International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 4, Issue 10 - 2017

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.10.025

Expression of some genes associated with aggressivity at the different Broomrape Races

Duca Maria, Boicu Adriana, Abdușa Daniela
Centre of Functional Genetics, University of the Academy of Sciences of Moldova, Chisinau, Republic of Moldova
*Corresponding author: *abdusadaniela@yahoo.com*

Abstract

To reveal the genetic-molecular mechanisms which determine the virulence growth from one physiologic race to another, gene expression from 16 broomrape populations with different race status and geographical origin was estimated. The genes which encode peroxidase (*PRX*), pectin metilesterase (*PME*), polygalacturonase (*PGU*) and chalcone synthase (*CHS*) where evaluated on the level of germinated seeds, bulbs, underground and aerial sprouts. The study has highlighted the transcription correlation level *PRX*, *PME*, *PGU* and *CHS* with the virulence at the different phases of ontogenetic development of broomrape phyto-pathogen, and in some cases – even with the attack frequency and degree, the higher level of these genes expression being revealed at the virulent race H, as compared to G and E, the less virulent ones. At all 16 populations of *O. cumana* the higher gene expression values were observed at the phase of aerial sprout, as compared to the germinated seeds, tubercles and underground sprouts. The comparative analysis of transcriptions contents has shown the differential involvement of studied genes, in dependence of the organ: in germinated seeds an increased transcriptional activity of *PGU* gene predominates, in tubercle – *PRX*, in underground sprouts – *PRX* and *CHS*, whereas in the aerial sprout - *PGU, CHS, PRX*, followed by *PME*. The results obtained are useful in elucidating of the physiological and genetic-molecular mechanisms associated with the *O. cumana* virulence and aggressivity.

Keywords: genes expression, broomrape, physiological varieties, development phases.

Introduction

Broomrape (*Orobanche cumana Wallr.*) is the main parasite which attacks the sunflower on the root system level, causing the significant damages to this strategic oleaginous crops in Turkey (Kaya *et al.*, 2004a), Spain (Fernandez *et al.*, 2009), Romania, Ukraine, Bulgaria etc. (Masirevic and Malidza, 2006; Parker, 2009) including the Republic of Moldova (Duca *et al.*, 2011; Gisca *et al.*, 2013; Glijin *et al.*, 2009, 2012; Rotarenco, 2010).

The first mentioning of the physiological races of *O. cumana* species dates back to the beginning of the XXth century. For the first time, in 1920ies, the researchers of the Saratov Experimental Station have initiated and recommended the creation of the sunflower sorts genetically resistant towards the bromrape (*Kruglik A-41; Saratovski 169; Fuxinka 3; Zelanka 10; Cerneanka 35*) (Placek, 1921). These sorts were resistant to the *O. cumana* races in the regions of Saratov and Voronezh, but not those from the regions of Rostov and Krasnodar, therefore, the presence of two varieties was detected, with different geographical distribution: the race A frequently attested in Saratov and Voronezh and the race B – in

the Rostov and Krasnodar regions (Sharova, 1977). The broomrape race B was identified in Moldova and Ukraine in 1930ies (Burlov and Kostyuk, 1976; Sharova, 1969). At the beginning of 1970ies, a new broomrape race has appeared for the first time in Moldova, more virulent than A and B races, which started to infect the resistant sorts and was rapidly spreading in all the regions of sunflower cultivation, especially in the Northern Caucasus (Antonova, 2009). This variety was named the C race, or Moldovan race (Burlov and Burlov, 2010; Sharova, 1977).

Later on, Vranceanu and his team (1980) have performed the comprehensive analysis of phyto-pathogen varieties, identifying 5 physiological boomrape races (from A to E) and, accordingly, a set of differentiating sunflower lines with 5 dominant resistant genes (*Or1, Or2, Or3, Or4,* and *Or5*). The new broomrape races (F, G and H), which exceeded the resistant gene *Or5* were identified and described starting from 1990ies in the most infected areas by *O. cumana* in Spain (Alonso, 1996; Fernandez-Martinez and Dominguez, 2008), Romania (Păcureanu *et al.*, 2004, 2009), Turkey (Kaya *et al.*, 2004b), Bulgaria (Shindrova, 2006), Ukraine (Burlov and Burlov, 2010), Russia (Antonova *et al.*, 2013), etc.

Thus, during the rather short time period, the considerable changes in virulence have been detected within the broomrape races, as well as the consequitive appearance of eight physiological races, capable to overcome the immune system of the resistant sorts and hybrids.

While examining the phyto-parasite retrospectively, the presence of three periods of appearance and spreading of the *Orobanche Cumana* races can be discerned.

The first period began in the USSR and was determined by the appearance of broomrape populations which started affecting the sunflower plantations. Later, the first records appeared concerning the broomrape races occurrence, attested especially in the areas with the intensive soil improvement and sorts creation activity, concentrated on Russian territory. However, the situation was relatively stable at this period, the most aggressive race being the E type. After the cytoplasmatic androsterility was discovered (Leclercq, 1969) and ASC-Rf system was applied in using the heterosis effect (Vranceanu *et al.*, 1980), a rapid growth in parasite aggressivity occurred in the areas of culture located around the Black Sea, the virulence explosion taking place in the Thrace region in Turkey, later on extending towards the South-East of Bulgaria and Romania, Moldova, Ukraine and Russua, intermediated by using the imported hybrids and because of the easy transfer of the miniscule seeds of *Orobanche* from one region to another (Fernandez *et al.*, 2009). Therefore, with the deplacement of the major centers of hybrids creation in Europe (Romania, France and Spain), the centers of the new bromrape races appearances have been moved, too.

These data confirm the theory of conjugated host-parasite evolution, taking as an example the sunflower and broomrape, and demonstrate that the appearance and rapid development of new physiologic races of *O. cumana* is determined by the pression of selection, generated by the permanent search for the new resistance gene.

Among the main genes associated with parasitism, there are specific members of the genes' families which codify the modifying enzymes of cell wall: cellulase, glycosyl hydrolase, pectin methylesterase (Losner-Goshen *et al.*, 1998); peroxidase (Antonova and Ter-Borg, 1996) and proteins which are known to be involved in the process of parasite invation (Perez-de-Luque, 2013; Singh and Singh, 1993; Yang, 2015). It is obvious, that many of phytopathogens have been evolved and generated the specific agents (efectors) and virulence factors capable to enter the host cell and suppress the resistance mechanisms of host plant or avoid their control (Boller and He, 2009; Torto-Alalibo *et al.*, 2009).

Parasite *Orobanche spp.* fanerogamas at the early stages of penetration secrete the enzymes such as peroxidase, pectin methylsterase (Ben-Hod *et al.*, 1993; Losner-Goshen *et al.*, 1998), polygalacturonase, pectin lyase (Losner-Goshen *et al.*, 1998), cellulase etc. (Joel *et al.*, 1996), which can modify the composition of host cells and medium lamellas, making them thinner and more vulnerable during the attack.

Proceeding from the aforesaid, the scope of this research consists in identification of some aspects related to the physiological mechanisms associated with the broomrape virulence and aggressivity, through the prism of gene *PRX, PME, PGU* and *CHS* expression evaluation.

Materials and Methods

Biological material

To identify and select some representative populations of *Orobanche cumana*, corresponding to the study objectives, the pathogen accessions have been selected from the different geographical regions of the Republic of Moldova (North, Center and South), as well as those from the sunflower cultivated areas cultivated from the other countries such as Spain, Romania and Ukraine. For the studies, three broomrape populations were used (Cazanesti, Buteni, Singera) which represent a race ≤ **E**, eight broomrape populations (Soroca, Verejeni, Costuleni, Gura Galbenei, Ermoclia, Corteni, Manta and Sevilia) – race **G**; and five populations (Ciocilteni, Sarata-Mereseni, Taraclia, Fundulea and Ismail) attributed to the **H** race, described in our previous research (Acciu, 2016).

RNA isolation

RNA isolation from germinated seeds, bulbs, underground and aerial sprouts has been carried out with the chemical TRI Reagent 46 (Ambion, Applied Biosystems), according to the recommendations (Orozco-Cardenas *et al.*, 2001). DNA quantification was performed spectrophotometrically at λ=260 nm and 280 nm, and quality determination – by electrophoretic analysis in the 1% agarose gel.

Gene expression analysis

The studied gene expression was quantified quantitatively in real-time regime with PCR, in the amplifier with automatic detection of fluorescence DT-96 (DNA technology, Russia) using the Maxima SYBR Green/ROX PCR Master Mix (*Thermo Scientific*, 2X). For each DNA sample the reaction was mounted in three analytical replicates.

Primer design

The design of specific primers (Table 1) was performed using the programme *Primer3Web v. 3.0.0.* (http://bioinfo.ut.ee/primer3-0.4.0/primer3/input.htm), and their verification with regard to the presence of secondary structures was made with *OligoAnalyzer 3.1* (http://eu.idtdna.com/analyzer/applications/oligoanalyzer/).

Table 1. Characteristics of primers used in the study

Gene symbol	Access Nr.	Sequence 5' -> 3'	Tm (°C)	L., bp
PRX peroxidase	AY353721.1	F GGACTCTACCACGGCTAACC (20) R GCTTGACCCAGTGTGTGTGA (20)	61,4 59,4	150
PGU polygalacturonase	AY353722.1	F ACACCGATGGAATTCACATCAC (22) R CGGGGCCAAACACTATGCTT (20)	58,4 59,4	153
PME pectin metilesterase	AY072720.1	F GAGTACGAGTGGGAGAGTGA (20) R TCCAAACATCCCCGGACAAA (20)	59,4 57,3	101
CHS chalcone synthase	AF074401.1	F CCGGCAAAACATCGTGGTC (19) R GGGCATATCGACACCACTGG (20)	58,8 61,4	136
α-tubulin	AF402679.1	F GATTATGAGGAGGTCGGGGC (20) R CAGAACCCCAAATACCAGACCA (22)	61,4 60,3	200

Note: ***F*** – sense nucleotide sequence; ***R*** – antisense nucleotide sequence; in round brackets ( ) the length of the primer's sequence is shown; ***Tm*** – denaturation temperature; ***L*** – amplicon length; ***bp*** – basic pairs.

Statistical analysis

As a reference gene, *α-tubulin* was used (access number to the database GenBank: AF402679.1).

The transcriptional gene activity was calculated according to Livak and Schmittgen (2001): $2^{-\Delta Ct}$, where $\Delta Ct = Ct_{(gene\ of\ interest)} - Ct_{(\alpha\text{-}tubulin)}$. The values $2^{-\Delta Ct}$, given in conventional units (c.un.), has been used for the diagrams building.

Results and Discussion

The study of the relative expression of genes *PRX, PME, PGU* and *CHS* involved into the penetration facilitation into the epidermal cells and cortex of the host plant at the different phases of ontogenetic development of phyto-pathogen, has revealed the correlation between the transcription level and virulence (Figure 1), and in some cases – even with the frequency (F, %) and the attack degree (A.D., %) by the broomrape. At all 16 populations of *O. cumana* the higher values of genes expression were revealed on the phase of aerial sprouts, as compared to the germinated seeds, tubercles and underground sprouts.

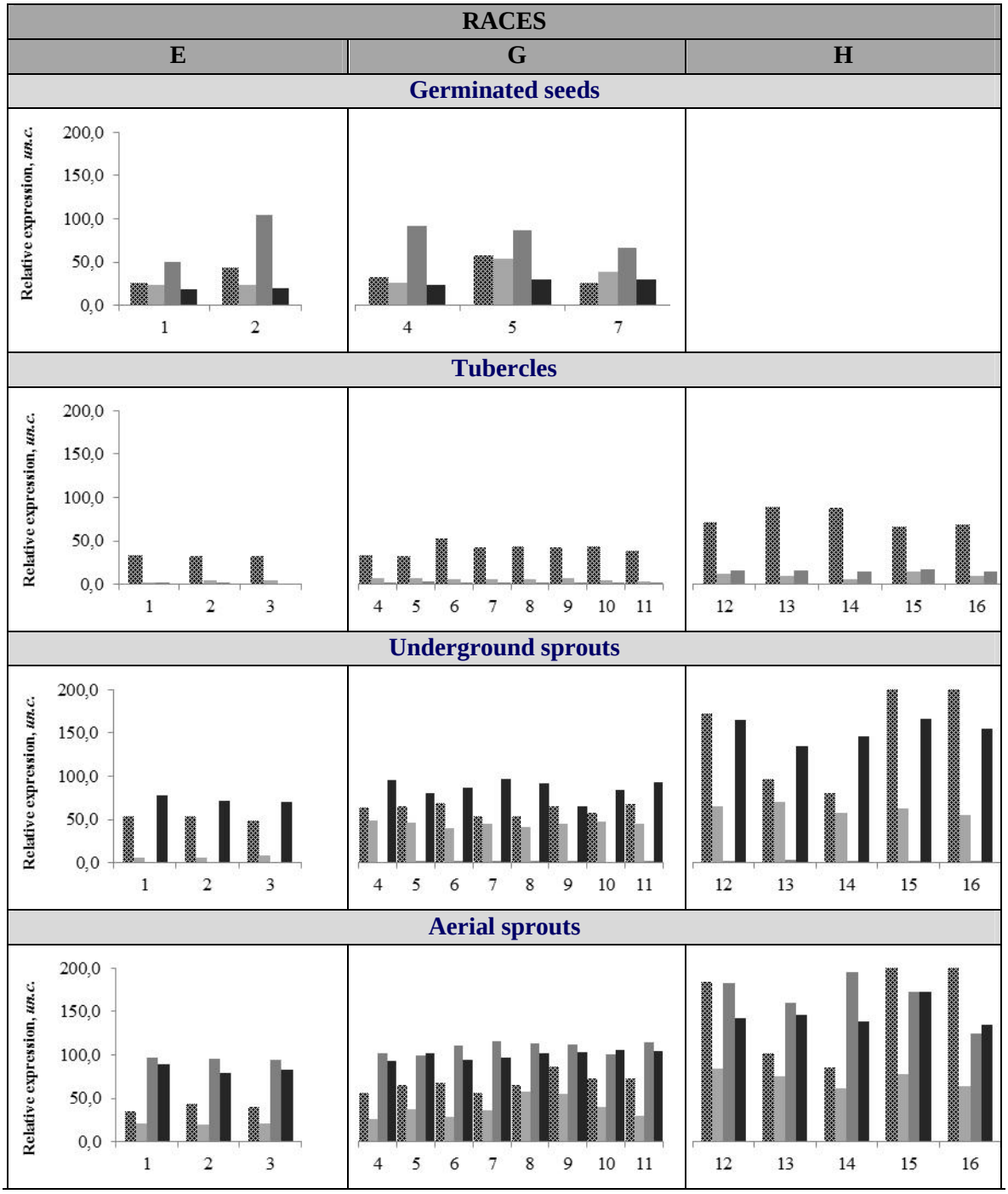
Among the four studied genes, the gene *PRX*, which encode the different peroxidase types, suppressing the host plant resistance during the penetration (Antonova and Ter-Borg, 1996), have demonstrated the predominant transcriptional activity in the tubercles. It was found that the gene expression in the germinated seeds (25.2 c.un. – 57.6 c.un.), in tubercles (31.9 c.un. and 88,5 c.un.), in underground sprouts (48.1 c.un. – 237.0 c.un.) and in aerial ones (34.2 c.un. – 261.7 c.un.) are varying in dependence on population and broomrape race. Thus, the activity of *PRX* gene was shown to be higher at the most virulent and aggressive race – a race H (85.5 c.un. Taraclia – 261.7 c.un. Fundulea), as compared to G and E – the less virulent ones (25.6 c.un. Costuleni – 85.9 c.un. Ermoclia, respectively, 25.2 c.un. Singera – 53.6 c.un. Cazanesti) in 100% of cases at the phase of tubercles, underground and aerial sprout. These data correlate with the attack frequency and degree, revealed at the Fundulea and Ismail populations (race H, 261.7 c.un., F 100%, respectively, 237.0 c.un., F 100%, A.D. 2.1%), Corteni and Sevilia (variety G, 72.5 c.un., F 100%, A.D. 1 %), established in the previous experiments carried out under the control conditions (Duca, *et al.*, 2016). The population from Ermoclia, which manifested the highest expression level of gene *PRX* (85.9 c.un.) among the populations of G race, did not show any correlation with the afore-mentioned parameters.

The results obtained are consistent with those established at *Orobanche ramosa* at the stage of the attachment organ differentiation (Gonzalez-Verdejo, *et al.*, 2004), thus supporting the idea that these enzymes are present in the absence of the host plant on the top of the *Orobanche* appressoria, before, during and after the haustorial penetration (Gonzalez-Verdejo *et al.*, 2006).

The relative expression of gene PME, involved in the methabolic pathway of pectin degradation during the haustorial penetration (Bar *et al.*, 1996; Losner-Goshen *et al.*, 1998), which, by means of esterification reaction of carboxyl methylate groups of D-galacturonidase, facilitate the activity of polygalacturonases (Mayer, 2006), have manifested a higher level at the phase of aerial sprout, followed by the underground sprout. Expression values of pectin methylesterase are between 23.5 c.un. and 53.7 c.un. in the germinated seeds, from 2.7 c.un. to 14.5 c.un. in tubercles, 5.4-69.7 c.un. – underground sprout and 20.1-84.6 c.un. in aerial sprouts.

The presence of *PME* was found at the pre- and haustorial phases at *O. cumana* (Veronesi *et al.*, 2005) and *C. pentagona* (Ranjan *et al.*, 2014).

Similarly with gene *PRX*, a positive correlation was established between the gene expression and virulence, found previously by Veronesi and team (2011, 2005) at the brromrape, which is supported by the over-expression of this gene at *C. pentagona* (Ranjan *et al.*, 2014). The highest concentration of *PME* transcriptions was attested at the race H (61.7 c.un. Taraclia – 84.6 c.un. Ciocilteni) in 100% cases at the phase of tubercle, underground and aerial sprout, as compared to G and E (3.5 c.un. Sevilia – 85.9 c.un. Ermoclia and, respectively, 2.7-23.8 c.un. Cazanesti), less virulent. The results obtained correlate with the data of previous studies (Duca *et al.*, 2016) concerning the frequency of broomrape attacks on the populations of Ciocilteni (race H, 84.6 c.un., F 100%) and Gura-Galbenei (race G, 53.5 c.un., F 100%).



Broomrape populations: *1 – Cazanesti, 2 – Singera, 3 – Buteni, 4 – Soroca, 5 – Gura Galbenei, 6 – Verejeni, 7 – Costuleni, 8 – Manta, 9 – Ermoclia, 10 – Corteni, 11 – Sevilia, 12 – Ciocilteni, 13 – Sarata-Mereseni, 14 –Taraclia, 15 – Fundulea, 16 – Ismail.*

Figure 1. Expression level pf genes *PRX, PME, PGU* and *CHS* in dependence on physiologic race and development phases of phyto-pathogen *O. cumana.*

The results related to the analysis of *gene PGU expression*, involved in the establishment of link between the haustoria with the vascular system of the host (Perez-de-Luque, 2013), have shown the maximal values at the stage of aerial sprout, even in the germinated sprouts at 5 (100%) between the populations for which the experimental data were available, gene activity was predominating, as compared to the other genes taken for study.

The expression values vary from 50.4 c.un. to 104.3 c.un. in the germinated seeds, between 1.2 c.un. and 2.8 c.un. – underground sprout, 94.2-194.6 c.un. – aerial sprout. Polygalacturonases, along with the other enzymes of cell wall degradation such as cellulose and xylanase, were identified in tubercles of *P. aegyptiaca* (Sing and Sing, 1993), and β-expansin – in the haustorium between the pathogen *T. versicolor* and its host (Honaas *et al.*, 2013).

The analysis of relative expression of gene *PGU* at the different broomrape populations has revealed the higher values at the populations which belong to the race H (2.5 c.un. Ciocilteni – 194.6 c.un. Taraclia), as compared to G and E (0.001-116.3 c.un. Costuleni and 0.01 c.un. Buteni – 104.3 c.un. Singera) in 100% of cases at the phase of underground and aerial sprout. These results, denoting a correlation of the transcription level with virulence, have been revealed and in the other studies (Veronesi and Thalouarn, 2001; Veronesi *et al.*, 2005).

The expression level of gene *PGU* positively correlates with the broomrape attack frequency and degree at the populations in Taraclia and Ciocilteni (race H, 194.6 c.un., F 100%, A.D. 3.92%, respectively, 182.6 c.un., F 100%). Among the populations of race G, Costuleni, the highest expression level of gene *PGU* (116.3 c.un.) was manifested, which, however, did not show any correlation with the attack frequency and degree with phyto-pathogen *O. cumana*.

On the opposite to the results of relative expression analysis of genes *PME* and *PRX*, the gene *PGU* has manifested the higher transcriptional activity at the phase of aerial sprout, expression values being between 124.1 c.un. – Ismail and 194.6 c.un. – Taraclia.

The gene *CHS* codify the calcon synthase, the key enzyme of methabolic pathway of flavonoids/isoflavonoids synthesis. The activity of gene CHS estimated at the different development stages of broomrape phyto-pathogen was shown to be high at the stage of aerian sprout. The transcripts concentration of gene *CHS* varies from 18.9 c.un. to 29.7 c.un. in the germinated seeds, between 1.3 c.un. and 17.6 c.un. in tubercles, between 64.7 c.un. and 165.9 c.un. – underground sprout and from 79.3 c.un. to 172.3 c.un. – aerial sprout. Inducing of gene *CHS* expression was observed in the roots of *Medicago truncatula* infected with *Orobanche crenata* in 35 days of inoculation, being marked with the relative expression level of 120.41 c.un. (Die *et al.*, 2009).

The transcriptional activity of gene *CHS*, analogue with three studied genes, correlates with regard to virulence, the expression level being higher at the race H, as compared to the race G and E, in all studied cases. The relative expression of gene *CHS* positively correlates with the broomrape attack frequency and degree at the populations of Fundulea, Ciocilteni, Sarata-Mereseni, (race H, 172.3 c.un., F 100%; 142.7 c.un., F 100%; 145.5 c.un., F 100%, A.D. 1.2%), Corteni and Sevilia (race G, 106.2 c.un., F 100%, A.D. 1%; 104.3 c.un., F 100%, A.D. 1%).

Given that there are no studies devoted to the transcriptional activity of gene *CHS* in the different organs of parasitic broomrape, and the gene *CHS* expression is strongly induced by the variety of factors of environment, both biotic and abiotic, which provoke the mechanical damages to plant and determine the accumulation of phytotoxins - flavonoids/isoflavonoids (Dao *et al.*, 2011; Djordjevic *et al.*, 1997), the conclusion can be made that the higher level of *CHS* transcriptions detected in the aerial and underground sprouts of *O. cumana* reflect the reaction of parasite to the abiotic and/or abiotic stress. Considering, that the host plant can demonstrate the active defense reactions towards the pathogens attack (Runyon *et al.*, 2010), the parasite is under the biotic stress, and, consequently, can recruit its own defense mechanism to overcome the counter-attack. The genes associated with the response to stress have been identified as overexpressed in the roots of a riso-parasite after the contact with the host plant (Honaas *et al.*, 2013; Torres *et al.*, 2005). Therefore, it can be suggested that the parasitic plants recruit the defense groups to recognize the host.

The results obtained related to the comparative analysis of the transcripts contents of studied genes, on the level of different organs of holoparasite broomrape phanerogam, have revealed the predominant transcriptional activity in the germinated seeds – of gene *PGU*, in tubercle – *PRX*; at the further phase of underground phase the overexpression is dominated

by the genes *PRX* and *CHS*, and at the aerial sprout phase – *PGU, CHS, PRX*, followed by *PME*. Proceeding from the hypothesis, that genes manifesting the highest transcription level could be directly involved in the genetic-molecular mechanisms, which raise the virulence from one physiologic race to another, the results of the present study suggest a differential involvement of genes, in dependence on the development phase of a phyto-pathogen.

These findings, in combination with the classical hystologic and immune-cytochemical studies *in situ*, which show the importance of cell walls relaxation complexes of host plants, suggest the importance of the cell walls modifications in inducing and penetration of host tissue by pathogen haustoria, as well as its rapid expansion in the tissue (Nagar *et al.*, 1984; Vaughn, 2002, 2003).

Conclusions

The study of transcriptional activity of genes involved in facilitation of pathogen penetration in the epidermal cells and cortex of the host plant at the different phases of onthogenetic development of phyto-pathogen (germinated seeds, bulbs, underground and aerial sprouts) have demonstrated a positive correlation with the virulence, the highest expression level of *PRX*, *PME*, *PGU* and *CHS* being revealed being at the virulent race H, as compared to G and E, the less virulent ones.

The transcriptional activity of four genes analysed at all 16 populations of *O. cumana* was proved to be higher at the phase of aerial sprout, as compared to the germinated seeds, tubercles and underground sprouts.

The comparative analysis of transcripts contents have elucidated the differential involvement of the genes of interest, in dependence of the organ: in the germinated seeds the enhanced transcriptional activity of gene *PGU* predominates, in the tubercle – *PRX*, in underground sprout – *PRX* and *CHS*, and in the aerial sprout - *PGU*, *CHS*, *PRX*, followed by *PME*.

The association was revealed of the expression level of genes *PGU*, *CHS*, *PRX* and *PME* with the broomrape attack frequency and degree, at the populations belonging to the race H – Fundulea, Ismail, Ciocilteni, Taraclia, Sarata-Mereseni and race G – Corteni, Sevilia, Gura-Galbenei.

Acknowledgments

The authors bring their sincere thanks to Mrs. Dr., Assoc. Prof. Angela PORT for the assistance in planning and carrying out the laboratory experiments and Mrs. Dr. Stela CLAPCO for the valuable advice concerning the data analysis and interpretation.

This research was performed under the Institutional Project 15.817.05.03F, financed by the Supreme Council for Science and Technology Development (SCSTD) of ASM.

References

1. Acciu A. 2016. Virulence pattern of *Orobanche cumana* populations from Moldovan sunflower agroecosystems. In: Proceedings of International Scientific Conference of Ph.D. Students „The contemporary trends of science development: view of young researchers". Chisinau, Vth edition, p. 115 – 120.
2. Alonso L.C., Fernández-Escobar J., López G. Rodríguez-Ojeda M, Sallago F. 1996. New highly virulent sunflower broomrape (*Orobanche cernua Loefl.*) pathotype in Spain. Advances in Parasitic Plant Research. Proc. 6th Int. Symp. Parasitic Weeds. Córdoba, Spain, p. 639-644.
3. Antonova T.S., Araslanova N.M., Guchetl S.Z. et al. 2009. Virulence of sunflower broomrape (*Orobanche cumana Wallr.*) in some regions of Northern Caucasus. HELIA - NOVI SAD-, vol. 32(51), p. 101-110.
4. Antonova T.S., Araslanova N.M., Strelnikov E.A., Ramazanova S.A., Guchetl S.Z., Chelyustnikova T.A. 2013. Distribution of highly virulent races of sunflower broomrape (*Orobanche cumana Wallr.*) in the Southern regions of the Russian Federation. Russian Agricultural Sciences, nr. 3, p. 46-50.
5. Antonova T.S., Ter-Borg S.J. 1996. The role of peroxidase in the resistance of sunflower against *Orobanche cumana* in Russia. Weed Research, nr. 36, p. 113-121.
6. Bar Nun N., Ben-Hod G., Lavi E., Mayer A.M. 1996. Purification of pectin methyl esterase from *Orobanche aegyptiaca*. Phytochemistry, vol. 41, p. 403–406.
7. Ben-Hod G., Losner D., Joel D.M., Mayer A.M. 1993. Pectin methyl esterase in calli and germinating seeds of *Orobanche aegyptiaca*. Phytochemistry, vol. 32, p. 1399–1442.

8. Boller T., He S.Y. 2009. Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. Science, vol. 324, p. 742–744.
9. Burlov V. and Burlov V. Breeding of sunflower resistant to new races of broomrape (*Orobanche cumana Wallr*.). Helia, 2010, vol. 33, p. 165-172.
10. Burlov V.V., Kostyuk S.V. 1976. Inheritance of resistance to a local race of broomrape (*Orobanche cumana Wallr*.) in the sunflower. Genetica (The Hague), nr. 12, p. 151–155.
11. Dao T.T.H., Linthorst H.J.M., Verpoorte R. 2011. Chalcone synthase and its functions in plant resistance. Phytochemistry Reviews, vol. 10(3), p. 397-412.
12. Die J.V., Verdejo C.I.G., Dita M.A., Nadal S., Roman B. 2009. Gene expression analysis of molecular mechanisms of defense induced in *Medicago truncatula* parasitized by *Orobanche crenata*. Plant Physiology and Biochemistry, vol. 47, p. 635–641.
13. Djordjevic M.A., Mathesius U., Arioli T., Weinman J.J., Gartner E. 1997. Chalcone synthase gene expression in transgenic subterranean clover correlates with localised accumulation of flavonoids. Aust J Plant Physiol, vol. 24, vol. 119–132.
14. Duca M., Acciu A., Clapco S. 2017. Geographical distribution and characteristics of some populations of *O. cumana* in the Republic of Moldova. Newsletter of the Academy of Sciences of Moldova. Life Sciences. (Bulletinul Academiei de Ştiinţe a Moldovei. Ştiinţele vieţii. 2017, (Rom.) (*in press*).
15. Duca M., Manolache C., Chilari R. 2011. Cultura florii-soarelui (*Helianthus annuus L*.). Repere istorice. Akademos, nr. 3(22), p. 68-76.
16. Fernandez Escobar J., Rodriguez Ojeda M. I., Fernandez Martinez J. M., Alonso L.C. 2009. Sunflower broomrape (*Orobanche cumana Wallr*.) in Castillaleon, a traditionally non-infested area in northern Spain. Helia, nr. 32(51), p. 57-64.
17. Fernandez-Martinez J.M., Dominguez J. 2008. Update on breeding for resistance to sunflower broomrape. Helia, nr. 48, p. 73-84.
18. Gisca I., Acciu A., Glijin A., Duca M. 2013. Highly virulent races of sunflower broomrape in the Republic of Moldova. Proc. Biotech. Congress. Current Opinion in Biotech., v. 24(1). p. S132.
19. Glijin A. 2009. Interaction between *Orobanche cumana Wallr*. and *Helianthus annuus L.* at the level of root system. Journal Environment, Chisinau. nr. 5(47), p. 31-36.
20. Glijin A. 2012. Sunflower hybrids response to natural broomrape infection in Republic of Moldova. In: Proc. III Vavilov InternationalConference “N. I. Vavilov’s ideas in the modern world”. St. Petersburg. p. 119-120.
21. Gonzalez-Verdejo C. I., Barandiaran X., Moreno M. T., Cubero J. I., Di Pietro A. 2006. A peroxidase gene expressed during early developmental stages of the parasitic plant Orobanche ramosa. J. Exp. Bot. 57, 185–192
22. Gonzalez-Verdejo C.I., Moreno-Yanguela M.T., Cubero J.I. and DiPietro A. 2004. Cloning and analysis of a peroxidase gene expressed during early infection stages of the parasitic plant *Orobanche ramosa*. Cost- 849 Meeting (Nitra, Slovakia).
23. Honaas LA, Wafula EK, Yang Z, Der JP, Wickett NJ, Altman NS, Taylor CG, Yoder JI, Timko MP, Westwood JH, et al. 2013. Functional genomics of a generalist parasitic plant: laser microdissection of host-parasite interface reveals host-specific patterns of parasite gene expression. BMC Plant Biol., vol. 13, p. 9.
24. Joel D., Losner-Goshen D., Hershenhorn J., Goldwasser Y., Assayag M. 1996. The haustorium and its development in compatible and resistant host. In: Moreno M, Cubero J, Berner D, Joel D, Musselman L, Parker C, editor. Advances in parasitic plant research. Sevilla, Spain: Junta de Andalucía, Consejería de Agricultura y Pesca, p. 531–541.
25. Kaya Y., Demerci M., Evci G. 2004a. Sunflower (*Helianthus annuus L*.) breeding in Turkey for broomrape (*Orobanche cernua Loeffl*.) and herbicide resistance. Helia, nr. 27(40), p. 199-210.
26. Kaya Y. et al. 2004b. Determining new broomrape-infested areas, resistant lines and hybrids in Trakya region of Turkey. Helia, vol. 27, p. 211-218.
27. Leclercq P. 1969. The sterile male cytoplasmic chezie tournesoli. Annals de l’Amelioration, des plantes, vol. 19: 99-106.

28. Livak K.J., Schmittgen T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods, vol. 25(4), p. 402-408.
29. Losner-Goshen, D., Portnoy, V.H., Mayer, A.M., Joel, D.M. 1998. Pectolytic activity by the haustorium of the parasitic plant *Orobanche* L (*Orobanchaceae*) in host roots. Ann. Bot., vol. 81, p. 319–326.
30. Masirevic S., Malidza G. 2006. Problem and control of broomrape (in Serbian). Biljni Lekar (Plant Doctor), vol. 34, p. 353-360.
31. Mayer A.M. 2006. Pathogenesis by fungi and by parasitic plants: Similarities and differences. Phytoparasitica, vol. 34(1), p. 3–16.
32. Nagar R., Singh M., Sanwal G.G. 1984. Cell wall degrading enzymes in *Cuscuta reflexa* and its hosts. J Exp Bot., vol. 35, p.1104–1112.
33. Orozco-Cardenas M., Narvaez-Vasquez J., Ryan Clarence A. 2001. Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. The Plant Cell, vol. 13, p. 179-191.
34. Păcureanu Joiţa M., Jose M. Fernandez-Martinez M., Sava E., Raranciuc S. 2009. Broomrape (*Orobanche cumana Wallr.*), the most important parasite in sunflower. I.N.C.D.A. Fundulea, VOL. LXXVII, Genetică şi ameliorarea plantelor, p. 49-56.
35. Păcureanu M., Veronesi C., Raranciuc S., Stanciu D. 2004. Parasite-Host plant interaction of *Orobanche cumana Wallr. (Orobanche cernua Loefl.)* with *Helianthus annuus*. In: Seiler, G.J. (Ed.), Proc. 16th Int. Sunflower Conf., Fargo, ND. Int. Sunflower Assoc., Paris. p. 171-177.
36. Parker C. 2009. Observations on the current status of *Orobanche* and *Striga* problems worldwide. Pest Manage. Sci. vol. 65, p. 453-459.
37. Perez-de-Luque A. 2013. Haustorium invasion into host tissues. In: Joel DM, Gressel J, Musselman LJ, editors. Parasitic *Orobanchaceae* - parasitic mechanisms and control strategies. Heidelberg (Germany): Springer, p. 82–83.
38. Placek E.M. 1921. Imunnitet podsolnecnika k porazeniju zarazihoj (*Orobanche cumana*). Izvestija Saratovskoj S H. Saratov, v. 3(1-2), p. 1-20.
39. Ranjan A., Ichihashi Y., Farhi M., Zumstein K., Townsley B., David-Schwartz R., Sinha N.R. 2014. De novo assembly and characterization of the transcriptome of the parasitic weed *Cuscuta pentagona* identifies genes associated with plant parasitism. Plant Physiol., vol. 166, p. 1186–1199.
40. Rotarenco V. 2010. Morpho-physiological and genetic aspects of host-parasite interaction (*Helianthus annuus L. – Orobanche cumana Wallr*.). Autoreferate of Ph.D.Thesis, State University of Moldova, Chisinau. 24 p.
41. Runyon J.B., Mescher M.C., Felton G.W., De Moraes C.M. 2010. Parasitism by *Cuscuta pentagona* sequentially induces JA and SA defence pathways in tomato. Plant Cell Environ., vol. 33, p. 290–303.
42. Sharova P.G. 1969. Selectia podsolnecinika na imunitet k agresivnim rasam moldavskoi populiatii zarazihi. P.G. Sharova, Trudi Vsesoiuznogo sovescianiia po imunitetu rastenii. Kiev, p. 28-31 (in Russian).
43. Sharova P.G. 1977. Zaraziha – opasnii parazit podsolnecinika. Chisinau. 42 p. (in Russian).
44. Shindrova P. 2006. Broomrape (*Orobanche Cumana Wallr*.) in Bulgaria: Distribution and race composition. Helia. v. 29(44), p. 111-120.
45. Shomer-Ilan A. 1993. Germinating seeds of the root parasite *Orobanche aegyptiaca Pers*. excrete enzymes with carbohydrase activity. Symbiosis, 15:61–70.
46. Singh A., Singh M. 1993. Cell-wall degrading enzymes in *Orobanche aegyptiaca* and its host *Brassica campestris*. Physiol Plant, vol. 89, p. 177–181.
47. *T*orres M.J., Tomilov A.A., Tomilova N., Reagan R.L., Yoder J.I. 2005. Pscroph, a parasitic plant EST database enriched for parasite associated transcripts. BMC Plant Biol., vol. 5, p. 24.
48. Torto-Alalibo T., Collmer C.W., Lindeberg M., Bird D., Collmer A., Tyler B.M. 2009. Common and contrasting themes in host cell-targeted effectors from bacterial, fungal, oomycete and nematode plant symbionts described using the Gene Ontology. BMC Microbiol, 9.
49. Vaughn K.C. 2002. Attachment of the parasitic weed dodder to the host. Protoplasma, vol. 219, p. 227–237.

50. Vaughn K.C. 2003. Dodder hyphae invade the host: a structural and immunocytochemical characterization. Protoplasma, vol. 220, p. 189–200.
51. Veronesi C. and Thalouarn E. 2001. Virulence variability among different *Orobanche cumana Walls.* races. Proc. 7th Int. Parasitic Weed Symposium (Nantes, France), p. 245.
52. Veronesi C., Bonnin E., Benharrat H., Fer A., Thalouarn P. 2005. Are pectinolytic activities of *Orobanche cumana* seedlings related to virulence towards sunflower? Israel J. Plant Sci., vol. 53, p. 19-27.
53. Vranceanu, AV., Tudor, F.M. Stoenescu, and N. Pirvu. 1980. Virulence groups of *Orobanche cumana Wallr.*, differential hosts and resistance sources and gens in Sunflower. Proc. 9th Int. Sunflower Conf. Torremolinos, Spain, vol. 1, p. 74-82.
54. Yang Z., Wafula E.K., Honaas L.A. et al. 2015. Comparative Transcriptome Analyses Reveal Core Parasitism Genes and Suggest Gene Duplication and Repurposing as Sources of Structural Novelty. Molecular Biology and Evolution, vol. 32(3), p. 767-790.

Access this Article in Online	
	Website: www.ijarbs.com
Quick Response Code	Subject: Genetics
DOI:10.22192/ijarbs.2017.04.10.025	

How to cite this article:
Duca Maria, Boicu Adriana, Abduşa Daniela. (2017). Expression of some genes associated with aggressivity at the different Broomrape Races. Int. J. Adv. Res. Biol. Sci. 4(10): 194-203.
DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.10.025