PHYSIOLOGICAL PARTICULARITIES OF SUNFLOWER LINES AND HYBRIDS RESISTANCE TO BROOMRAPE

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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.

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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**

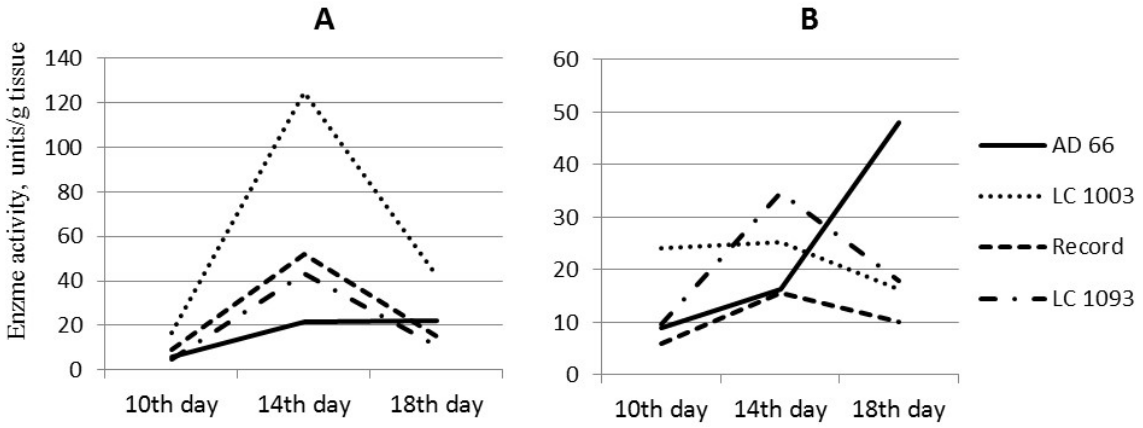

Fig. 1. Polyphenoloxidase activity dynamics in leaves (A) and roots (B) of sunflower resistance differentiators infected by broomrape

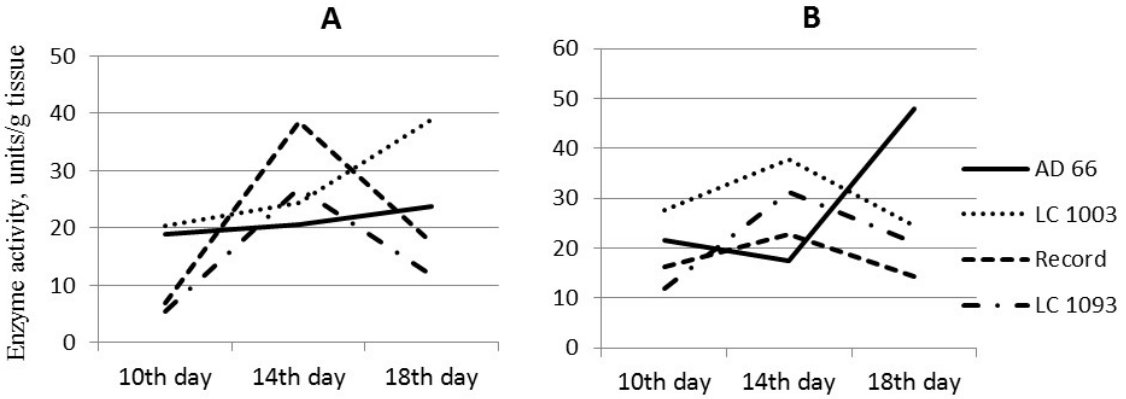

Fig. 2. Peroxidase activity dynamics in leaves (A) and roots (B) of sunflower resistance differentiators infected by broomrape

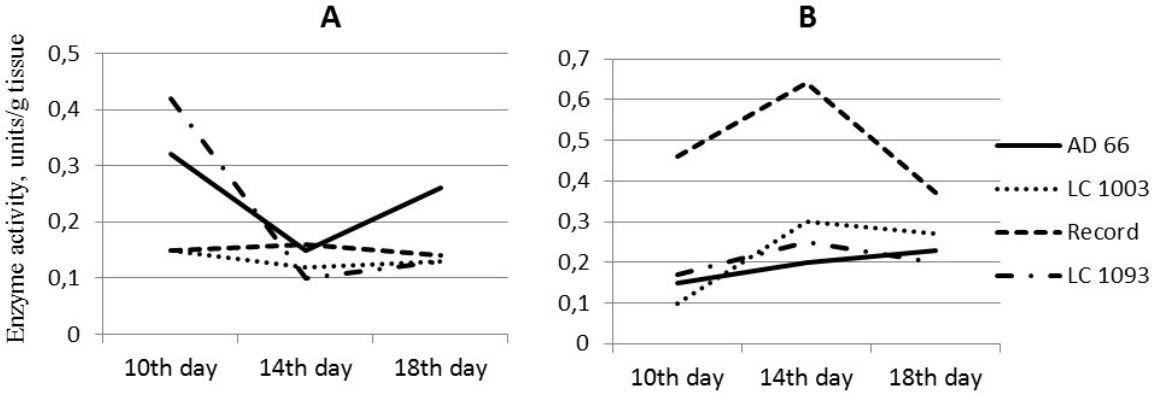

Fig. 3. Catalase activity dynamics in leaves (A) and roots (B) of sunflower resistance differentiators infected by broomrape