EXPRESSION OF SOME ANTIOXIDANT GENES IN SUNFLOWER INFECTED WITH BROOMRAPE

TATIANA ŞESTACOVA[1*], ION GISCĂ[2], ALIONA CUCEREAVÎI[2], OLESEA TABĂRĂ[1], ANGELA PORT[1], MARIA DUCA[1]

Abstract Expression levels of ROS-scavenging genes (*MnSODI, APX3* and *AOX1A*) in leaves (R5 stage; 90 days after sowing) of seven sunflower genotypes infected with three *Orobanche cumana* Wallr. populations were assayed in plants with/without broomrape aerial shoots and control group. Five lines were highly susceptible to all three populations. MS-2161A was resistant and MS-2039A was tolerant to broomrape populations. The expression of studied genes was much more altered in highly susceptible genotypes than in those resistant. Significant differences in number of cases of ROS-scavenging genes with modified transcriptional activity in infected and non-symptomatic plants were not ascertained. The transcriptional activity of *MnSODI, APX3* and *AOX1A* genes was weakly influenced by infection with broomrape (67 % cases) or was down-regulated (24 % cases). Some up-regulation cases (9 %) for *MnSODI* (MS-2039) and *AOX1A* gene (MS-2067) were revealed. *AOX1A* was the most responsive gene, especially when infection was produced by population from Anenii Noi.

INTRODUCTION

Sunflower broomrape (*Orobanche cumana* Wallr.) is an obligatory chlorophyll-lacking root parasite (Eizenberg et al, 2003). It is considered one of the most devastating pathogens of sunflower in Eastern Europe and Mediterranean regions (Echevarria-Zomeno et al, 2006), including Republic of Moldova (Duca, 2014). *O. cumana* uses nutritional resources of the host plant, thereby significantly reducing crop development, yield, (up to 50 %) and seed quality (Glijin, 2014). Mechanical, biological, and chemical methods have been applied for control of sunflower broomrape, but genetic resistance is considered as the most efficient, easy, economically and environmentally friendly solution. Cultivation of broomrape monogenic resistant varieties (*Or₁-Or₇*) is followed by the appearance of new more virulent races of the pathogen (Letousey et al, 2007), which determined a special attention of many authors to focus on the better understanding of the non-specific quantitative polygenic mechanisms (Letousey et al, 2007; Labrousse et al, 2010).

One of the most important steps in plant pathogen recognition and beginning of defense response is rapid production of the reactive oxygen species (ROS), referred as "oxidative burst" (Torres, 2010). The ROS possesses the toxic effects on plant cells and their organelles, and existence of antioxidant system for detoxification of these compounds in plants is crucial for the protection against the oxidative stress (Apel and Hirt, 2004). Flexibility of the antioxidant system is determined by the presence of enzymatic and non-enzymatic antioxidants, which members have different subcellular localization, biochemical properties and responses in gene expression and are able to control the optimum ROS levels (Scandalios, 2005; Caverzan et al, 2012).

Enzymatic antioxidants include a large and versatile set of enzymes (superoxide dismutase –SOD, ascorbate peroxidase – APX, catalase – CAT, glutathione peroxidase – GPX and peroxiredoxin – PrxR), which are present in all subcellular compartments of the plant cell (Scandalios, 2005; Caverzan et al, 2012; Mittler et al, 2004).

SODs are the first defense line in cell against ROS. Differential expression and localization of SODs have been studied in different plant species, especially model plants – Arabidopsis and rice (Marques et al, 2014; Mishra et al, 2013).

There are few data regarding the SODs structure and expression in cultivated plants. Fernández-Ocaña A. and coauthors (2011) for the first time demonstrated that sunflower has two mitochondrial *Mn-SOD* genes with the expression 1000-fold lower than that of the cytosolic and chloroplastic CuZn-SODs, estimated in different parts of 9-day-old sunflower seedlings. It was showed differential regulation of these *SOD* genes and formation of complex expression pattern in response to biotic (*Plasmopara halstedii* infection) and abiotic stimuli (high and low temperature, mechanical wounding) (Fernández-Ocaña et al, 2011).

Another gene implicated in prevention of oxidative damage induced under biotic and abiotic stress is alternative oxidase (AOX), which branches from the main respiratory chain at the level of ubiquinone after cytochrome c and catalyzes reduction of oxygen to water. *AOX* genes were studied under normal and specific stress or growth conditions using biochemical and molecular approaches (transcript and protein levels) in different plant species: rice, wheat, maize, soybean, cowpea and Arabidopsis (Clifton et al, 2006; Polidoros et al, 2009).

A broad range of studies showed that *AOX1* expression is associated with extreme environmental conditions, whilst *AOX2* is expressed under normal conditions of growth and development (Saisho et al, 2001; Juszczuk and Rychter, 2003). Thus, *AOX* potentially serve as a functional marker in molecular plant breeding for resistance (Polidoros et al, 2009).

Furthermore, it was found that *AOX* can act in resistance to pathogens. For example, up-regulation of *AOX* was ascertained at local and systemic level in tobacco and tomato leaves infected with tobacco mosaic virus (TMV) (Lennon et al, 1997) and in *Nicotiana attenuata* infected with *Pseudomonas syringae pv tomato* DC3000 (Zhang et al, 2012).

Hydrogen peroxide is essential ROS, which actively participate in defense responses (Torres, 2010) and the main system of its detoxification in plant chloroplasts and cytosol is the ascorbate-glutathione cycle, in which APX is a key enzyme (Caverzan et al, 2012).

APX (EC 1.11.1.11) take part from the class I heme-peroxidases and was studied in different plant species such as *Arabidopsis thaliana*, *Oryza sativa*, *Solanum lycopersicum*, *Spinacia oleracea*, *Vigna unguiculata*, *Eucalyptus grandis* (Caverzan et al, 2012).

Nine types of APX were identified in *Arabidopsis* according to their subcellular localization (Caverzan et al, 2012). The expression of *APX* genes is tissue and stage dependent and it is regulated by various abiotic and biotic stimuli: drought and salt stress, excessive light, high and low temperatures, pathogen attacks, H_2O_2 and abscisic acid (Yoshimura et al, 2000; Agrawal et al, 2003; Fryer et al, 2003; Menezes-Benavente et al, 2004; Bonifacio et al, 2011).

Although gene expression studies have great importance for understanding of defense response in plants, there are few data regarding transcriptional activity in sunflower-broomrape pathosystem. Letousey et al. (2007) conducted comprehensive research using molecular, biochemical, proteomic and histological approaches on *in vitro* co-culture of sunflower and broomrape. Expression patterns of 11 defense-related genes involved in different metabolic pathways in roots of resistant and susceptible genotypes showed strong induction of expression of methionine synthase, glutathione S-transferase and quinone oxidoreductase, possibly involved in detoxification of ROS and suggesting apparition of oxidative burst in resistant genotypes (Letousey et al, 2007).

Existing studies of sunflower-broomrape pathosystem especially are focused on host reponse during early phases of infection and there is lack of data regarding gene expression in advanced phases of interaction, mainly for antioxidant system genes and non-infected parts of infected plants (e.g. leaves).

In the present work, attention was focused on expression of *Mn-SODI, APX3* and *AOXIA* sunflower genes during the advanced stages of infection with broomrape, in order to gain new insights about the adaptation to biotic stress of sunflower and its defensive response to *O. cumana* Wallr. Furthermore, expression of *APX3* and *AOX1A* was not previously studied in sunflower infected with broomrape.

MATERIALS AND METHODS

Biological material. Seven CMS sunflower lines (MS-2161A, MS-2098A, MS-2039A, MS-2091A, MS-2077A, MS-2067A, MS-1589A) created and offered by AMG-Agroselect Company were used as host plant biological material. To produce infection three broomrape populations, collected from different geographical regions: two from Republic of Moldova (Soroca and Anenii Noi) and other one from Romania (Tulcea), were used. Broomrape seeds were gathered from 100 plants grown in the same field.

Growth conditions. Sunflower was grown in greenhouse at 18 – 20 °C during the day and 12 – 14 °C at night under 14 h photoperiod. Seeds were planted in individual pots (volume 35 – 40 l) with soil artificially infected with broomrape seeds (inoculum density 150 mg broomrape seeds per 1 kg of soil). The control samples were sowed in the same conditions but on the normal soil (without infection).

Phytopathological evaluation of broomrape occurrence. Evaluation of the attack frequency and the attack degree was carried out by direct counting of infected plants and the number of broomrape nodules per sunflower plant.

Frequency of attack was estimated using formula: $F(\%) = \dfrac{N \times 100}{N_t}$, where N – number of attacked plants n, Nt – total number of observed plants.

Attack intensity was calculated as follows: I = total number of broomrape nodules/ total number of infected plants. *Attacking rate* was estimated by the formula AR (%) = (F x I) /100.

Analysis of these three parameters highlighted virulence groups of pathogen and resistance of investigated genotypes. Plants which have attack frequency 0 - 5 % and attacking rate 0 - 0,5 % are considered *resistant (R)*; with F = 5 – 20 % were accepted to be *tolerant (T)*; 20 – 50 % – *susceptible (S)* and these with frequency higher than 50 % *highly susceptible (SS)*(Kaya et al, 2004). For estimation of phytopathological parameters were used 150-180 plants of each line.

Sample collection, RNA extraction and cDNA synthesis. For genes expression analysis, the total RNA was extracted from leaves of plants collected at R5 stage of development (Schneiter and Miller, 1981) and immediately frozen in liquid nitrogen. Samples were collected from plants with aerial shoots – *infected*, plants grown on infected soil but

without aerial shoots at surface – **non-symptomatic** and without infection – **control** group. Because of the sensitivity of qPCR, the plant-to-plant variation was reduced by pooling of total RNA extracted from three plants within each experimental case (infected, nonsymptomatic and control). The pooled RNA sample has represented one biological replicate. Three biological replicates (9 plants for each studied variant) in three technical repetition performed in different runs were studied.

The total RNA was extracted using TRI Reagent (*Ambion, Applied Biosystems*) following the manufacturer's instructions. Prior to cDNA synthesis using RevertAid RT Kit (*Thermo Scientific*) 0,6 μg of RNA were treated with DNAse I, RNAse-free (*Thermo Scientific*).

<u>Gene expression analysis via qRT-PCR.</u> Primers (Table 1) for quantitative real-time RT-PCR were designed using web based primer designing tool Primer3Web v.3.0.0. (Untergrasser et al, 2012).

Table 1. Genes/ESTs and primers used in study

Gene/EST	GeneBank ID	Primer sequence		Amplicon length, bp
		Forward	Reverse	
Mn-SOD I	DQ812551.2	5'- ataaggaaagcccgattttg-3'	5'- ttgcgattacaaacggtgaa-3'	107
*APX3**	BU032190.1	5'- cccaaatgctaccaaaggtg-3'	5'- atgtgctcttccaagggtgt-3'	112
*AOX1A**	GE502151.1	5'- gttagaagaagccgagaacgaa-3'	5'- gctagtttaggggaagcaaggt-3'	150
Actin	AF282624.1	5'- gctaacagggaaaagatgactc-3'	5'- actggcataaagagaaagcacg-3'	96

*primers were designed using ESTs highly similar to Arabidopsis gene sequences

The mRNA expression levels of selected samples were estimated by quantitative real-time PCR (qPCR) using Maxima SYBR Green/ROX PCR Master Mix (*Thermo Scientific*) according to the manufacturer's protocols on DT-96 (*DNA Technology, Russia*). Each sample was analysed in three technical replicates performed in three different runs. To normalize the target gene expression, the ΔCt value was calculated, which presents difference between the Ct of the target gene and the Ct of sunflower actin (constitutive control) for the respective template according to Livak and Schmittgen (2001): $2^{-\Delta Ct}$, where $\Delta Ct = Ct_{target\ gene} - Ct_{constitutive\ control\ gene}$ (Livak and Schmittgen, 2001).

Fold changes (FC) in gene expression between experiment and control were estimated as follows: $FC=2^{-\Delta\Delta Ct}$.

Amplification of gene-specific products was confirmed by melting curve analysis, followed by agarose gel electrophoresis. Statistical analysis was performed according to Dospekhov (Dospekhov, 1985). Standard t-test was used for estimation of significant differences. Fold changes greater than 1.5 or lower than -1.5 were considered biologically significant.

RESULTS

Screening of three studied broomrape populations revealed the highest values of phytopathological parameters in case of broomrape population from Tulcea, Romania. All investigated genotypes demonstrated high susceptibility to this population of broomrape. The most affected genotype MS-2091A was characterized by 100 % attack frequency, 12.85 attack intensity and 12.85 % attacking rate. MS-2161 was the weakest infected genotype: F = 4.24 %; I = 32.71; AR = 1.39 % (Table 2).

In contrast with broomrape population from Romania, populations from Republic of Moldova were less aggressive. Population of *Orobanche* from Soroca, similarly with those from Tulcea and Anenii Noi, determined maximal values of attack frequency – 100 % in two genotypes MS-2091A (15.53 intensity and attacking rate) and MS-1589A (12.19 intensity and attacking rate). Genotype MS-2161A showed resistance to this population too (F = 3.88 %; I = 3.86; AR = 0.15 %) (Table 2).

The lowest pathogenicity was registered in case of population from Anenii Noi region. The MS-2161A genotype, which showed resistance to other two populations, did not demonstrate some symptoms of infection, being resistant too in case of Anenii Noi population. Other genotype MS-2039A demonstrates low values of phytopathological parameters (F = 7.06 %; I = 3.0; AR = 0.21 %) and was tolerant. The rest of genotypes showed high susceptibility to this population (Table 2).

In conclusion, based on values of attack frequency, intensity and degree (Table 2) MS-2161A CMS line was classified as resistant, MS-2039A as tolerant and other lines as highly susceptible to broomrape. MS-2091A and MS-1589A are the most susceptible lines to all three broomrape populations used in investigations.

Table 2. Phytopathological parameters estimation on investigated genotypes, artificially infected with three broomrape populations

Genotype	Total number of plants	Number of infected plants	Total number of broomrape shoots	Attack frequency (F, %)	Attack intensity (I)	Attacking rate (AR, %)	Resistance degree*
Population from Soroca							
MS – 2161A	180	7	27	3,88	3,86	0,15	R
MS – 2098A	165	160	715	96,97	4,47	4,33	SS
MS – 2091A	170	170	2640	100,00	15,53	15,53	SS
MS – 2077A	180	170	1790	94,44	10,53	9,94	SS
MS – 2067A	175	165	905	94,29	5,48	5,17	SS
MS – 2039A	175	18	129	10,29	7,17	0,74	T
MS – 1589A	180	180	2195	100,00	12,19	12,19	SS
Population from Anenii Noi							
MS – 2161A	180	0	0	0,00	0,00	0,00	R
MS – 2098A	165	130	419	78,79	3,22	2,54	SS
MS – 2091A	180	170	1551	94,44	9,12	8,61	SS
MS – 2077A	180	160	586	88,89	3,66	3,25	SS
MS – 2067A	180	95	225	52,78	2,37	1,25	SS
MS – 2039A	170	12	36	7,06	3,00	0,21	T
MS – 1589A	180	170	1234	94,44	7,26	6,86	SS
Population from Tulcea							
MS – 2161A	165	7	229	4,24	32,71	1,39	R
MS – 2098A	170	150	675	88,24	4,50	3,97	SS
MS – 2091A	160	160	2056	100,00	12,85	12,85	SS
MS – 2077A	185	165	1921	91,67	11,64	10,67	SS
MS – 2067A	180	160	985	91,43	6,16	5,63	SS
MS – 2039A	170	26	551	15,29	21,19	3,24	T
MS – 1589A	180	175	2014	97,22	11,51	11,19	SS

*R – resistant; T – tolerant; SS – highly susceptible

Gene expression under infection induced with three broomrape populations.

Tulcea broomrape population. Infection with broomrape population from Tulcea region did not modified substantially the transcriptional activity of studied genes (*MnSODI, APX3* and *AOX1A*) with the exception of some single cases.

Thus, in infected plants with broomrape aerial shoots it was revealed up to 2 fold down-regulation of *APX3* gene expression (2.22 and 1.53 fold in MS-2067A and MS-1589A respectively). In contrast to *APX3*, *AOX1A* gene showed changes in expression caused by genotype and it was determined more than 1.5 fold down-regulation in MS-2039A (1.69 fold) and in MS-2067A (3.28 fold) and up-regulation in MS-1589A. Also, the stimulatory effect was observed for *MnSODI* in MS-2039A genotype (2.22 fold increase) (Fig. 1).

Similarly with infected plants, in non-symptomatic plants, grown on infected soil but without aerial shoots, was detected variation of gene expression associated with genotype. Thus, it was observed up to 2 fold down-regulation of gene expression for *MnSODI* (in MS-2098A and MS-1589A), 1.59 fold for *APX3* (in MS-2039A) and 2.05 fold for *AOX1A* (in MS-2161A). In some

cases *AOX1A* and *MnSODI* genes were up-regulated (1.75 and 2.42 fold in MS-2039A and MS-2067A lines respectively) (Fig.1).

It could be mentioned that *APX3* gene were not up-regulated neither in infected nor in non-symptomatic plants.
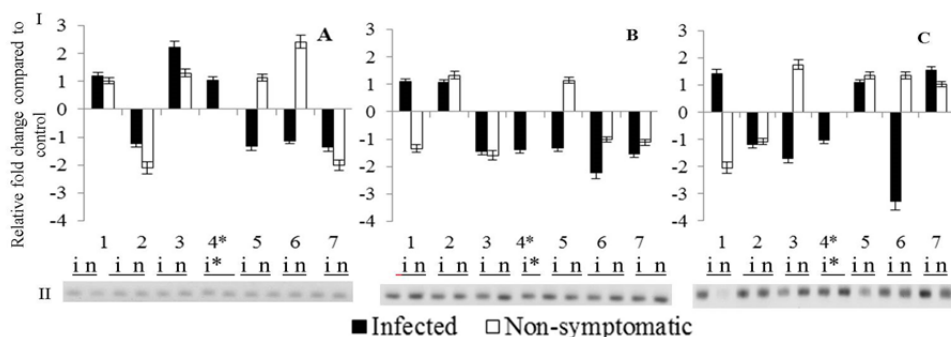

Figure 1. Fold change experiment/control in gene expression under influence of broomrape population from Tulcea, Romania (I) and 1,4 % agarose gel electrophoresis of amplicons (II): A – MnSODI, B – APX3, C – AOX1A

1-MS-2161A, 2-MS-2098A, 3-MS-2039A, 4-MS-2091A, 5-MS-2077A, 6-MS-2067A, 7-MS-1589A

Values represent fold change relative to control, n =3.
*in case of MS-2091A genotype frequency of attack was 100 % and there are no plants without symptoms of broomrape attack

Soroca broomrape population. As well as population from Romania (Tulcea) those from RM (Soroca) induced insignificant modifications or down-regulated the expression of studied genes (Fig. 2).
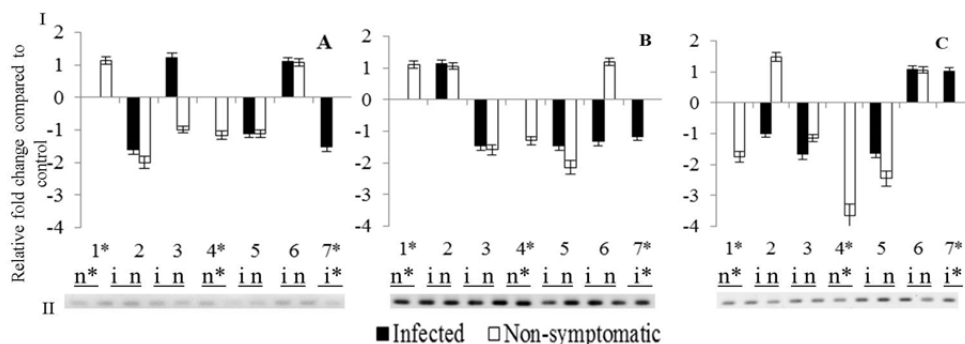

Figure 2. Fold change experiment/control in gene expression under influence of broomrape population from Soroca, RM (I) and 1,4 % agarose gel electrophoresis of amplicons (II): A – MnSODI, B – APX3, C – AOX1A

1-MS-2161A, 2-MS-2098A, 3-MS-2039A, 4-MS-2091A, 5-MS-2077A, 6-MS-2067A, 7-MS-1589A

Values represent fold change relative to control, n =3.
*in case of MS-1589A genotype frequency of attack was 100 % and there are no plants without symptoms of broomrape attack; for MS-2161A (tolerant) and MS-2091A lines there are lack of samples from infected plants

The strongest down-regulation both in intensity as well as number of cases was detected for *AOX1A* gene (five cases from 11 studied). Thus, the transcriptional activity of *AOX1A* gene was decreased both in infected and non-symptomatic plants up to 1.6 fold in MS-2039A and MS-2077A and up to 4 fold (1.76, 3.65 fold and 2.46 fold in MS-2161A, MS-2091A and MS-2077A) respectively. Like *AOX1A* gene *MnSODI* was up to 2.0 fold down-regulated only in some cases (in both infected and non-symptomatic plants of MS-2098A and MS-1589A) lines.

Transcript levels for *APX3* gene undergone insignificant changes in infected plants and decreased only in non-symptomatic plants of MS-2039A and MS-2077A genotypes (1.59 and 2.15 fold respectively) (Fig. 2).

Comparatively with other two studied populations, there are no cases of up-regulation of the investigated genes.

Anenii Noi broomrape population. In plants infected with broomrape from Anenii Noi the expression of studied genes was much more altered than in case of infection produced by other investigated populations, especially in highly susceptible genotypes – MS-2098A, MS-2077A, MS-2067A and non-symptomatic plants of MS-2091A.

Similarly with the populations from Tulcea and Soroca, broomrape from Anenii Noi mostly influenced *AOX1A* gene expression. Thus, it was showed down-regulation of *AOX1A* gene in infected plants of MS-2098A (3.64 fold) and both infected and non-symptomatic plants of MS-2077A (2.38 and 1.56 fold respectively). The most significant changes were observed in the case of non-symptomatic plants of MS-2091A genotype, where *AOX1A* gene was 70.42 fold down-regulated.

It was ascertained significant up-regulation of this gene in MS-2067A (3.0 fold in infected plants and 2.84 in non-symptomatic plants) and also, it was observed up to 2 fold increase in transcriptional activity of other two studied genes (1.98 fold for *MnSODI* in infected and 1.96 for *APX3* in non-symptomatic plants). In contrast to MS-2067A, in MS-2098A *MnSODI* showed more than 2 fold down-regulation (Fig. 3).
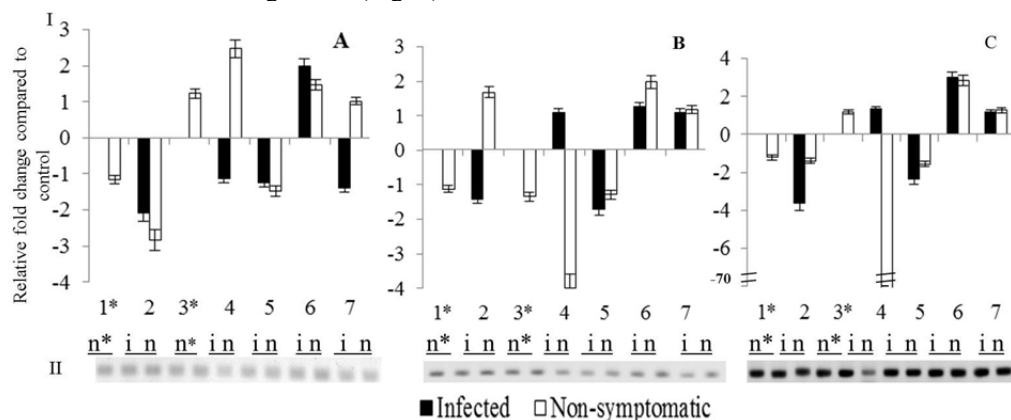

Figure 3. Fold change experiment/control in gene expression under influence of broomrape population from Anenii Noi, RM (I) and 1,4 % agarose gel electrophoresis of amplicons (II): A – MnSODI, B – APX3, C – AOX1A

1-MS-2161A, 2-MS-2098A, 3-MS-2039A, 4-MS-2091A, 5-MS-2077A, 6-MS-2067A, 7-MS-1589A

Values represent fold change relative to control, n =3.

*MS-2161A and MS-2039A were resistant and tolerant to this broomrape population and there are no infected samples for these lines

Unlike other two populations, in case of broomrape from Anenii Noi *APX3* gene was not only down-regulated (1.74 and 4.01 fold in infected plants of MS-2077A and non-symptomatic plants of MS-2091A respectively) but showed in some cases significant up-regulation (1.66 and 1.96 fold in non-symptomatic plants of MS-2098A and MS-2067A lines).

Two genotypes MS-2161A resistant and MS-2039A tolerant to broomrape population from Anenii Noi did not show any significant changes in transcripts levels of all three studied genes.

DISCUSSION

One of the most important mechanisms which occurs in resistant plants after perception of infection caused by avirulent pathogens is elicitation of a multiple defenses often accompanied by the collapse of challenged plant cells in the so-called hypersensitive response (HR). The HR is the outcome of specific interaction between ligand and receptor ensured by paired plant resistance (*R*) and pathogen avirulence (*avr*) genes and it determines restricted lesion at the site of attack clearly delimited from surrounding healthy tissue. Furthermore, systemic acquired resistance (SAR) to subsequent attack by virulent pathogens is triggered in the rest of the plant (Ryals, Uknes, Ward, 1994).

Many inducible defense responses including phytoalexin synthesis and expression of pathogenesis-related (PR) genes are regulated transcriptionally, and avirulence signals cause significant switches in host gene expression (Lamb and Dixon, 1997).

HR occurrence in plants induces increase in expression of ROS-scavenging genes: superoxide dismutases (SOD), ascorbate peroxidases (APX), glutathione peroxidase (GPX), and catalases (CAT), especially in early stages of infection. Thereby, there are not sufficient data regarding antioxidant gene expression in advanced stages of infection, when plants are adapted to biotic stress.

Reported data demonstrated, that five genotypes were highly susceptible to studied populations with the exception of MS-2161A – resistant and MS-2039A tolerant to broomrape. The transcriptional activity of *MnSODI, APX3* and *AOX1A* was weakly influenced by infection with broomrape populations (67 % cases), or was down-regulated (24 %), with the exception of some up-regulation cases for *MnSODI* (MS-2039) and *AOX1A* gene (MS-2067) (9 %). Significant differences in number of cases of ROS-scavenging genes transcriptional activity alteration in infected and non-symptomatic plants were not observed.

The plant defence reaction identified in this study, confirm previously data (Fernández-Ocaña et al, 2011) showed that mitochondrial *Mn-SOD* gene expression acts like an early signal in the prevention of oxidative damage in response to environmental stress in sunflower plants. This conclusion could explain unchanged or decreased transcript level of *MnSODI* gene in investigated lines, which were analyzed in late stages of infection and development (R5 developmental stage, beginning of flowering).

In addition, according to the theory of adaptation process (Udovenco, 1995) after long exposion to stress plants enter in adaptation stage with insignificant changes in metabolism.

Expression of studied genes was much more altered in highly susceptible genotypes than in resistant. For example, the MS-2161A resistant genotype had insignificant changes in transcriptional activity of investigated genes with exception of *AOX1A*, which was down-regulated in non-symptomatic plants under the influence of Soroca and Tulcea broomrape populations.

Furthermore, analysis of different SODs genes activity in *Arabidopsis* showed that MSDI (*MnSODI*) had minimal changes in response to any treatments (light regimes, ozone fumigation, UV-B irradiation) at mRNA and protein levels and did not alter in response to virulent or avirulent *Pseudomonas syringae pv maculicola.* The authors supposed that: ROS levels did not significantly increased in mitochondria under abiotic and biotic stress or MSD is not a primary mechanism of ROS-scavenging in mitochondria (Kliebenstein et al, 1998). These data could confirm minimal changes in transcriptional activity of *MnSODI* in sunflower during infection with broomrape.

Our study showed that *APX3* similarly with *MnSODI* generally was characterized by insignificant changes in expression under the broomrape infection that suggests about low participation of this gene in defense or active ROS detoxification.

The similar conclusions were done in investigations performed on *Arabidopsis APX3* knockout mutant under normal and several stress conditions showed. Thus, *APX3* loss of function does not affect *Arabidopsis* growth and development, suggesting that *APX3* may not be a crucial antioxidant enzyme in *Arabidopsis*, at least under the chilling and heat treatments or the function of *APX3* could be compensated by other ROS-scavenging enzymes or APX isoforms (Narendra et al, 2006). Another hypothesis that could account for insignificant changes in *APX3* expression in sunflower is decreased peroxide accumulation during advanced stages of infection (Torres, 2010).

In the conducted research *AOX1A* mostly demonstrated strong down-regulation or non-significant changes in expression. In some cases it showed up-regulated expression – especially on non-symptomatic and infected plants of MS-2067 genotype infected with Anenii Noi broomrape population.

Our findings regarding *AOX1A* gene expression matches with data of other authors. Accordingly to several studies performed for Arabidopsis *AOX* genes, *AOX1A* is the most stress responsive (Clifton et al, 2006) gene and its activity is altered under influence of different stress factors (Apel and Hirt, 2004).

Generally, it was ascertained that in case of infection with the broomrape population from Anenii Noi with lowest pathogenicity were observed the most significant changes in expression of studied genes in highly susceptible lines. However, in genotypes that showed resistance and tolerance to this population MS-2161 and MS-2039 respectively, expression of studied genes was modified insignificantly. It could be explained through lack of oxidative stress or HR at late stages of infection or high stability of metabolic activity and higher adaptation capacity of resistant genotypes (Torres, 2010).

CONCLUSIONS

The results obtained in this work showed that in sunflower infected with broomrape ROS-scavenging genes (*MnSODI, APX3* and *AOX1A*) did not significantly change their transcriptional activity, pointing out that in the late stages of infection phenotypic adaptation of plants is correlated with molecular one.

Further investigations regarding ROS accumulation, enzymatic activity and isozyme patterns are required for a better understanding of molecular events in sunflower-broomrape pathosystem in different stages of development.

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[1] University Center of Molecular Biology, University of the Academy of Science of Moldova, Chisinau, Republic of Moldova
[2] AMG-Agroselect Comerţ Company, Soroca, Republic of Moldova
[*] tatiana.shestacova@gmail.com