SOME ASPECTS OF HOST-PARASITE INTERACTION IN THE SYSTEM SUNFLOWER (*HELIANTHUS ANNUUS* L.) – BROOMRAPE (*OROBANCHE CUMANA* WALLR.)

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Introduction

Sunflower broomrape (*Orobanche cumana* Wallr.) is an obligate, holoparasitic angiosperm that connects to the vascular system of their host plant through a specialized structure known as haustorium and lives attached to the roots of sunflower, depleting the plant of nutrients and water. This parasitic plant is regarded as one of the most important constraints on sunflower production in areas of eastern and southern Europe, the Middle East, Russia, Ukraine and China [20], including Republic of Moldova. A high infestation level can cause total yield loss.

The plant defense system. To be successful, pathogens have to breach several lines of defense, the first of which includes passive defenses such as the leaf wax layer, cell wall, and preformed antimicrobial compounds. Those pathogens able to penetrate beyond this barrier will try to establish a relationship with the plant. Within minutes of contact between the pathogen and the host, reactive oxygen species (ROS) are produced that activate defense gene expression in adjacent cells, for example by causing the accumulation of transcripts of oxidation stress-protective glutathione S-transferases, by initiating the hypersensitive response (HR) or by induction of pathogenesis-related proteins [10]. HR is a form of programmed plant cell death which results from a reprogramming of metabolism in cells surrounding the infection in order to stop the spread of the pathogen. This localized response can trigger a long-lasting systemic response, systemic acquired resistance (SAR), which has been shown to be effective against a broad range of pathogens [35]. SAR is associated with accumulation of salicylic acid, H_2O_2 and a pathogenesis-related (PR) proteins belonging to the chitinase family. Recent reports suggest that sunflower resistance to broomrape can be enhanced by treating seeds with benzothiadiazole (BTH), a structural analogue of salicylic acid, a chemical inducer of systemic acquired resistance (SAR) [30]. Plant defense against pathogens is regulated by multiple signal transduction pathways. These signaling pathways involve three major endogenous plant signal molecules: salicylic acid, jasmonic acid and ethylene. Over the course of the infection, changes were observed across functional categories of genes including those involved in metabolic enzymes, cellular organization, signal transduction, control of gene expression, stress response-related and other unknown genes [11].

Sunflower response to *Orobanche* parasitism. Plant resistance to parasitic plants is a complex multifactorial process and depends on the host (species, varieties and populations), the parasite (species and races or populations), and biotic and abiotic environmental factors. Although much is known about the parasite's biological cycle and the effect of broomrapes on susceptible hosts less is known about the molecular basis of host resistance to these parasites. A better knowledge of the mechanisms responsible for resistance to broomrape is necessary to improve the production of sunflower with long-lasting resistance. Recent studies have revealed that *pre-haustorial* mechanisms (prevents parasite penetration into the host central cylinder of plant) and *post-haustorial* mechanisms (parasite reaches the host central cylinder of plant, but it is killed before developing a haustorium) are involved in resistance to parasitic plants [17].

Therefore, several groups of sunflower responses to broomrape parasitism have been described: synthesis, accumulation and excretion of toxic compounds (7-hydroxylated simple coumarins) by the host plant prevents successful germination, penetration and/or parasitic plant connection to the host vascular system [68]; production and accumulation of phytoalexins (phenolic compounds) in the roots of host plants [6; 8; 16; 17; 31]; host cell browning around the penetration site and necrosis of developing tubercules [8]; cell wall phenolic deposition, vessel occlusion, lignification of host endodermis and xylem vessels, and sealing of host xylem vessels by deposition of mucilage [3; 14; 23]; hypersensitive response (HR) or pathogenesis-related protein induction [10; 21]; high levels of peroxidase activities [8; 9]; callose deposition and reactive oxygen species (ROS) production and accumulation [21].

Skoric and Pacureanu (2010) highlighted three important types of sunflower resistance to broomrape that can be used in sunflower breeding:
1) resistance acting at an early stage in broomrape development (*H. debilis* ssp. *debilis*), when broomrape seedlings were present on the sunflower root, but an impassable encapsulation layer blocked the intruding parasite, which then died;
2) resistance found in the resistant line LR1, which involves two types of action: a) decreased stimulation of broomrape germination (a three-fold reduction compared to susceptible line 2603); and b) rapid necrosis that appeared as early as stage 2 of parasite development;
3) resistance observed at a later stage of broomrape development in the line 92B6 (necrosis developing prior to broomrape flowering).

A potential strategy in sunflower breeding for resistance to *Orobanche* might be directed at interrupting specific developmental stages as germination, appressorium formation, haustorial induction, attachment, penetration and connection to the vascular system, development, emergence and flowering [31]. Most of the studies have focused on identifying the host signals that induce germination and appressorium formation. It has also been reported that once parasite seeds have germinated and become attached to the host root, either root tissue penetration and connection to the vascular tissue or tubercle development are prevented in resistant hosts [16].

Genetics of sunflower resistance to *Orobanche.* Genetic resistance to broomrape has been introduced into sunflower cultivars from early sunflower breeding programs in the former USSR [26]. The sources of resistance to *Orobanche* races found in the early sunflower breeding work in the former USSR originated from land races of cultivated sunflowers, but genetic resistance was also introduced into susceptible sunflower from wild *Helianthus* species [7; 26]. Sources of resistance to recent virulent races have been scant in cultivated germplasm [7]. However, the widespread use of resistant cultivars has led to the appearance of new races of the parasite that overcome the resistance genes, and a continuous need for new resistance sources. Thus it appears necessary to introgress several resistance mechanisms in one sunflower line to prolong broomrape resistance in the field. Most of the resistant sources have been found to be controlled by major genes (vertical resistance), although quantitative (horizontal resistance) resistance and epistatic effects have has also been reported [7]. In a classic study, Vranceanu et al. (1980) identified five physiological races, named A through E, of the parasite and suggested the presence of five different dominant resistance genes, *Or1* to *Or5*, based on the five different hosts. These genes have also been used in breeding programs and reported by other authors who agreed with monogenic and dominant inheritance of resistance to sunflower broomrape [12; 33], although two dominant genes and one recessive gene [28] have also been reported. However, the mechanisms linked with these monogenic resistances have never been clearly identified.

In 1995, a new race F, overcoming all the known resistance genes, *Or1* through *Or5*, was identified in Spain and it spread rapidly [7]. When race F subsequently appeared in Romania and resistance to it was discovered in the line LC-1093 (*Or6*) by Pacureanu-Joita et al. (1998), this cycle of genetic research was completed. Resistance to this new race was found in germplasm of both cultivated and wild sunflower [7; 28; 33]. Genetic resistance to race F in the germplasm sources P-96 and KI-534, derived from cultivated sunflower, was found to be controlled by recessive alleles at two loci [1; 28]. These recessive genes also controlled resistance to race E in the case of KI-534 [28], while resistance to race E in P-96 was conferred by the dominant gene *Or5* [25]. Dominguez (1996), identified the line R-41 having resistance to broomrape controlled by two independent dominant genes. However, a more virulent race (designated race G), attacking the cultivars resistant to race F, has been identified [7].

Dominance relationships and genetic control of broomrape resistance in sunflower is highly on the race of broomrape, the source of resistance, and also the susceptible parental line used for the cross [24].

Quantitative trait loci (QTL) mapping and marker-assisted selection (MAS) breeding. Resistance of simple inheritance, acting after parasite penetration, has been exploited in breeding and single dominant genes for resistance *Or1–Or5* have been progressively introduced in commercial sunflower hybrids as soon as new *O. cumana* races appeared. Resistance of complex inheritance that was neglected in the past in favour of monogenic resistance has also been identified in sunflower [25]. Thus, the resistance to broomrape in sunflower is controlled by a combination of a qualitative, race-specific resistance com-

ponent affecting the presence or absence of broomrape, and a quantitative, non-race specific resistance component affecting the number of broomrape stalks per plant [7; 32].

Sunflower breeders realised the need to accumulate levels of quantitative resistance together with qualitative resistance to avoid breakdown of resistance due to new races of the parasite [25]. Combination of these different resistance mechanisms into a single cultivar should provide a more durable resistance. This can be facilitated by the adoption of marker-assisted selection (MAS) techniques [25], together with the use of *in vitro* screening methods that allow dissecting parasitic weed resistance into highly heritable components [29].

Molecular studies aimed to map genes conferring resistance to race E have been carried out. The identification of RAPD (Random Amplified Polymorphic DNA), SCAR (sequenced characterized amplified region) and SSR (simple sequence repeat) markers, linked to the *Or5* gene conferring sunflower resistance to race E of broomrape, have been proposed to assist breeding for sunflower resistance [18; 34]. The *Or5* gene conferring resistance to race E has been mapped to a telomeric region of linkage group (LG) 3 of the sunflower genetic map [18; 25; 34]. *Or5* has not been placed on the SSR map of sunflower and few DNA markers are presently found in the region surrounding *Or5*. Thus, the five SCAR markers, RTS05, RTS28, RTS40, RTS29 and RTS41, were significantly linked to the *Or5* gene and mapped separately at 5.6, 13.6, 14.1, 21.4 and 39.4 cM from the *Or5* locus on one side, while the RAPD marker, UBC120_660, was found at 22.5 cM on the opposite side [18].

The combination of genetic mapping with gene expression studies, should clearly help in understanding the molecular bases and genetic control of broomrape resistance in sunflower and speeding up the development of resistant inbred lines though marker-assisted selection [7; 25].

The objectives of our research were: to estimate the infestation degree with broomrape of different sunflower genotypes and to test these genotypes for resistance; to screen the presence of the *Or5* gene in the studied sunflower genotypes using PCR-SCAR analysis and to identify molecular SCAR markers, linked to this gene; to study the protein metabolism in sunflower genotypes infested with broomrape.

Materials and methods

Plant materials and growth conditions. The plant materials used for the study were 53 genotypes of sunflower, including: 3 hybrids and their parental lines (*Drofa, Valentino* and *Xenia*), 11 male sterile lines (*ASC 1-11*); 6 hybrids (*Olea, Oxana, Performer, Alcazar, Favorit, Turbo*) and 27 inbred perspective lines (FS_1 - FS_{27}) has been used as a research objects. Broomrape seeds were collected from the South of Moldova. The behavior of sunflower genotypes regarding resistance to broomrape was studied under artificial infestation conditions.

Pot experiments. The characterization of the resistant/susceptible phenotype of sunflower genotypes was conducted by pot infection experiments under glasshouse and natural light conditions. Sunflower plants were grown in a sand/compost mixture (1:1, v/v) in 0,2; 1,0 and 10,0 kg plastic pots. The soil of each pot was mixed with 30-40 mg of broomrape seeds per 200 g of soil [37].

Morpho-physiological parameters such as: plants height, roots lenght and leaf area were analysed according to Duca et al. (2001).

DNA isolation and PCR. DNA was isolated from young leaves using CTAB [4]. For amplification of SCAR RTS05 marker was used the following primers: 5´-TGGTCGCAGATGGACGTGTGGGTG and 5´- GTCGCAGAGAGTGAGAGAG AGTGT (Alpha DNA, Canada).

Isolation of total proteins. The soluble proteins were isolated from leaves during 30 min in tampon solution: Tris-HCl 0.628 mM, pH=6.8 (1g sample : 5 ml), ascorbic acid 0.03%, EDTA 1 mM, glycerin, 6% β-mercaptoetanol. After that the solution was centrifuged at 6000 rotations/min in 15 min. Protein sediment has been purified according to Reva et al. (2001).

SDS-PAGE electrophoresis. Electrophoresis was performed in vertical plates of polyacrylamide gel under denaturing conditions, according to standard method [15]. Molecular mass of polypeptides has been determined according to molecular weight of some standard proteins (SDS Molecular Weight markers Proteins, USA).

Protein lab Chip technology. Protein analysis based on Protein lab Chip technology and 2100expert_Protein 230 program has been performed at Bioanalzyer (Agilent 2100 Bioanalyzer, Germania) according to Kuschel (2000) recomandations.

Statistical analysis of data was carried out according to standard methods [36].

Results and discussions

Phenotypic evaluation of broomrape infestation at different sunflower genotypes. One of the objectives of the present work was to study the reaction of several sunflower genotypes to the broomrape attack. Upon germination, stimulated by specific root host-exuded chemical signals, broomrape seed develops a small radicle – appressorium (fig. 1,A), that attaches itself to the host root and differentiates into a haustorium, the infective attachment organ (fig. 1, B). First attachments were observed after 15 days cultivation at inbred lines of hybrids Xenia and Valentino (the most of all were detected at maternal line of hybrid Valentino). After host tissue penetration and connection to the vascular system, the parasite begins to use the host resources, gradually forming a tubercle (fig.1, C, D). The next developmental stages of broomrape biological cycle are adventitious root and shoot formation and growth on the tubercle (fig.1, E); shoot emergence (fig. 1, F); flowering; setting of seeds.

The tubercles with adventitious roots were appeared on the sunflower roots for the first time at 30 days post-infestation. The maximal number of the tubercles was reported at hybrid Xenia and paternal line of hybrid Valentino. From 40 day cultivation the underground broomrape shoots were observed on the host roots. The maximal number of underground broomrape shoots was noted at parental lines of hybrid Valentino (♀ - 5,2; ♂ - 5,6 underground shoots/plant). The first emerged shoots were registered at genotype Valentino ♀ at 48 day post-infestation and at hybrid Xenia and maternal line of hybrid Xenia at 50 day after infestation.

The most affected genotypes were hybrid Xenia and inbred lines Valentino. The maximal number of attachments (23,5 attachments/plant) at hybrid Xenia was reported at 40 day after inoculation and 43,7 attachments at inbred lines Valentino was noted at 50 day post-infestation. One of the most common incompatible host-parasite interactions described in the literature is the darkening or necrosis of developing tubercles [14; 22] (fig. 1. G, H). Histological studies have revealed that initial vascular connections are established and tubercles develop, but they then become dark and the parasite dies at an early developmental stage [14; 22]. The presence of substances containing carbohydrates and polyphenols inside host vessels has been associated with the darkening of broomrape tubercles [14; 22; 23] and it is possible that these substances block the vessels and interfere with the nutrient flux between host and parasite [22; 23]. Darkening of established tubercles has been usually asociated to a hypersensitive response (HR) [14]. It has been suggested that a hypersensitive reaction (HR) takes place after invasion of sunflower by broomrape and prevented penetration of haustorium into the cortex and the further development of the parasite.

The highest proportion of necrotic tubercles was found at 60 days post-infestation at heterozygous genotypes (0,6-0,63 necrosis/plant) and at one of inbred line of these hybrids (Xenia ♀ - 0,2 necrosis/plant and Valentino ♂ - 0,7 necrosis/plant).

Established broomrapes were quantified and classified according to their different development stages from day 15 to day 60 after infestation. The highest degree of broomrape infestation with the maximal number of attachments was observed in the maternal genotype Valentino and the lowest degree of infestation was confirmed in the paternal genotype Xenia.

It should be noted that 10 genotypes were totally resistant to broomrape (FS_9, FS_{12}, FS_{17}, FS_{21}, FS_{26}, FS_{27}, ASC_1, Alcazar F_1, Favorit F_1, Turbo F_1,), 5 genotypes were tolerant (FS_4, FS_8, FS_{11}, ASC_2, Xenia ♂), one genotype was susceptible (FS_{10}) and 37 genotypes were extremelly susceptible. The Valentino hybrid has related The highest level of infestation has been note dat the Valentino hybrid. So, the presence of parasite attachments with further development of broomrape was reported at 43 sunflower genotypes (higher than 80%).

The results of the present study indicate that according to their resistance to broomrape, 53 sunflower genotypes might be classified in four groups according to IASAS scale [2]: resistant (infestation degree is below 5%), medium-resistant (infestation degree is 5-20%), susceptible (infestation degree is 20-50%) and fully susceptible (infestation degree is higher than 50%).

Screening the presence of the *Or5* gene in the studied sunflower genotypes. Few DNA markers are presently found in the region surrounding *Or5* gene that confers resistance to a broomrape race E. The nearest marker, RTS05, was estimated at 5,6 cM from the *Or5* locus. The presence of this marker in genome is associated with presence of *Or5* gene. This marker should facilitate the efficient transfer of the *Or5* gene among sunflower breeding lines and could be used in a MAS procedure for the selection of new resistant lines to broomrape [18].

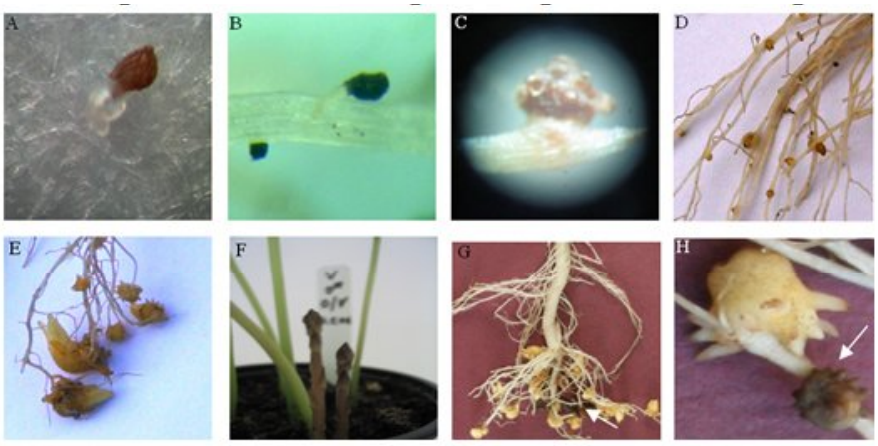

Fig. 1. The development of broomrape on the sunflower roots.
A – seed germination; B – attachment; C, D – tubercle development; E – adventitious root and underground shoot formation on the tubercle; F – shoot emergence; C, G, H – necrosis of tubercles.

Molecular screening of 53 sunflower genotypes has shown the presence of RTS05 locus in the most of samples, except maternal line Drofa, the line ASC_7 and inbred lines FS_7, FS_{14}, FS_{16}, FS_{24}. Lack of this marker suggests that these lines do not contain the *Or5* gene and are susceptible to race E of broomrape.

The authenticity of PCR product was checked by restriction analysis with enzyme *Taq* I (5' T/CGA 3'). The restriction analysis of the 650 bp amplicon has detected two digested products of 530 bp and 120 bp, at resistant genotypes (fig. 2 A.), at susceptible and extremely susceptible genotypes (fig. 2 B.). The data obtained here confirm the dominant inheritance mode of the RTS05 locus according to *Taq* I polymorphism in all 47 analyzed resistant genotypes.

Protein metabolism in sunflower − broomrape system. The hypersensitive response (HR) to a pathogen and systemic acquired resistance (SAR) are the efficient plant defense mechanisms that assures adaptation to stressful environment and leads to the induction of numerous plant genes encoding defense proteins. A group of plant-coded proteins induced by different stress stimuli is pathogenesis-related proteins (PR) which represent major quantitative changes in soluble protein during the defense response [35]. In this context, one of the goals of our research was to analyze polypeptide spectrums of total soluble proteins at different sunflower genotypes infested with broomrape.

The electrophoretic spectrums of total soluble proteins extracted from sunflower roots at different ontogenetical stages were examined at hybrids Xenia, Valentino and their paternal lines. Approximately 30-40 protein components with molecular weight located in 10,0-120,0 kDa range were detected at each ontogenetical stages − 15, 20, 30, 40, 50, 60 days post-infestation. Broomrape infestation changes insignificantly the number of polypeptide tracks at studied genotypes. But, at different ontogenetical stages, modification of electrophoretic tracks colour was observed, which means that the quantity of polypeptides was different at infested samples. So, at 15 and 20 days post-infestation, intensification of synthesis 16,5; 26,8; 28,5 and 36,2 kDa polypeptide fractions at hybrid Xenia and its parental lines has been established. Since 30/40 days post-infestation, intensification of synthesis the same polypeptide components − 16,5; 26,8; 36,2 kDa has been observed only at Xenia matern line.

According to our results Valentino genotypes were less susceptible to broomrape infestation than Xenia genotypes at polypeptide level. Valentino genotypes showed the first reaction of susceptibility to broomrape infestation at polypeptide level at 30/40 days post-infestation and after 10 days the sunflower response was decreased in intensity.

Broomrape attack also caused early synthesis of high molecular weight polypeptides (68,5; 70,3 and 111,0 kDa), small and medium molecular weight polypeptides (11,3; 12,0; 16,5; 21,0; 33,8 şi 35,5 kDa) in roots and leaves at studied genotypes. The present data confirm that most of detected polypeptides have a molecular weight (small and medium) characteristic of pathogenesis-related proteins (PR) which show the intensity of plant defense response.

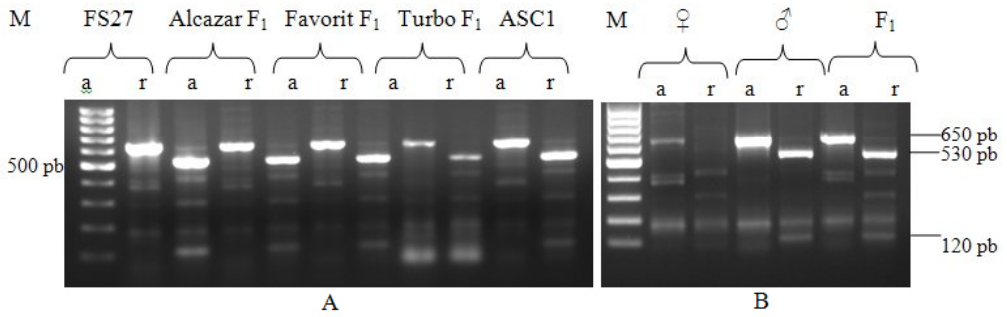

Fig. 2. Restriction profile of RTS 05 locus at genotypes resistant to broomrape (A) and at Xenia hybrid and its parental forms (B).
M – DNA marker (100-1000 pb), a – amplicon, r – restriction products.

Quantitative variation of some polypeptide components at infested sunflower genotypes identified using SDS-PAGE electrophoresis was confirmed by quantification of components using Protein lab Chip technology.

After infestation with broomrape of ♂ Xenia line the amount of 28,2 kDa polypeptide component was reduced from 2327,8 ng/µl to 12,61 ng/µl. The 58,5 kDa polypeptide fraction of ♀ Xenia line was decreased from 389,79 ng/µl to 111,9 ng/µl after infestation. There were no significant differences in the quantity of polypeptides at infested hybrid Xenia F_1 and non-infested control sample.

It has been noted the quantitative and qualitative changes in polypeptide tracks at parental lines of hybrid Valentino in comparison with parental lines of Xenia hybrid. So, it has been reported at ♀ Valentino line the lack of 90,3 kDa polypeptide and the presence of some specific polypeptides: 12,2 kDa (500ng/µl), 25,6 kDa (117,42 ng/µl), 41,3 kDa (177,3 ng/µl); 78,0 kDa (220,78 ng/µl), 137,7 kDa (51,73 ng/µl) and 148,6 kDa (28,86 ng/µl).

Three specific polypeptide tracks − 46,6 kDa (18,76 ng/µl), 78,0 kDa (46,67 ng/µl), 148,9 kDa (1122,7 ng/µl) have been identified at infested ♂ Valentino line. It has also been observed at the same line the lack of two polypeptide components − 90,3 kDa (17,74 ng/µl) and 128,2 kDa (191,93 ng/µl).

Polypeptide electroforegrams of hybrids F_1 have been suggested higher degree of physiological homeostasis and a higher level of adaptability.

In fact, the biochemical analysis data presented here corresponds morphological evaluation of broomrape infestation and indicate that the hypersensitive response (HR) seems to be an important defensive mechanism especially at Xenia genotypes. HR reprogrammed a protein metabolism at early stages of infestation with broomrape, increase the resistance to broomrape and Xenia genotypes was less phenotypic affected by parasite than Valentino genotypes.

Conclusions

The impact of broomrape attack on different sunflower genotypes has been studied at morphological, genetic and protein levels. The obtained data bring new contribution in explication of physiological and molecular mechanisms of sunflower resistance to broomrape.

Seven sunflower genotypes resistant to broomrape − FS9, FS12, FS17, FS21, FS26, FS27, ASC1− have been identified, which can be used in further research and breeding. The resistant Romanian hybrids to broomrape − Alcazar, Favorit şi Turbo − can be recomended for cultivation in affected areas in Moldova.

Transitory changes of sunflower protein metabolism associated with host-parasite interaction have been detected during broomrape infestation at tubercle formation stage. Qualitative analysis of polipeptyde spectrums has been showed early synthesis of high molecular weight polypeptides (68,5; 70,3 and 111,1 kDa), small and medium molecular weight polypeptides (11,3; 12,0; 16,5; 21,0; 33,8 şi 35,5 kDa) in roots and leaves at studied infested genotypes. Most of polypeptides have small and medium molecular weight specific to pathogenesis-related proteins (PR) which show the intensity of plant defense response.

The relationship between the presence of RTS05 locus in sunflower genotypes and its phenotypic resistance to broomrape was tested. It has not been detected correlation between presence of this genetic marker and phenotypic resistance to broomrape in the most of analyzed sunflower lines. It is known that *Orobanche* races change frequently and

although no public reports have been made yet, we suppose there are at least seven races of the broomrape in the Republic of Moldova and the sunflower genotypes could be infested by more aggressive races F or G. In this context it is necessary to investigate all sunflower cultivars and races of broomrape in Moldova using screening in field, greenhouse conditions and at the molecular level.

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RAPD ANALYSIS OF THE GENETIC VARIATION AMONG *CMS*, *Rf* LINES AND HYBRID SUNFLOWER GENOTYPES

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Introduction

One of the most important breeding milestones in sunflower has been the development of hybrid cultivars that made possible the utilization of heterosis. Hybrid vigor in higher plants is often obtained through the use of complete sets of stable male-sterile, maintainers and efficient restorer lines. The first stable source of cytoplasmic male sterility (CMS) was discovered by Leclercq in 1968 [18] from an interspecific cross involving *H. petiolaris Nutt.* and *H. annuus L.* Subsequent identification of genes for fertility restoration in wild species [17] and in certain sunflower cultivars [32, 33] allowed the efficient obtaining of hybrid seed, thus intensifying the interest of seed companies, which led to a considerable increase of sunflower production in many countries.

A total of 72 unique CMS sources have been identified [27] and fertility restoration genes are available for 34 of them. Most of the CMS sources require at least two genes for fertility restoration, although some of them show single-gene restoration [8, 16]. Despite of these achievements, only the first CMS PET1 developed by Leclercq has proved to be very stable and used almost exclusively in breeding programmes throughout the world since late 1970s. Moreother, inheritance studies indicated that practically all restoration lines for this type of CMS carry the same nuclear restorer gene Rf_1 [26].

As it is known, frequent use of the same sterile cytoplasm increases the genetic vulnerability of the present sunflower hybrids to diseases and pests. In order to improve the hybrid performance and the introduction of resistance to different pathogens from wild species into the cultivated species, new lines has to be continuous developed, which is time- and cost – intensive.

Thus, the main objectives of sunflower breeding projects remain focused on inbreeds development with broader genetic base and high combining ability to be converted either into CMS or fertility restorer lines for their future use in hybrid production. While a CMS line can be relatively easily produced and a number of mitochondrial genes associated