II. RESISTANCE MECHANISMS MECHANISMS TO OROBANCHE CUMANA IN SUNNFLOWER

4th International Symposium
on Broomrape in Sunflower

RESISTANCE MECHANISMS TO *OROBANCHE CUMANA* WALLR. IN SUNFLOWER

Alberto MARTÍN-SANZ

Pioneer Hi-Bred Agro Servicios Spain SL, Sevilla, Spain
Corresponding author: alberto.martinsanz@pioneer.com

Abstract

Breeding for resistance is regarded as the most effective, feasible, and environmentally friendly solution to control sunflower broomrape. However, breeding for resistance is challenging as new races have evolved and the existing sources of genetic resistance are defeated. The use of resistant hybrids, of monogenic nature in many cases, is followed by the appearance of new more virulent races of the parasite that overcome the existing resistance genes. Because of this frequent development of more virulent races, it would be desirable to pay more attention to quantitative resistances and to accumulate different mechanism of resistances in a single sunflower hybrid, resulting in a resistance more likely to be durable. Hence, a detailed knowledge of sunflower–broomrape interaction and the mechanisms underlying resistance is mandatory to reach this goal. The *Orobanche cumana* biological cycle comprises welldefined steps, separated both spatially and temporally, that are potential targets for host defence strategies. Upon germination, stimulated by host root-exuded chemical signals, broomrape seed develops a small seedling that attaches to the host root and differentiates in the attachment organ (appressorium). After host tissue penetration and connection to the vascular system through the haustorium, the parasite becomes a major sink for plant photosynthates, gradually forming a tubercle from which a shoot arises to emerge from the soil to flower and produce seeds. A review of the different mechanism of resistance described so far in the different steps of the biological cycle will be presented. They can operate at the pre-attachment, pre-haustorial or post-haustorial stage of the host-parasite interaction. Practical examples about the combination of pre-haustorial and post-haustorial resistance in a single cultivar will be described as way to provide durable and sustainable genetic resistance.

Keywords: broomrape, genetic resistance, race, sunflower

SEED PRETREATMENT WITH BRASSINOLIDE INDUCES THE ANTIOXIDANT DEFENSE SYSTEM OF *HELIANTHUS ANNUUS* AGAINST SUNFLOWER BROOMRAPE INFECTION

Na ZHANG[1], Jiansu WANG[1], Luyang HU[1,2],
Wenjian SONG[1], Jinwen ZHU[3], Weijun ZHOU[1*]

[1]*Institute of Crop Science and Zhejiang Key Lab of Crop Germplasm, Zhejiang University, Hangzhou 310058, China*
[2]*Laboratory of Plant-Microorganism Interactions, University of Toulouse, INRA, CNRS, Castanet-Tolosan, France*
[3]*Department of Plant Protection and Ministry of Agriculture Key Lab of Molecular Biology of Crop Pathogens and Insects, Zhejiang University, Hangzhou 310058, China*
[*]*Corresponding author:* wjzhou@zju.edu.cn

Abstract

Sunflower (*Helianthus annuus*), an economically important crop species, can be specifically infected by the root holoparasitic angiosperm sunflower broomrape (*Orobanche cumana*), resulting in a severe growth retardation and yield loss, globally. This study was conducted to examine the protective effects of brassinolide (BR) application on the seeds of susceptible sunflower (cultivar TK0409) against *O. cumana* infestation. Sunflower seeds were primed with different concentrations of BR (0, 0.005, 0.05, 0.5 mg L^{-1}) for 24 hours. The primed seeds of sunflower were grown along with *O. cumana* for 4 weeks. Results showed that *O. cumana* infection contributed to an inhibition of plant growth, accompanied by notable chlorophyll loss and protein degradation. Furthermore, *O. cumana* infection induces oxidative stress by enhancing the production of reactive oxygen species (hydrogen peroxide and superoxide), which led to the lipid peroxidation and activation of antioxidant defense system. Enhanced expression of antioxidant enzymes (superoxide dismutase, peroxidase, ascorbate peroxidase, glutathione reductase) as well as their transcript levels under *O. cumana* infection were confirmed by quantitative Real-Time PCR (RT-qPCR) assays. Pretreatment of sunflower seeds with 0.05 mg L^{-1} BR significantly increased the full plant height (27.5%), fresh weight (63.1%) and dry weight (51.9%) compared with control, respectively. BR application also reduces the number and biomass of established *O. cumana*. Morphological observations, supported by ultrastructural analysis revealed exogenous application of BR significantly modified the damaged organelles caused by infection of *O. cumana*. The impairment in the photosynthetic efficiency affected by *O. cumana,* was significantly recovered with the application of 0.05 mg L^{-1} BR as compared with other BR treatments. The findings of the present study revealed that BR improves the plant growth and biomass, photosynthetic efficiency and antioxidant defense system against *O. cumana*-induced oxidative stress in the leaves and roots of susceptible sunflower (TK0409).

Keywords: *Orobanche cumana*, sunflower, brassinolide, antioxidants, gene expression

AUTOMATIC PHENOTYPING OF SUNFLOWER FOR THEIR RESISTANCE TO *OROBANCHE CUMANA* AT EARLY STAGES OF THE INTERACTION

Mireille CHABAUD[1*], Guillaume IBARCQ[1], Aurélie BAUSSART[2],
Marie-Claude BONIFACE[1], Nicolas POUILLY[1],
Nicolas LANGLADE[1], Stéphane MUNOS[1]

[1]*LIPM, Université de Toulouse, INRA, CNRS, Castanet-Tolosan, France*
[2]*Maïsadour Semences, Haut-Mauco, France*
[*]*Corresponding author: mireille.chabaud@inra.fr*

Abstract

Orobanche cumana (the sunflower broomrape) is a parasitic plant that specifically infects sunflower and is one of the main constraint of sunflower crop in Europe. Quick and high throughput phenotyping of sunflowers for their resistance to broomrape is a challenge for breeding varieties with new resistances. Various biological mechanisms, such as the fixation of *O.cumana* on sunflower roots before emerging overground as a flower shoot and the fact that *O. cumana* populations differ by their virulence and aggressiveness depending on their geographical locations make high throughput phenotyping difficult in field. Therefore, developing new phenotyping tools in controlled conditions is a good alternative to better characterize the physiological effect of the infection on sunflower development and to screen for resistant sunflowers at various stages. A collaborative project between Maïsadour Semences and INRA (LIPM-Toulouse) has been set up with the objective to develop a mini-rhizotron-based phenotyping system to access to sunflower roots infected by the parasitic plant at early stages. We will present biological and technical optimization of this growth culture system such as the nutritive solution, the pre-conditioning of the *O. cumana* seeds before inoculation and the type of solid substrate used in the rhizotron. With the goal of a high through put system, we are developing an automatic image analysis tool to accelerate sunflower genotype screening for resistance at early stages.

Keywords: screening, rhizotron, image analysis

PHYSIOLOGICAL PARTICULARITIES OF SUNFLOWER LINES AND HYBRIDS RESISTANCE TO BROOMRAPE

Tamara SAKHNO *, Vera PETRENKOVA, Yaroslava SHARYPINA, Irina BOROVSKAYA, Ekaterina MAKLIAK

Yuriev Plant Production Institute of NAAS, Ukraine

**Corresponding author: tamara.sakhno@gmail.com*

ABSTRACT

Physiological and biochemical processes that occur at the initial stages of sunflower infection with broomrape and cause its resistance to the parasite still extremely poorly investigated. However, such knowledge is very important for describing the biological nature of plant resistance to a flowering pest – broomrape – and for developing methods of its determination. In literature there are only separate reports about the connection between oxidoreductases activity and intensity of phenolic compounds synthesis with sunflower infection by pathogenic fungi.

The investigation aimed to study the morphogenesis, oxidases activity and phenolic compounds content in sunflower genotypes during infection with broomrape (*Orobanche cumana* Wallr.). It is shown that sunflower plants growth was inhibited by infection with broomrape. However, it doesn't affect the morphological parameters of resistant sunflower genotypes. It has been established that resistant and susceptible genotypes differ by phenols level in plants under the broomrape influence. Phenols level in resistant samples was higher by 1,5-3 times than in susceptible ones. It was found that inoculation of sunflower plants with broomrape significantly affects the enzymes activity. The clearest patterns were observed on the 14th day after infection, indicating that it is expedient to conduct further studies precisely in this phase. It was determined that polyphenoloxidase and catalase activity in leaves of control plants is on the same level as in the resistant standard sample (71,3 units/g tissue and 5,6 units/g tissue respectively), and at the same time exceed the susceptible line-standard parameters by 1,5-3 times. This may indicate a potential resistance to broomrape.

Key words: *sunflower, broomrape, morphometric parameters, phenolic compounds, enzyme activity*

INTRODUCTION

Now we have clarified the important aspects of the morphological features of *Orobanche cumana* Wallr. (Păcureanu-Joita et al., 2008; Antonova et al., 2009). Sunflower genes of resistance to broomrape were identified, molecular genetic methods for their labeling were developed as well (Molinero-Ruiz, 2005; Atanasova et al., 2005; Imerovski, 2011; Guchetl et al., 2011). At the same time, physiological and biochemical processes that occur at the initial stages of infection of sunflower with broomrape and cause its resistance to the parasite remain extremely poorly investigated. However, such data are very important for deepening existing ideas about the biological nature of plant resistance to a parasite and for developing methods of its determination.

In literature there are only separate reports about the connection between the oxidoreductases activity and the intensity of phenolic compounds synthesis with the infection of sunflower by pathogenic fungi (Luhová, 2003; Hermandez et al., 2006; Buze-Dragomir, 2010) and broomrape (Panchenko, 1975).

Proceeding from the established protective role of oxidoreductases (Bindschedler et al., 2001; Glyan'ko, 2009) and phenol compounds (Voytsehivska, 2015) in the plant biotic stress, we assume that characteristics of changes in phenolic compounds biosynthesis and oxidases activity during sunflower infection by broomrape, can be useful in understanding of parasite resistance developing processes. The investigation aimed to study the morphogenesis, oxidases activity and phenol compounds content in sunflower genotypes during infection with broomrape (*Orobanche cumana* Wallr.).

MATERIAL AND METHODS

Sunflower fertile male lines, sterile female lines and hybrids created on their basis with different resistance level to broomrape, breeded in Yuriev Plant Production Institute of NAAS (Ukraine), were used as research material. The sterile female line Cx 908 A was used as a susceptible standard, the hybrid PR64A71 (Pioneer DuPont) - as a resistant standard. Specific differentiators AD 66 (susceptible), Record (race C), LC 1003 (race E), LC 1093 (race F), PR64A71 (race G) were used as model objects to determine the sunflower vegetative phase when broomrape infestation occurs.

All sunflower samples were grown in vegetation containers in stable greenhouse conditions. Each sample plants were grown in two variants: control without broomrape infestation and infected ones, which were planted along with the broomrape seeds at the rate of 1 g per 5 kg of soil (Panchenko, 1975). Broomrape seeds were collected on the Kharkov region and the Donetsk region territory, where the broomrape population was characterized as high aggressive and virulent (Makliak, 2012; Hablak, 2013).

The total phenol content in plant tissues was determined on a ULAB spectrophotometer 101 at 760 nm (Priecina, 2013), enzyme activity was determined in leaves and roots spectrophotometrically as well - at 420 nm for polyphenoloxidase, 470 nm for peroxidase (Ermakov, 1987) and 405 nm for catalase (Luhová et al., 2003).

RESULTS AND DISCUSSION

It was shown that infection with broomrape inhibited the growth and development of sunflower plants. However, it doesn’t affect the morphological indicators of resistant sunflower genotypes (Sakhno, 2016, Kharkiv).

The results of conducted investigation showed significant broomrape infection effect on phenol content in sunflower material.

When infected by broomrape, the phenol level in all sunflower male lines increased significantly (Table 1). Sterile female lines showed a slight excess of the phenol compounds level in infected plants comparing to control. At the same time, the resistant standard indices increased by 31% and were 2-3 times higher than the susceptible (Cx 908 A) ones. According to the results of the phenol content determination in all hybrids (Table 2), it was noted its increasing in leaves (by 10-30%) and roots (2-3 times) of infected plants versus the control ones. While, the phenols level in the susceptible line Cx 908 A was decreasing in infected plants leaves (by 4%) and in roots (almost in 3 times) in comparison with the control.

Thus, the results indicate that the phenol compounds content in sunflower roots and leaves depends on the resistance level of genotype to flowering parasite, which is particularly clearly manifested in conditions of infestation by broomrape.

The activity of polyphenoloxidase (Fig.1), peroxidase (Fig. 2) and catalase (Fig.3) in 10-, 14- and 18-day seedlings of sunflower resistance differentiators to broomrape was studied. When infected by broomrape, it was observed a significant increase of polyphenoloxidase activity in 14-day-old sunflower seedlings and its further decrease in 18-day-old seedlings both in leaves and roots.

According to the analysis of peroxidase activity dynamics (Fig.2) in the leaves and roots of the differentiators with broomrape infestation, it was established a gradual enzyme activity increase in the susceptible line AD 66 and in the LC 1003 during the vegetation, while in the LC 1093 and Record a peak of peroxidase activity was shown on 14th vegetation day, and the decline - on day 18.

Regarding the catalase activity dynamics (Fig. 3) in the leaves of sunflower differentiators infected by *Orobanche cumana* Wallr., it was found that the catalase activity decreases in most cases, except Record, on the 14th vegetation day, and further the enzyme activity rises to the initial level on the 18th day. The Record showed a slight increase in the enzyme activity of the on day 14 and its decrease by day 18. In roots of all differentiators infected by broomrape, a significant catalase activity increase on 14th vegetation day and its further decline on 18th day was observed.

Thus, according to the study's results of oxidases activity in the differentiators of resistance to flowering parasite (Sakhno, 2016, Sumy), it was established that significant changes in enzymatic activity in response to parasite action occur on the 14th vegetation day after sunflower plants infection by broomrape, which, in turn, confirms histological studies (Antonova, 1978) and indicates penetration *Orobanche cumana* Wallr. in sunflower roots right in this phase.

Differences between resistant and susceptible sunflower genotypes have been shown by the oxidase enzymes activity indices (Sakhno T., Petrenkova V., 2017).

The polyphenoloxidase activity in leaves and roots of sunflower plants infected by broomrape was studied. There was a tendency of enzyme activity decreasing in most sunflower lines and the susceptible standard (by 2-15 % compared to control plants). At the same time, indices of the resistant standard and hybrids increased in comparison with control by 8 % and 4-6 % respectively (Table 3, 4).

Analyzing the peroxidase activity in sunflower lines and hybrids infected by broomrape, a strong indices variability and a general tendency of enzyme activity declining were discovered.

Studying catalase activity in sunflower material affected by broomrape, it was disclosed a general tendency of its rising. The catalase activity in leaves (Table 3) of the infected plants increased in most cases versus control ones (fertile male lines - by 26-85 %, sterile female lines - by 33-130 %, hybrids - by 15-25 %). The resistant and the susceptible standard enzyme activity indices increased by 16 % and 47 % respectively comparing with control plants. However, catalase activity significantly varied in roots (Table 4). In general, there was a tendency of its declining in the majority of sunflower lines comparing with control, whereas catalase activity in hybrids and standards increased almost by two times versus control.

CONCLUSIONS

It was proved that the sunflower plants infestation by broomrape significantly affects the phenol compounds content and enzymes activity in the material. The phenol compounds level increases both in leaves and roots of infected sunflower plants, except some samples, which phenol level decreased as well as in the susceptible standard. It has been established that resistant and susceptible sunflower genotypes differ in the phenol level in plants infected by broomrape. Phenols level in resistant samples was higher by 1,5-3 times than in susceptible ones. The clearest patterns of

enzymes activity were observed on the 14th vegetation day, indicating that it is expedient to conduct further studies precisely in this phase. It was determined that polyphenoloxidase and catalase activity in leaves of control plants is on the same level as in the resistant standard sample (71,3 units/g tissue and 5,6 units/g tissue respectively), and at the same time exceed the susceptible standard parameters by 1,5-3 times. This may indicate a potential resistance to broomrape. Analyzing oxidase activity in sunflower plants infected by broomrape it was discovered the general tendency of decreasing polyphenoloxidase and peroxidase activity and increasing of catalase activity in comparing with control plants.

REFERENCES

1. Antonova T. S., Sitalo G. M., Araslanova N. M.,Guchetl S. Z., Ramazanova S. A. Chelyustnikova T. A. Distribution and virulence of broomrape (*Orobanche cumana* Wallr.) on sunflower in the Rostov region. Oil crops. Scientific and technical bulletin of the All-Russian Research Institute of Oil Crops. Issue 1 (140), 2009. P. 51–60.
2. Antonova T. S. Development of broomrape haustorium (*Orobanche cumana* Wallr.) and protective reaction in immune forms of sunflower. Ph.D thesis in biological sciences. Krasnodar, 1978. 146 p.
3. Atanasova R., Batcharova R., Todorovska E. and Atanassov A. Molecular study of broomrape (*Orobanche Spp.)* by RAPD analyses. Biotechol. and Bionechnol. Eq. 2005. V. 19 (3). P. 51–60.
4. Bindschedler L.V., Minibayeva F., Gardner S. L. et al. Early signaling events in the apoplastic oxidative burst in suspension cultured French bean cells involve cAMP and Ca^{2+}. New Phytol. 2001.V. 151, № 1. P. 185-194.
5. Buze-Dragomir L., Niculescu M. Researches on the catalase and Peroxidase activity at sunflower plants, infected by phytopatogenic fungi. Annals of the University of Craiova. 2010. Vol.15. P.1–8.
6. Ermakov A. I. Methods of biochemical plant investigation. Leningrad: Kolos, 1987. 430 p.
7. Glyan'ko A. K., Ischenko O. O., Mitanova N. B., Vasil'eva G. G. Plants NADPH-oxidase. KhNAU Bulletin. Series "Biology". 2009. Issue 2 (17). P. 6–18.
8. Guchetl S. Z., Chelyustnikova T. A., Ramazanova S. A. Interpopulation variability of *Orobanche cumana* Wallr., infecting sunflower in the south regions of Russia, revealed by molecular-genetic markers. Oil crops. Scientific and technical bulletin of the All-Russian Research Institute of Oil Crops. 2011. Issue 1 (146–147). P. 119–122.
9. Hablak S. G., Abdullaeva Ya. A. The racial composition of broomrape (*Orobanche cumana* Wallr.) in sunflower crops on the Northern steppes of Ukraine. Ukrainian Black Sea region agrarian science journal. 2013. Issue 3 (73). P.116-121.
10. Hermandez J. A., Diaz-Vivancos P., Rubio M. et al. Long-term plum pox virus infection produces an oxidative stress in a susceptible apricot, Prunus armeniaca, ucltivar but not in a resistant cultivar. Physiologa Plantarum. 2006. V. 126. Is.1. P.140–152.
11. Imerovski I., Dimitrijevic A., Miladinovic D., Dedic D., Jocic S., Miklic V. Molecular profiles of sunflower lines resistant to broomrape (*Orobanche cumana* Wallr.). International Symposium on Broomrape (*Orobanche spp*.) in Sunflower. Chisinau, Republic of Moldova. August 25-27. 2011. P. 25.
12. Luhová L., Lebeda A., Hedererová D. et al. Activities of amine oxidase, peroxidase and catalase in seedlings of *Pisum sativum* L. under different light conditions. Plant Soil Environ. 2003. Vol. 49. №4. P.151–157.
13. Makliak K. N., Kyrychenko V. V. Resistance of sunflower source material to new broomrape races (*Orobanche cumana* Wallr.). Selection and seed industry, 2012. Issue 102. P. 16–21.
14. Molinero-Ruiz M. L. and Melero-Vara J. M. Virulence and aggressiveness of sunflower

broomrape (*Orobanche cumana*) populations overcoming the Or5 gene.In: G.J. Seiler, (ed), Proc. Int. Sunflower Conf., Far go, ND, USA. 2005. P. 165–169.

15. Pãcureanu-Joita Maria, Raranciuc Steluta, Procopovici Emilia, Sava Elisabeta, Nastase Dumitru. The impact of the new races of broomrape (*Orobanche cumana* Wallr.) parasite in sunflower crop in Romania. Proc. 17th International Sunflower Conference. Cordoba. Spain. 2008. V.1. P. 225–230.

16. Panchenko A. Ya. Early broomrape resistance diagnosis in sunflower breeding and improving seed production. Bulletin of Agricultural Science. 1975. № 2. P.107–115.

17. Priecina Liga, Karlina Daina. Total polyphenol, flavonoid content and antiradical activity of dried parsley (*Petroselinum crispum*), celery (*Apium graveolens*) and dill (*Anethum graveolens* L.). Journal of international scientific publications: Agriculture and Food. Vol. 1. Part 1. 2013. P. 279–286.

18. Sakhno T. V. Morphometric indices and phenol content in sunflower lines and hybrids infected by broomrape. Selection and seed industry. Issue 110. № 15. Kharkiv. 2016. P. 117-122.

19. Sakhno T. V. Dynamics of redox enzymes activity in sunflower differentiators infected by broomrape. SNAU Bulletin. Series "Agronomy and Biology". V. 9 (32). Sumy, 2016. P. 3-9.

20. Sakhno T. V., Petrenkova V. P. Activity of redox enzymes in sunflower lines and hybrids infected by broomrape. Uman National University of horticulture bulletin. Series “Agricultural sciences”. V. 90 (1). Uman, 2017. P. 112-121.

21. Voytsehivska O. V., Sitar O. V., Taran N. Yu. Phenolic compounds: diversity, biological activity, prospects of using. KhNAU Bulletin. Series “Biology”. 2015. Issue 1 (34). C. 104–119.

Table 1. Total phenols content in leaves and roots of sunflower lines infected by broomrape

Line	Phenols content, mg/100 g dry weight			
	in leaves		in roots	
	control	infected	control	infected
X 114 B	567,0*±5,6	1148,8*±8,7	147,0*±7,1	403,2*±4,6
X 526 B	882,0*±7,6	1300,2*±8,8	229,1*±6,0	252,0*±4,5
X 711 B	478,8*±4,9	819,0*±6,9	214,2*±5,7	579,6*±5,3
X 720 B	163,8*±4,6	945,0*±5,8	31,5*±3,4	850,5*±5,8
X 762 B	667,8±6,8	730,8*±7,3	162,8*±5,1	277,2*±3,7
Cx 1002 A	730,8*±8,6	787,5*±8,5	162,0*±4,8	163,8±3,2
Cx 1006 A	655,2±5,9	844,2*±7,8	113,4*±3,9	239,4*±4,2
Cx 1010 A	1071,0*±7,9	1073,9*±6,6	420,0*±6,1	340,2*±3,1
Cx 1012 A	982,8*±8,5	1211,5*±7,4	280,0*±4,2	157,5±2,8
Cx 503 A	655,2±7,4	781,2*±6,9	88,2*±4,0	333,5*±4,1
Cx 2111A	840,0*±6,8	570,0*±5,7	75,0*±3,7	177,7±3,3
Cx 4021 A	1096,2*±9,1	1348,2*±7,8	264,6*±5,2	718,2*±5,3
PR64A71	**945,0*±5,4**	**1234,8*±9,3**	**189,0*±6,1**	**667,8*±6,6**
Cx 908 A	**680,4±4,8**	**655,2±5,2**	**403,2±3,9**	**138,6±3,7**

Note. * - the difference with the susceptible standard indices is significant at P≤0.05

Table 2. Total phenols content in leaves and roots of sunflower hybrids infected by broomrape

Hybrid	Phenols content, mg/100 g dry weight			
	in leaves		in roots	
	control	infected	control	infected
Borey (Cx 503 A/X 114 B)	579,6*±5,8	1021,0*±6,6	189,0*±5,8	825,0*±6,5
Kiy (Cx 908 A/X 762 B)	768,6*±7,2	830,5*±7,2	252,0*±4,7	1386,0*±7,9
Oskil (Cx 1006 A/X 720 B)	712,2*±7,3	821,7*±5,4	151,2*±5,4	1260,0*±7,7
Sayt (Cx 1012 A/X 526 B)	592,2*±6,8	1239,0*±6,5	88,2*±3,7	151,2±5,1
Poglyad (Cx 2111 A/X 711 B)	655,2±7,1	844,2*±6,8	37,2*±3,2	403,2*±4,6
Svitoch (Cx 1006 A/X 711 B)	693,0±6,2	705,6*±4,9	163,8*±5,4	264,5*±4,4

PR64A71	**945,0*±5,4**	**1234,8*±9,3**	**189,0*±6,1**	**667,8*±6,6**
Cx 908 A	**680,4±4,8**	**655,2±5,2**	**403,2±3,9**	**138,6±3,7**

Table 3. Oxidase activity in leaves of sunflower genotypes infected by broomrape, units/g tissue.

Sample	Poliphenoloxidase activity		Peroxidase activity		Catalase activity	
	control	infected	control	infected	control	infected
Sterile female lines						
Cx 503 A	76,3±2,4*	78,7±0,8*	10,1±1,3*	12,4±0,7*	6,3±1,4*	8,8±0,5*
Cx 1002 A	79,1±2,4*	80,2±0,9*	13,6±1,6*	17,1±1,1*	4,0±0,5*	4,0±0,8*
Cx 1006 A	82,2±2,4*	73,5±1,2*	19,1±1,4	24,4±1,2*	2,6±0,4	6,0±0,7*
Cx 1010A	77,2±2,2*	73,9±1,3*	9,4±0,8*	8,8±0,6	3,5±0,6*	3,6±0,6
Cx 1012 A	70,0±3,0	73,5±1,2*	15,6±1,7	15,1±1,5*	2,7±0,6	3,6±0,7
Cx 2111 A	69,1±1,3	83,5±2,1*	7,7±0,5*	7,7±1,0	3,4±0,6*	5,8±0,6*
Cx 4021 A	75,6±3,0*	78,7±0,8*	11,1±0,7*	13,6±0,8*	8,8±0,7*	8,2±1,1*
Fertile male lines						
X 114 B	72,0±3,3	69,4±2,3*	9,2±1,0*	31,5±1,6*	3,4±0,5	4,3±0,5*
X 711 B	72,0±3,3	70,2±3,0*	28,3±3,6*	20,4±1,1*	2,8±0,4	1,3±0,2*
X 720 B	78,0±1,4*	71,4±2,7*	20,5±2,4	13,3±0,6*	7,1±1,3*	8,0±0,6*
X 762 B	71,3±4,7	76,3±2,4*	8,3±1,1*	7,0±0,6*	7,7±2,0*	4,5±0,6*
X 526 B	73,0±5,0	70,5±1,8*	5,7±1,0*	7,5±0,8*	1,9±0,3	3,5±0,4
Hybrids						
Borey	78,7±0,8*	81,6±1,5*	20,1±1,2	16,1±0,8*	3,2±0,4*	2,7±0,5
Kiy	80,2±0,9*	85,4±0,9*	7,0±1,6*	19,6±1,1*	4,6±0,5*	5,6±0,7*
Oskil	73,9±1,3*	76,7±0,8*	19,2±7,3	13,7±1,1*	3,1±0,3*	3,5±0,5
Sayt	73,5±1,2*	75,5±1,6*	8,1±0,9*	15,4±1,0*	6,6±0,4*	4,9±0,5*
Poglyad	83,5±2,1*	87,8±1,2*	13,9±2,4*	16,2±1,4*	6,2±0,5*	6,1±0,6*
Svitoch	83,5±2,1*	87,8±1,2*	18,5±1,3	17,7±1,1*	5,9±0,4*	5,3±0,5*
PR64A71	**71,3±2,3***	**77,3±1,5***	**12,8±2,9***	**11,7±0,8***	**5,6±0,6***	**6,5±0,7***
Cx 908 A	**66,9±3,1**	**56,7±2,4**	**17,6±2,4**	**8,7±0,7**	**2,1±0,4**	**3,1±0,5**

Table 4. Oxidase activity in roots of sunflower genotypes infected by broomrape, units/g tissue.

Sample	Poliphenoloxidase activity		Peroxidase activity		Catalase activity	
	control	infected	control	infected	control	infected
Sterile female lines						
Cx 503 A	29,9±3,1*	43,5±2,7	28,1±1,4*	12,8±1,2*	1,5±0,3*	2,2±0,3*
Cx 1002 A	31,4±2,2*	29,8±2,0*	39,8±1,88	37,0±2,0*	3,2±0,3	2,0±0,3*
Cx 1006 A	33,5±2,1*	29,2±1,7*	41,5±1,9*	39,8±1,8*	3,5±0,5	2,3±0,4*
Cx 1010A	32,7±1,7*	28,4±1,4*	39,7±1,5*	39,5±1,8*	2,5±0,4*	2,3±0,4*
Cx 1012 A	31,4±1,6*	31,6±1,1*	41,0±1,5*	38,5±1,7*	2,8±0,4	2,6±0,4*
Cx 2111 A	29,5±1,2*	27,4±1,5*	39,8±1,4*	38,2±1,6*	3,1±0,5	2,5±0,4*
Cx 4021 A	36,6±1,8*	27,0±1,5*	45,3±2,1*	42,2±1,9*	2,7±0,3	2,7±0,3*
Fertile male lines						
X 114 B	20,1±1,6*	19,5±1,3*	24,8±1,3*	36,6±1,4*	2,3±0,3*	3,6±0,4*
X 711 B	18,6±1,4*	12,7±1,1*	21,1±1,1*	24,5±1,1*	4,1±0,5	2,5±0,3*
X 720 B	27,8±1,2*	16,7±1,1*	45,5±2,0*	27,5±1,0	4,8±0,4*	3,0±0,3*
X 762 B	38,9±2,2*	16,2±1,0*	38,5±1,4	18,9±0,9*	2,8±0,3	1,9±0,2*
X 526 B	26,8±1,0*	11,8±0,8*	32,5±1,2	17,6±0,8*	3,7±0,3	3,6±0,3*
Hybrids						
Borey	29,9±1,5*	40,9±2,1	50,6±3,1*	73,2±3,7*	3,3±0,4	5,7±0,5
Kiy	33,3±2,6*	41,3±3,3	42,2±2,1*	47,7±1,7*	2,6±0,3	3,7±0,4*
Oskil	33,7±1,8*	39,7±1,8	46,6±0,9*	41,2±0,6*	3,4±0,4	4,7±0,5*
Sayt	30,4±1,9*	43,3±2,5	44,8±1,8*	55,9±2,7*	2,5±0,3*	3,5±0,4*
Poglyad	38,8±2,4*	41,9±1,9	51,4±1,7*	53,5±0,9*	2,3±0,3*	8,8±0,6*
Svitoch	33,5±1,7*	46,9±1,5*	45,6±1,7*	47,9±1,5*	2,6±0,3	5,8±0,5
PR64A71	**35,7±1,2***	**38,9±1,4**	**41,5±1,3***	**42,3±1,1***	**3,7±0,6**	**6,8±0,5**

Cx 908 A	**44,1±0,9**	**41,9±1,2**	**35,4±0,9**	**27,1±0,7**	**3,4±0,4**	**6,0±0,6**

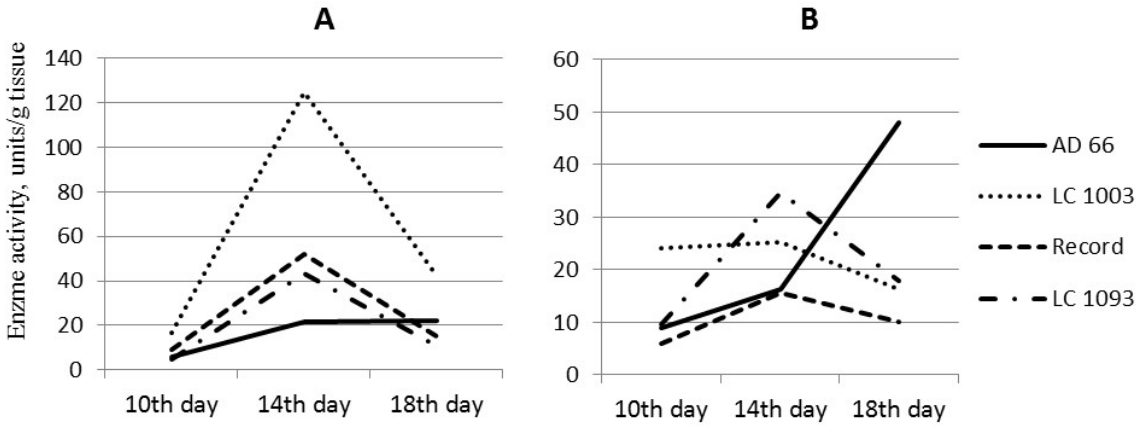

Fig. 1. Polyphenoloxidase activity dynamics in leaves (A) and roots (B) of sunflower resistance differentiators infected by broomrape

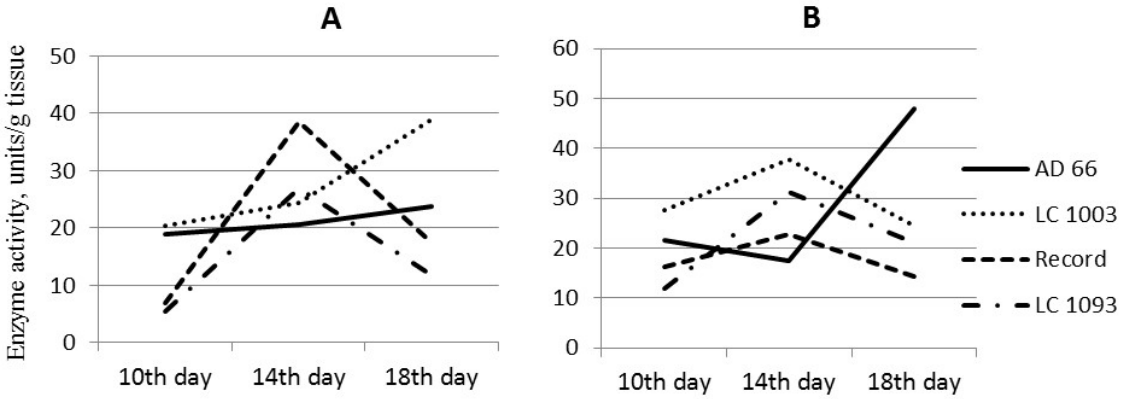

Fig. 2. Peroxidase activity dynamics in leaves (A) and roots (B) of sunflower resistance differentiators infected by broomrape

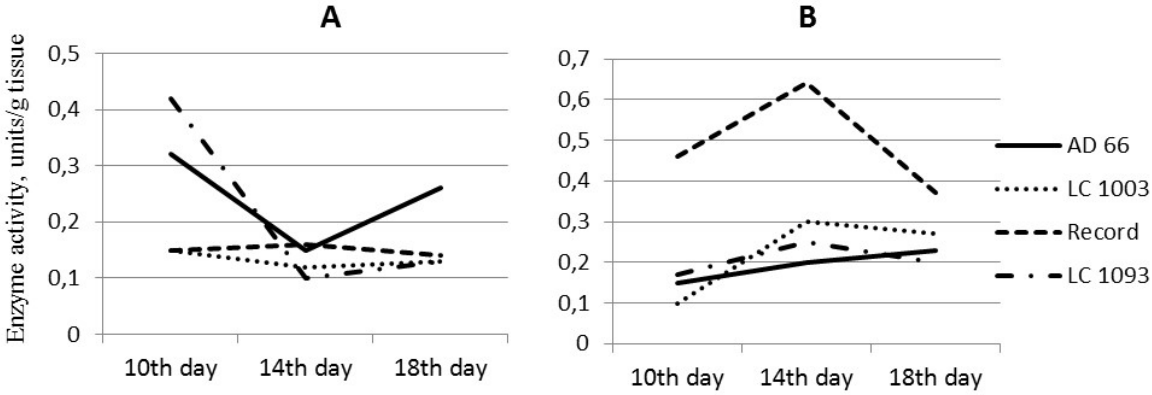

Fig. 3. Catalase activity dynamics in leaves (A) and roots (B) of sunflower resistance differentiators infected by broomrape

PRE-HAUSTORIAL AND POST-HAUSTORIAL RESISTANCE OF SUNFLOWER INFECTED WITH BROOMRAPE

Olesea TABARA*, Angela PORT, Maria DUCA

State University "Dimitrie Cantemir", Chişinău, Republic of Moldova
**Corresponding author: teolesea@gmail.com*

Abstract

The parasitic weed *Orobanche cumana* Wallr. is an obligatory and non-photosynthetic root parasitic plant of the sunflower. During the broomrape life cycle, several resistance mechanisms of the parasite-host interaction have been reported. The studies were focused on the transcriptional activity of some genes involved in reinforcement of cell walls and oxidative stress correlated with the analysis of three enzymes activity and histochemical investigations of root in sunflower resistant (Favorit and PR64LE20) and susceptible (Performer) genotypes at post-attachment and post-haustorial resistance mechanisms.

The experiences have been realized in pots with sterilized soil – uninfected and artificial infected with broomrape seeds. The biologic material was collected in temporal dynamics during the four development phases (attachments, tubercles, undergrounds and flowering shoots) of pathosystem, during the 67 days. The deposition of callose and lignin was analyzed by histochemical methods. The enzymes activity was measured spectrophotometrically and transcript accumulation of genes was assayed by real-time PCR using specific primers.

The comparative analysis of two experimental models in the background of infection: pathogen-host incompatibility (Favorit, PR64LE20 - uninfected) and pathosystem (Performer - infected) was revealed different expression profiles for the same physiological reaction (resistance). In the incompatible combination of the hybrid Favorit - *O. cumana* the biological response to stress in the background of infection beginning with 35 days after cultivation through increasing of lignin and callose content, genes expression and PAL activity. The second resistant genotype PR64LE20 has displayed a defense response at the last experimental stage 67 days by accumulation of compounds, the high mRNA synthesis of the eight genes and the PAL activity. The pre-haustorial resistance in the case of pathosystem Performer - *O. cumana* was poorly manifested. Significant intensification of the stress state was manifested at the period of underground stems and flowering shoots resulting of post-haustorial mechanism activation.

Keywords: *Orobanche cumana*, resistance mechanisms, sunflower