resistant to new races of broomrape. It is known that the detection of wild species resistant to *Orobansha* is unreliable due to the fact that it takes a long time, requires intensive labor and is related to environmental conditions. In addition, it is not always possible to find the source and factors of the disease. However, determining whether the genotypes existing today contain genes for resistance to these diseases and blight; In addition, the way to understand whether they are dominant or not is through molecular markers, etc. The key is to use biotechnological methods effectively. Recently, great efforts have been made, especially by private sector organizations, to develop sunflower varieties that are resistant to the borer parasite and herbicide. For this, inbred parental lines with different genetic resources are needed. These different genes and these new gene resources existing in annual or perennial wild sunflower species need to be transferred to the cultivated annual *Helianthus annuus* L. species. However, this transfer is possible through interspecies hybridization and is extremely difficult to achieve using classical methods. However, it is possible to carry out this gene transfer effectively and economically in a shorter time with MAS, which increases the success of classical breeding studies and plays an auxiliary and complementary role in these studies.

In this study, disease tests were carried out using wild sunflower species and genetic plant materials obtained from TTAE, as well as parasite seeds known to contain the latest races of broomrape collected by TTAE from different regions of Thrace. The disease test was carried out to confirm the accuracy of the markers that were found to be able to discriminate at the molecular level at the end of the study. After the disease test, sensitive and resistant genotypes were determined and hybrid varieties with or without Or genes and wild sunflower species were screened with markers known to be in the LG3 region. The aims of the study are to understand the marker-gene relationship by detecting resistance to the Barrowworm parasite at the molecular level and to contribute to MAS studies. When we look at the molecular marker studies on the blowfish parasite, it is seen that the studies are mostly related to the Or5 gene, which is the resistance gene (Kaya et al., 2012).

It is known that the markers planned to be used to determine orobanche resistant sunflower genotypes are markers related to the Or5 gene, which provides resistance in races A to E (Radwan et al., 2009). Or5 is one of several alleles or tightly linked orobanche resistance loci (Or1-Or5) located at the upper end of LG3. It was observed that the RGA172, RGA192, RGA181 markers identified from *H. tuberosus* and the RGA206 marker identified from wild *H. annuus* were not completely separated by Or5. RGA181, the marker we used in our study, is 2.6 cM away from the Or5 locus, while the RGA172 marker is 7.5 cM away from the Or5 locus. RGA206 and RGA192 markers were not included in the study because they were far from the locus (33 cM). Amino acid sequence analysis of RGA markers reveals that they are members of the TIR-NBS-LRR subfamily of R genes. In the article by Radwan et al., (2009); He stated that known resistance genes located on LG3 can distinguish NBS-LRR genes. It has been reported that these NBS-LRR genes play a role in triggering host defense responses and recognition of the broomrape by encoding R proteins that confer resistance specific to the broomrape races.

Since linkage group 3 (LG3) is a region rich in resistance genes (Imerovski et al., 2013), in his study, he tested SSR markers from this linkage group to investigate whether these markers could be used to identify different Or genes. It was previously found that the ORS1114 marker may be useful for MAS (Iuoras et al., 2004). In the study of Iuoras et al. (2004), the LC1093 line used as a resistant source in hybrid varieties that are resistant or sensitive to blueberry contains the resistant allele in the Or5 locus. The ORS 1114 marker, matching 74.3 cM proximal to the Or5 locus, identified the 280 bp band in 56% of the genotypes considered resistant. The



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ORS 1036 marker, reported to map 7.5 cM proximal to the Or5 locus, 245-246 bp in 81% of the genotypes characterized as resistant. defined the band.

Imerovski et al. (2013) reported in their study that the ORS1114 marker did not correlate with Or5. In the study, a relationship was found between the ORS1114-260bp alleles of the Or2 gene, between the ORS1114-264bp, ORS665-281bp alleles of the Or4 gene, and a high rate of relationship was found between the ORS1114-265bp, ORS1036-240bp alleles of the Or6 gene. Again, ORS1036 marker is the closest SSR marker in the telomeric region of LG3, with a distance of 7.5 cM to Or5 (Tang and Knapp, 2003).

In this study, Imerovski et al. (2013) study, it is known that the ORS665 marker used produces a selective band in the 281bp region of the Or4 gene region. In the agarose gel electrophoresis images, it is seen that the PCR products work and the desired band formation is observed. 67 of the individuals tested for the disease were found to be resistant, and 281bp band formation was observed in 44 individuals. It was predicted that these 44 morphologically resistant individuals contained the Or4 resistance gene.

It has been reported that some perennial wild species and annual wild species *H. argophyllus*, *H. agrestis*, *H. anomalus* are resistant to blight (Fernández-Martínez et al., 2000). *H. exilis*, *H. cusickii*, *H. eggertii*, *H. strumosus*, *H. decapetalus*, *H. glaucophyllus*, *H. resinus*, *H. hirsutus*, *H. laevigatus*, *H. laetiflorus*, *H. tuberosus*, *H. smithii*, *H. divaricatus*, *H. simulans*, *H. pumilus* and *H. californicus* species are known to be resistant to blight. Again, *H. grosseserratus*, *H. giganteus*, *H. mollis*, *H. maximiliani*, *H. multiflorus* and *H. salicifolius* species are slightly affected by blight (Fernández-Martínez et al., 2008; Fernández-Martínez et al., 2000; Ruso et al., 1996).

According to the evaluation made based on gel images in the study conducted with ORS665 marker, *H. agrestis*, *H. eggertii*, *H. strumosus*, *H. hirsutus*, *H. tuberosus*, *H. smithii*, *H. divaricatus*, *H. simulans*, *H. grosseserratus*, *H. A* selective band formation of 281bp in size was observed in *H. giganteus* species. In the *H. mollis* wild type, one horn nodule formation was observed in the horn test tests, but a 281 bp selective band formation was also seen in the gel images. This shows that *H. mollis* is slightly affected by blight, as explained above. Although *H. maximiliani* from Kentucky, USA, appeared to be resistant in the test results, it did not show the formation of a 281bp selective band in the gel images. However, except for the species found in the state of North Dakota, USA (species found in Illinois, USA, Montana, USA, and Iowa, USA), selective band formation was also observed in the gel images of those that were found to be resistant as a result of the test. Based on the gel images, *H. cusickii*, *H. decapetalus* and *H. mollis* originating from Ohio, USA, *H. grosseserratus*, originating from Pennsylvania, USA, *H. maximiliani*, originating from Montana, USA, *H. nuttalli* subsp., originating from North Dakota, USA. *rydbergii*, *H. paradoxus* from New Mexico, USA, *H. pauciflorus* from New Jersey, USA, *H. winteri* from California, USA, and SLT\_009 x *H. deb-581*, SLT\_Ay\_146 x *H. bol-673141*, SLT\_009 x *H. annuus-9* It can be said that the genomic DNAs of LC1002 hybrids cannot be accessed.

It is known that ORS1036 and ORS1114 markers are related to the Or6 gene region. It forms a selective band in the region of ORS1036-240bp and ORS1114-265bp (Imerovski et al., 2013). In the agarose gel electrophoresis images, it is seen that the PCR products work and the desired band formation is observed. 68 of the individuals tested for the disease were found to be resistant. As a result of the study conducted with the ORS1036 marker, 41 of them gave results supporting this result, and it is thought that these 41 individuals contain the Or6 resistance gene. As for the ORS1114 marker, 52 of them gave correct results for the 265bp allele, so it is thought that these 52 individuals contain the Or6 resistance gene.

According to the evaluation made based on gel images in the study conducted with the ORS1036 marker, wild *H. annuus*, *H. decapetalus*, *H. divaricatus*, *H. eggertii*, *H. floridanus*, *H. laciniatus*, *H. nuttalli* subsp. 240bp selective band formation was observed in *H. nuttalli*, *H.*

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resinosus, *H. simulans*, *H. strumosus*, *H. tuberosus*, *H. maximiliani* species. It can be said that these species are related to the Or6 gene, which is resistant to the last races of the broomrape. In the study conducted with the ORS1114 marker, according to the evaluation based on gel images, wild *H. annuus*, *H. atrorubens*, *H. bolanderi*, *H. divaricatus*, *H. eggertii*, *H. floridanus*, *H. giganteus*, *H. heterophyllus*, *H. hirsutus*, *H. laciniatus*, *H. nuttallii*, *H. nuttallii* subsp. *nuttallii*, *H. occidentalis* subsp. 265bp selective band formation was observed in *plantagineus*, *H. porteri*, *H. resinosa*, *H. simulans*, *H. smithii*, *H. strumosus*, *H. tuberosus*, *H. maximiliani* species and it can be said that it is related to the Or6 resistance gene. *H. heterophyllus*, which did not show any orobanche nodule formation in disease tests, showed band formation only in gel electrophoresis with the ORS1114 marker.

It is known that the RGC172 marker is located 7.5 cM away from the Or5 gene region and produces selective bands of 550bp and 620bp (Radwan et al., 2009). In the agarose gel electrophoresis images, it is seen that the PCR products work and the desired band formation is observed. 68 of the individuals tested for the disease were found to be resistant. Since 45 of them gave confirmatory results for the 550bp allele of the RGC172 marker, it is thought that these 45 individuals contain the Or5 resistance gene. The 620bp DNA fragment of the RGC172 marker was observed in 44 individuals, and these 44 individuals are thought to contain the Or5 resistance gene.

According to the evaluation made based on gel images in the study conducted with the RGC172 marker, *H. atrorubens*, *H. bolanderi*, *H. divaricatus*, *H. eggertii*, *H. floridanus*, *H. hirsutus*, *H. laciniatus*, *H. nuttallii* subsp. were recorded as resistant in disease test studies. *H. nuttallii*, *H. occidentalis* subsp. *plantagineus*, *H. porteri*, *H. resinosa*, *H. simulans*, *H. smithii*, *H. strumosus*, *H. maximiliani* species and SLT\_009 x *H. arg\_1093*, SLT\_Ay\_146 x *H. arg17*, Solso x *H. winteri237*, SLT009 x *H. bol* Selective band formation was observed in both alleles in .28 hybrid varieties.

It is known that the RGC181 marker is located 2.6 cM away from the Or5 gene region and produces selective bands of 550bp and 600bp (Radwan et al., 2009). In the agarose gel electrophoresis images, it is seen that the PCR products work and the desired band formation is observed. 68 of the individuals tested for the disease were found to be resistant. Since 38 of them gave confirmatory results for both alleles of the RGC181 marker, these 38 individuals are thought to contain the Or5 resistance gene.

According to the evaluation made based on gel images in the study conducted with the RGC181 marker, *H. atrorubens*, *H. floridanus*, *H. giganteus*, *H. laciniatus*, *H. resinosa*, *H. simulans*, *H. strumosus*, *H. maximiliani* species were recorded as resistant in disease test studies. Selective band formation was observed in both alleles.

It has been reported that the ORS683 marker is 1.5cM away from the Orab-vl-8 gene region (Imerovski et al., 2016). As a result of the study conducted with this marker, it was observed that the PCR products did not work effectively and the desired band formation was not observed in the agarose gel electrophoresis images. No differentiation was observed in wild sunflower genotypes, hybrid varieties and individuals known to be resistant or susceptible to blight.

As a result, in this thesis study, it was aimed to obtain information about the markers associated with the resistance genes against blight resistance in wild species and hybrid varieties of sunflower, which is the most important oil crop in Turkey. Molecular screenings were performed on the genotypes tested for the disease to obtain information about the resistance of wild species and hybrid varieties to blight and to examine the potential of molecular markers to distinguish blight resistance genes. Molecular scans were used to determine whether these gene sources contain blight-resistant genes. However, the fact that the broomrape parasite used in disease tests was not a pure breed caused clear results not to be detected. The results were determined by taking into account that the orobanche seeds used in phenotypic analyzes contained the latest races in the region.

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In resistance breeding, molecular markers are a reliable, fast and accurate address since it takes a long time and is unreliable to phenotypically detect resistant varieties containing resistance to roobanche. In this regard, institutions and organizations carrying out breeding programs need to acquire sufficient knowledge and equipment for molecular studies and make investments or receive support in this regard. In order to carry out more effective resistance tests to sunflower, the blubber races in our country need to be distinguished and molecular markers specific to that race must be used.

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