INTERNATIONAL SUNFLOWER ASSOCIATION, FRANCE
ACADEMY OF SCIENCES OF MOLDOVA
UNIVERSITY OF ACADEMY OF SCIENCES OF MOLDOVA
SCIENTIFIC SOCIETY OF GENETICISTS AND BREEDERS FROM MOLDOVA
NATIONAL AGRICULTURAL RESEARCH AND DEVELOPMENT INSTITUTE, ROMANIA

INTERNATIONAL SYMPOSIUM ON BROOMRAPE (Orobanche spp.) IN SUNFLOWER

Program and Abstracts
August 25-27, 2011

Chisinau, Republic of Moldova

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WELCOME MESSAGE

It is a great honor and pleasure to the University of the Academy of Sciences of Moldova that the ***International Symposium on Broomrape (Orobanche spp.) in Sunflower*** is organized in the Republic of Moldova.

On behalf of Moldavian scientific community we express our warm welcome to all the participants. We would like to emphasize the importance of the Symposium by the participation of over 50 authors from cca 15 countries, who presented 21 scientific abstracts.

Sunflower broomrape (*Orobanche cumana* Wallr.) is a weedy parasitic organism that represents nowadays a serious constrain for sunflower production in many countries all over the world. It's evolution leads to appearance of new, more virulent, races, which cause yield and quality decrease. Besides, a strong accent must be put on the geographical distribution of the pest, with statistics on the rate of broomrape attack. Thus, materials represent results of recent activities in the field of investigation of sunflower resistance to broomrape, their biology and physiology, but also genetic basics, population studies and pest control.

The effort done for organizing this event is due to our strong desire to strengthen and develop UnASM as a national and international center of competences, education and research in modern directions of science.

This meeting should contribute to a fruitful exchange of experience and opportunity for creation of new collaboration networks on the investigation of broomrape – sunflower interaction.

On behalf of Scientific and Organizing committee,

Professor Maria DUCA

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SYMPOSIUM PROGRAM

Thursday, August 25, 2011	
Arrival day	
Accommodation	
Friday, August 26, 2011	
9:00 - 9:30	*REGISTRATION AND POSTER SETUP*
9:30 - 10:00	*WELCOME*
SESSION "**GROWTH AND DEVELOPMENT OF THE PARASITE**" Chair: **Dr. Miguel CANTAMUTTO** (***ARGENTINA***) **Dr. Yalcin KAYA** (***TURKEY***) **Dr. Maria JOIŢA-PĂCUREANU** (***ROMANIA***)	
10:00 - 10:20	***Some peculiarities of broomrape Orobanche cumana Wallr. ontogenesis on sunflower*** Dr. **Tatiana ANTONOVA** All Russia Research Institute of Oil Crops by the name of V.S. Pustovoit (VNIIMK), Krasnodar, RUSSIA
10:20 - 10:40	***Broomrape seed germination on nutritive media and possibility of it's biological control*** Prof. **Stevan MAŠIREVIĆ** Ministry of Science and Education, Faculty of Agriculture, Novi Sad, SERBIA
10:40 - 11:00	***Sunflower sesuiterpene lactones as germination stimulants for Orobanche cumana*** Prof. **Otmar SPRING** Institut fur Botanik, Fachgebiet Biodiversitat und pflanzliche Interaktion, Universitat Hohenheim, Stuttgart, GERMANY
11:00 - 11:20	***Morpho-physiologic and genetic aspects of host-parasite interaction (Helianthus annuus L. - Orobanche cumana Wallr.)*** Prof. **Maria DUCA**, University of Academy of Sciences of Moldova, Republic of MOLDOVA
11:20 - 12:00	**Coffee Break. Visit of the ASM Museum.**

Chair:	SESSION "**RESISTANCE TO OROBANCHE AND RESISTANCE BREEDING**" **Dr. Tatiana ANTONOVA (*RUSSIA*)** **Prof. Maria DUCA (*Republic of MOLDOVA*)** **Prof. Stevan MAŠIREVIĆ (*SERBIA*)**
12:00 - 12:20	***Investigation of possible sources for resistance to Orobanche cumana Wallr. in sunflower*** Dr. **Michail CHRISTOV** Dobroudja Agricultural Institute, General Toshevo, BULGARIA
12:20 - 12:40	***Results regarding the testing for resistance to broomrape (Orobanche cumana Wallr.) in sunflower crop, in Romania*** PhD st. **Răzvan ŞTIRBU** Biotech Foundation, Bucureşti, ROMÂNIA
12:40 - 13:00	***Broomrape control in sunflower production in Turkey*** Dr. **Yalcin KAYA** Trakya Agricultural Research Institute, Istanbul, TURKEY
13:00 -14:00	**Lunch**
14:00 - 15:00	**Poster Session and Discussions**
15:00 - 18:00	**Cultural program** Visiting **of the famous underground wine city**
Saturday, August 27, 2011	
Chair:	SESSION "**PROGRESS IN OROBANCHE CONTROL**" **Acad. Dragan ŠKORIČ (*SERBIA*)** **Dr. Michail CHRISTOV (*BULGARIA*)** **Prof. Otmar SPRING (*GERMANY*)**
9:30 - 9:50	***Exploring the environmental determinants of Broomrape (Orobanche cumana Wallr.) geographic distribution*** **Dr. Miguel CANTAMUTTO** Universidad Nacional del Sur, Bahia Blanca, Buenos Aires, ARGENTINA
9:50 - 10:10	***Chemical control of broomrape and key weeds with imidazolinone herbicide and resistant hybrids in sunflower production in Turkey*** **Dr. Yalcin KAYA** Trakya Agricultural Research Institute, Istanbul, TURKEY
10:10 - 10:30	***Broomrape (Orobanche cumana Wallr.) control, by breeding in sunflower*** **Dr. Maria JOIŢA-PĂCUREANU** National Agricultural Research and Development Institute, Fundulea, ROMANIA
10:30 - 11:00	**Close**
11:00 - 12:00	**Coffee Break**
12:00 - 14:00	**Visit to the experimental field**

ABSTRACTS

International Symposium on Broomrape (*Orobanche* spp.) in Sunflower

SOME PECULIARITIES OF BROOMRAPE *O. cumana* Wallr. ONTOGENESIS ON SUNFLOWER

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After two decades of absence of a problem connected with *O. cumana* in sunflower fields of the Russian Federation, it has appeared again. In the course of our recent research, new virulent *O. cumana* races (F, G, H) were recognized in different districts of the Rostov region. Diversity of morphological and some other traits of parasitic plants were identified. The purpose of the present research is to describe peculiarities of the diversity *O. cumana* ontogeny found in separate individuals from the most virulent populations in the Rostov region.

It is known, that vegetative propagation of *O. cumana* parasitizing on sunflower in the European countries is a reduced form of "perennial haustorial-root type" with very small potential for a multiplication of shoots in tissues of the tubercle. Rudimentary roots of initial broomrape tubercle, coming into contact to sunflower roots, penetrate into them, forming a haustorium. In the field of penetration a new tubercle forms and then adventive shoot develops from it.

We have found out, that rudiments of the reduced roots of *O. cumana* initial tubercle have also acquired another function. Root apex can develop an adventive stem at once, passing stages: penetrations into the root of sunflower, formation of a haustorium and a new tubercle. Loss of these stages and possibility at once to develop an adventive shoot promote considerable acceleration of additional seeds formation in reproductive process of the same individual.

Formation of shoots plural apexes in one tubercle also became a frequent phenomenon. In the broomrape populations examined, in addition to plants with single stems, there were many samples with 2-3 or 4-6 stems from one tubercle. Some multistem samples with 10-40 stems from one tubercle were observed. Some samples have a rather interesting peculiarity: while the main stem was at the ripening stage, other stems were at different stages of development and the weakest ones were forming buds which tended to flower even before reaching the soil surface. Additional shoots could develop in leaf scale axils on both the tubercle and the main shoot stem. Generally, damaged stems formed few new shoots from the axils of leaf scales. New shoots emerged from the basal and lateral parts of tubercles.

Also from the meristematic cells undifferentiated structures similar to somatic embryos have been observed on the basal surface of tubercle. We speculate that epidermal cells of the *O. cumana* tubercle have acquired the ability to form spontaneous somatic embryos. These adaptations reveal many opportunities for survival of genotype and species in the conditions of constant annihilation.

Usually normal spike-like inflorescence of *O. cumana* consists of friably placed flowers occupying from 1/2 to 2/3 of stem. Other part of stem to soil level is without flowers. Often some peculiarities were observed as all the overground part of the plant was an inflorescence. Such form of *O. cumana* on sunflower has not been described earlier. The length of some such inflorescences reached 90 cm.

In conclusion - *O. cumana* parasitizing on sunflower in the Rostov region of the Russian Federation has a highly developed potential for reproductive function. Individuals found there are capable of the following: forming adventitious shoots from rudimentary roots, increasing number of flowers on a stem from the soil level; simultaneously developing both stems from endogenous and exogenous apexes; developing several adventitious shoots from each tubercle; forming adventitious shoots not only from apical part of a tubercle but also from both basal parts and in axils of stem leaf scales.

TO THE QUESTION OF *O. cumana* Wallr. SEEDS GERMINATION UNDER THE INFLUENCE OF EXUDATES FROM ROOTS OF DIFFERENT CORN HYBRIDS

Araslanova N.M., Antonova T. S., Strelnikov E. A., Tchelustnikova T. A., Ramazanova S. A., Guchetl S. Z.
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At present in some areas in the South of the Russian Federation there have extended a high virulent races of *O. cumana*, affecting all native sunflower assortment and hybrids of foreign breeding. All aspects of its control became actual.

The idea is attractive to use for cleaning of soil from broomrape seeds the crops which are not its hosts, but capable to stimulate germination of seeds. It is known from the middle of the last century. In particular, it has been shown, that the corn can cause germination of broomrape seeds and is thus incompatible with it, as the host.

The purpose of this present research – is to study the germination ability of broomrape seeds from different populations under the influence of root exudations of different corn hybrids.

O. cumana seeds of 8 populations were used in different years gathering and differing by virulence, together with 22 corn hybrids of a different origin. Roots of two-day old seedlings of corn were placed in small volume of water and grew up within 3-5 days. Water was used further as a solution of corn seedlings roots exudates. Similarly sunflower roots exudations have been prepared and used for a control variant.

In 10 days after soaking in a solution of sunflower roots exudations seeds of all broomrape populations germinated on 98-100 %. Soaking of seeds in corn roots exudates has shown that their germination has not been connected with broomrape virulence. Because in all variants with corn hybrids exudation seeds of two populations have shown the greatest germination: most virulent (with the high content of races G and H) and less virulent (with predominance of race E). An exudates from roots of hybrid Arobase stimulated germination of seeds from the mentioned populations on 80 and 59 %, accordingly. But population from the Volgograd area has been germinated only on 5 %. Exudates from roots of other hybrids also almost did not stimulate the germination of seeds of the Volgograd population (from 0 to 10 %). The only way to explain this fact is by insensitiveness of seeds from this population to exudations of corn roots.

The percentage of the germinated seeds of broomrape increased, when they were in direct contact to roots of the corn plants which was grown up in model installations. For example, in 10 days in such an experiment the share of the germinated seeds from the most virulent population on roots of hybrid PR39R 86 reached 93 % (against 64 % - at soaking in exudates). Root shoots of broomrape seeds penetrated into corn roots, however not deeply and perished in external layers of cortex cells.

As a whole, corn hybrids differed by exudates ability of their roots to cause germination of *O. cumana* seeds from different populations. Hybrids Arobase, PR39R 86, ЗПСК 341, Dolar have differed from others by their high ability to stimulate a germination of broomrape seeds from all populations. These hybrids are most suitable as provocative for annihilation of *O. cumana* seeds in soil. However action of hybrids is selective and it is necessary to do preliminary testing as their roots exudates influence the ability of germination of broomrape seeds, collected on each concrete field.

Acknowledgements. The authors render thanks to the head of firm Agrozeminvest P. S. Kosov for the given seeds of corn hybrids.

THE STUDY OF INFLUENCE ON *Orobanche cumana* Wallr. INFECTION OF PEROXIDASE ACTIVITY IN VARIOUS GENOTYPES OF SUNFLOWER

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Plant resistance to phytopathogens is manifested by changes in activity regulation of enzyme systems involved in metabolic processes. Most reactions, including hydrolysis, dehydrogenation, oxidation and decomposition of hydrogen peroxide, are followed by activation of their influence phytopathogens. Since *Orobanche cumana* Wallr. is rhysoparasite perception (the receptor) and signal transmission (mainly through xilem and phloem) as a result of infection is initiated in the root system. Therefore we studied the peroxidase activity in leaves and roots of sunflower plants in normal growth and infection *O.cumana* background. Investigations at the root system showed obvious changes in peroxidase activity. Thus, the background of infection, peroxidase activity increases in maternal forming hybrids *Xenia* and *Valentino*. But note that these hybrids are characterized by very high activity, representing values from 32.5 to 44.2 UR g/mv in comparison to the control. Increased peroxidase activity in roots of infected plants could be determined by an enzyme involved in the synthesis of lignin and other phenolic polymers with training fenilpropanoli - predecessors of lignin, which in the presence of hydrogen peroxide polymerizes, thus contributing to strengthening the cell wall and respectively, blocked parasite invasion and decomposition of hydrogen peroxide which accumulate in cells in response to phytopathogenic. Peroxidase activity of interest year study and control plant leaves infected with *O. cumana*. The analysis results show an opposite phenomenon. There was a significant decrease in infected plants compared with controls. The highest values of the parameters studied was found in *Xenia* hydride forming genotypes grown under normal conditions (RH 47.5 g/mv.) and maternal (42 UR g/mv) and the lower hybrid (21 UR g/mv).

The results of the investigations found that peroxidase activity in leaves of infected plants varied witness and, accordingly, between 21-48 and 20-45 RH w/mv The highest values for forming hybrid genotypes studied in *Xenia*, grown under normal conditions, was found to paternal (RH 47.5 g/mv) and maternal (42 UR g/mv) and the lowest - the hybrid (RH 21 g / mv). Analysis of peroxidase activity in the leaf of plants infected system allowed us to see that this is about 1.2 to 2.5 times higher in leaves than in roots. This is determined by the intensity of metabolic processes occurring in the leaves and the need to maintain balance and homeostasis of cellular oxidation-reduction. Peroxidase activity in leaves varied within 21.43 to 47.95 UR / g / mv. Infection with *O. cumana* has caused a reduction in peroxidase activity of the 1,2 - 2,3 times compared to the control, their values ranging from 25.25 to 47.95 UR/g/mv, except, only, heterozygous genotypes which showed an increase in activity 17.6% - 30.7% in F1 and *Xenia - Valentino* in F1. Such data of peroxidase activity showed that the increase in the root system of sunflower genotypes to attack by the parasite, as a consequence of the increased accumulation of peroxidase and its conversion into nontoxic compounds as well as the possible involvement of peroxidase in cell wall lignifications. Peroxidase activity in leaves under normal conditions is 1.2 to 4.2 and the largest *O. cumana* infection induced reduction of these, we chose the parental lines, which is caused by intense involvement at the site of infection. Also mention that hybrid genotypes have an increased attack *O. cumana* more various.

Therefore, the results of investigations allow us to confirm that the enhanced peroxidase activity can be regarded as an indicator of response reaction of plants to *O.cumana* attack.

BROOMRAPE IN TOBACCO PLANTATION IN MOLDOVA

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Weeds are pests that compete with crops for the main factors of vegetation: water, air, light, heat, etc. Their danger is sometimes so great that they can compromise the production of tobacco raw material, and sometimes including the physical body of the plant.

Though there is a myriad of economic and ecological impediments in cultivating various crops, weeds are considered the paramount obstacle in this process which has to be dealt with. Moreover, the main step that has to be taken in order to win the battle for the crop is to know the plant both its biological and ecological features.

In recent years the tobacco plantations have been often attacked by broomrape, a parasitic plant. The research conducted during 1981-2006 demonstrated that one-flower cancer root (one-flowered broomrape) (*Orobanche ramoza* L.) penetrated the tobacco roots, whereas sunflower broomrape (*Orobanche cumana* Wallr.) is less often encountered in tobacco plantations. Most often broomrape was detected in the north and center of the country. In Edinet, Glodeni, Rezina etc. 10% of the land is attacked by broomrape while in Orhei 33% of the land under tobacco is affected by this vicious parasite. The tobacco plantations from the south of Moldova are less attacked by broomrape. Thus in Cimislia, Comrat and Taraclia broomrape was identified only on 90 hectares.

Through digging it was established that on average on June 18, 11 to 12 buds of 1-5 mm in height, whereas on June 24 the shoots were 5-6 cm in height, hence in this period the sprouting and intensive growth of broomrape take place. The table illustrates that leaf area of the affected plants decreases by 1.4 (30.07) and by 6.5 (1.07). Furthermore, the number of leaves per plant decreases from 1.4 to 3.8 accordingly; consistency was reduced by 1.23 times, and the yield dropped by 7.6 to 1.6 times.

Table 1. Influence of the period of broomrape appearance on the yield and quality of tobacco

Influence of the period of broomrape appearance	Leaf surface from the 2nd cropping, cm^2	Number of leaves on a tobacco plant	Percentage of plant death in the second half of August	Consistency of the leaves from the 3rd cropping	Yield t/ha	Yield of production types
June 29	91.7	6.6	88.5	-	0.55	75.9
July 7	215.0	8.1	80.2	54.80	0.84	92.8
July 15	255.0	9.7	46.2	54.97	1.08	96.2
July 20	330.0	13.6	8.8	57.74	1.80	98.7
July 30	428.0	18.0	0.0	62.61	2.64	99.3
Healthy Plants	600.0	25.5	0.0	67.53	4.17	100.0

In Moldova, some evaluation researches were conducted on broomrape combating methods: the germination of broomrape seeds was provoked with the help of the solutions of several acids (citric with 0.05, 0.025 and 0, 0125%; concentration of succinic, oxisuccinic and dioxisuccinic with 0.03% concentration), juice of tobacco, alfalfa, and rape roots (20-40g of roots diluted in 1 litre of distilled water). In Petri cup the seeds were evenly dispersed and watered with the same solution until germination. Investigations showed that the broomrape germination reached 70.9% only in the experience with the juice from the rape roots.

The results of the field evaluation demonstrated that the roots of the tobacco are parasitized by one-flowered cancer root and less by sunflower broomrape. The damage of this plant depends on the period of its appearance. The early appearance of the parasite leads to the reduction of yield by 5-7.6, whereas the late occurrence only by 1.6 – 2.3. The juice of rape seeds has a combating influence on the germination of broomrape seeds.

METHODS OF COMBATING BROOMRAPE IN TOBACCO PLANTATIONS

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The most inoffensive methods of combating parasitic plant broomrape (*Orobanche spp.)* in tobacco plantations are the technological and agricultural ones which include:

1. In June-July a study of all the cultures attacked by broomrape was carried out (tobacco, sunflower, vegetables and squashes). The observed outbreaks were registered in the field record and on the map of land management. Based on crop rotation, tobacco should be planted on the land that is least attacked by broomrape.

2. In case there is no land which would be free of broomrape the land which is allotted under tobacco should be ploughed in the autumn at a depth of 45 cm. The soils massively attacked by broomrape should be ploughed at the depth of 60 cm, but in subsequent years the depth of plowing should be 15-20 cm in order not to bring the broomrape seed layer to the surface.

3. *Crop rotation and rotation.* Tobacco is advisable to plant tobacco after straw grains, corn, peas, beets and alfalfa. The plantation of tobacco for four years in a row on the same land is forbidden due to harmful organisms (broomrape, diseases and pests), which lead to the reduction of yield per hectare.

4. *Soil fertilization* with mineral fertilizers in major proportions -1t/ha that include nitrogen, phosphorus and potassium significantly reduces broomrape damages.

5. *Planting tobacco.* The optimal timing for planting seedlings in the field is the third decade of April - I-II decade of May, depending on the area (in the south of the country from 28 April to 5 May, in the north from May 5 to May 15 and in the center May 1 to May 10).

6. To accelerate the development, growth and ripening of tobacco plants it is necessary to carry out all agricultural and technological procedures. From planting to harvesting tobacco plants must be properly taken care of. The first cultivation of the land should be performed 7-8 days after planting when plants are rooted, at the depth of 6-8 cm to destroy weeds. The two manual cultivations and two technical cultivations are performed at an interval of 10-15 days, depending on rainfall and weeding. Mound layering of plants of 20-30 cm in height, aid root formation, and increase resistance to broomrape and fall plants.

7. During the blooming period of broomrape the stems should be systematically pulled out and buried or burned at the edge of the field (especially on the fields where the technology of preparing soil was not observed). Floriferous ramifications with seeds that were not ripe should not be left in the field because the seed capsules are able to ripen and increase the harmfulness of the field.

8. Plant remnants from tobacco leaves after the last cropping are chopped with the combine or it is deeply buried in the soil. This speeds up their putrefaction and liquidation of fungi infection, mycelium which can keep the tissue alive, it also stops the development life cycle of trips and Green Scale, reducing their population in the future.

9. At the beginning of the calendar year the biological methods of controlling broomrape can be applied which involve seeding rape at the end of February, beginning of March. When rape sprouts, grows and develops, broomrape sprouts too. After the mowing of rape the necessary plant is planted. Thus the broomrape seeds in the soil are reduced.

10. One of the effective procedures to combat broomrape is sowing sunflower crop after grass forage (mixture of grasses with mash). In spring sunflower seedlings appear, but with them and broomrape appears too. Mowing for forage in the second half of June prevents ripe seed formation and plowing causes death of broomrape vegetation.

INVESTIGATION OF POSSIBLE SOURCES FOR RESISTANCE TO *Orobanche cumana* Wallr. IN SUNFLOWER

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This study was aimed to search opportunities for establishment and revealing the sources for resistance to the new races of the parasite broomrape (***Orobanche cumana*** **Wallr.**) in sunflower. Old populations, sunflower varieties and cultivars, wild *Helianthus* species, species from some genera of family *Compositae*, new sunflower mutant forms and materials, resistant to herbicides in the broomrape infection plots sprayed with the herbicide Pulsar were investigated. It was established that some of the studied material could be used in hybridization or by treatment with gamma rays for obtaining new sunflower forms, resistant to the parasite broomrape. Positive result was received by treatment of plants with different level of resistance to the parasite with the herbicide Pulsar.

Keywords: broomrape, *Helianthus*, *Compositae*, gamma rays, herbicide

OPTIMISATION OF LABORATORY METHOD FOR SUNFLOWER SCREENING FOR RESISTANCE TO BROOMRAPE

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Broomrape species *Orobanche cumana* Wallr. is an annual achlorophyllous plant. It is an obligate parasite attached to the sunflower root depleting it of water and nutrients. Substantial yield loss is often recorded in highly infested areas with virulent races of parasite. Therefore, growing of resistant hybrids as main control measure is necessary.

During the process of breeding, different tests have been developed for the assessment of resistance. The aim of this research was to optimize an in vitro test on filter paper. Main benefit of the test could be shortening of period needed for evaluation in comparison to field tests and test in pots. Broomrape seeds, collected from field where sunflower is tested for resistance, were carefully placed on filter paper and afterwards put in a nylon bag. Seedlings of sunflower line AD-66, chosen because of susceptibility to broomrape, were placed near the top edge of filter paper. MS medium was added two times a week, and plants were grown under constant illumination. However, the conditions set in chamber proved to not be adequate for growth of sunflower plants and plant decay was noticed as early as 7 days after starting the test. Slight improvement was achieved by additional sterilization of seeds with ethanol and sodium-hypochlorite. Further modifications will be made by setting a photoperiod, decreasing light intensity and by choice of a different medium.

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RESEARCH REGARDING THE VIRULENCE OF BROOMRAPE PARASITE *Orobanche cumana* Wallr. IN SOUTH-EASTERN PART OF ROMANIA

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Broomrape (*Orobanche cumana Wallr.*) becomes one of the most serious parasites for sunflower crop in Romania mainly in the south-eastern part of the country. During last two decades the aggressivity of the parasite increased significantly, the new physiological races appeared fast enough after a relative stable period of time on race E of broomrape. Research concerning races evolution carried out in different Institutes in Eastern Europe or Spain showed that the interaction host-parasite system the gene action was dominant type up to the level of race E beeing involved one single gene. Increasing the virulence of the parasite up to the race F or G, a new type of interaction was identified, being involved one or more genes for resistance dominant or recessive ones depending of the genetic material involved in the studies. This study carried out during 2009 and 2010 by Procera Agrochemicals in 5 location in South-eastern part of Romania identified the race G based on a differential set established on the characterization concerning the resistance of commercial hybrids available in commercial seeds catalog. It was included one hybrid without resistance genes (Performer), one race E resistant hybrid (PR64A89). The race F resistant hybrid was PRO229 (Procera) and the race G was PR64A71. This last one was characterized as „ resistant against races more aggressive than E" in the Pioneer Hi-Bred-Seeds Agro.srl Romania" and as „resistant against race G" by Pacureanu in 2009.

The susceptible check and the resistant one planted on the borders and in the middle of the field for a better tracking of field's infestation. Two reps planted for each differential in each location.

Definitely the race G was identified in Tulcea and Constanta counties based on Infestation values obtained after 2 years of trialing. In some location (no1, no3, no4 and no5) virulence above race G was found because PR64A71 presented a very low number of shoots. It seems to be a new race more aggressive than G and we named G+. The reason of infestation of PR64A71 could be the incomplete homozygocity of the hybrid compounds and in this situation broomrape shoots may appear. For the host spots infested with race G is not recommended to plant race E resistant hybrids because of the very high yield decrease. The hybrids race F resistant may register as well significant yield decreases in those areas.

Keywords: broomrape, virulence, races, genetic inheritance

Orobanche cumana IMPACT ON THE ONTOGENETIC DYNAMICS OF PROTEIN METABOLISM

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Host-parasite relationship between *Orobanche cumana* and sunflower involves specific molecular mechanisms, which depending on the particular genetic and morpho-physiological of genotypes, determine their susceptibility or resistance to parasite attack. A special role in establishing these relationships it has protein compounds. Data from literature show that accelerated biosynthesis of proteins (PR-related patogenesis) correlate with plant resistance to pest attack [1, 2, 3].

Attaching directly to the roots of sunflower, rizoparasite orobanche causes a metabolic imbalance: food, hormonal, energetic, both at the root and the overground organs, having an indirect impact on the processes of photosynthesis of the plant host [4]. Therefore, to characterize the pathosystem *Orobanche cumana - Helianthus annuus*, a special interest presented highlighting the biochemistry features of the host-parasite interaction on the polypeptide pattern of foliar system.

SDS-PAGE-electrophoresis of proteins extracted from leaves of parental lines and F_1 sunflower hybrid Xenia revealed a molecular diversity of protein component which had some degree of specificity determined, in particular, by the plant genotype and the ontogenetic stage.

Depending on the stage of development of host, electrophoretic spectra plant of genotypes witness revealed the presence of 20-40 polypeptides, distributed in a range of 11.3-142.6 kDa Mr kDa, of which 16 were common fractions F_1 hybrid, as for parental lines.

Between proteins that were expressed under the stress conditions in all investigated genotypes, but at different stages of cultivation, polypeptides of interest were those of about Mr 11, 12, 20 kDa, background expression of infection in all genotypes and polypeptide with Mr 35.5 kDa, which content decreases at F_1 hybrid Xenia, the impact of *O. cumana* on the sunflower leaf system, is however less pronounced compared to that exercised in the root system.

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PHENOTYPIC SCREENING OF BROOMRAPE RESISTANCE AT SUNFLOWER GENOTYPES CULTIVATED IN REPUBLIC OF MOLDOVA

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Sunflower broomrape (*Orobanche cumana* Wallr.) is a weedy parasitic angiosperm that nowadays represents a serious constrain for sunflower production, including in the Republic of Moldova. It keeps expanding its distribution area, forming new and more virulent races. This leads to considerable losses expressed, on the one hand, in yield decrease, and on the other in reduced quality of the obtained produce. With a view of limiting the parasite's distribution and decreasing the losses it causes, it would be preferable to know the distribution area of individual races, and the rate and percentage of broomrape attack. The aim of the present study included screening of analyzed sunflower genotypes, by the presence of parasite's attachments on roots to look for correlation between the presence of *Or5* gene and phenotypic resistance to broomrape.

Fifty three genotypes of sunflower were used as a research objects, including: 3 hybrids and their parental lines (*Drofa*, *Valentino* and *Xenia*), 11 lines with male cytoplasmatic androsterility (*ASC 1-11*); 6 hybrids (*Olea, Oxana, Performer, Alcazar, Favorit, Turbo*) and 27 perspective genotypes (*FS1 - FS27*). The test on broomrape susceptibility has been realized in laboratory conditions, and the level of infection was determined according to recommendations (Alexandrov V. and Dimitrov S., 2007). The evaluation of resistance to infection was performed after three months.

Molecular screening results have been compared with phenotypic resistance of sunflower genotypes to broomrape. Because broomrape attacks sunflower roots, as the affection level index was used the number of parasite attachments. Artificial infection of 53 genotypes of sunflower with *Orobanche cumana* Wallr., collected from the South part of Moldova showed the presence of parasite attachments with further development of pathogens in 43 genotypes, which constitute over 80%. The experience has allowed classifying the analyzed sunflower genotypes in four groups: *resisted*, *tolerant*, *sensitive* and *extremely sensitive*.

The most affected root was detected in cytoplasmic male sterility lines ASC3, ASC4, ASC8 and ASC11. The hybrids Alcazar, Favorit and Turbo are fully resistant to broomrape, results have been confirmed by other sources too (Vranceanu A., 200; Pacureanu-Joita M. et al., 2008). The Valentino hybrid has shown the highest level of parasite attachments. The sunflower genotypes FS9, FS12, FS17, FS21, FS26, FS27 and ASC1 were fully resistant too, while the genotypes FS4, FS8, FS11, ASC2, Xenia ♂ were tolerant to infection. Thirty two genotypes were attributed to sensitive and extremely sensitive groups, although they contain the RTS05 locus. Only genotypes Drofa ♀; ASC7; FS7; FS14; FS16; FS24 demonstrated the correlation between the lack of RTS05 locus and susceptibility to infection. It suggests that the sunflower genotypes could be infected not by race E, but probably by more aggressive race F, G or H.

BROOMRAPE CONTROL IN SUNFLOWER PRODUCTION IN TURKEY

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The main sunflower production area of Turkey is in Trakya Region of Turkey which is European part of Turkey and these areas are mostly infested with new races of broomrape. Sunflower farmers mostly use genetically resistant to broomrape and Imidazolinone (IMI) herbicide resistant hybrids in the production in Trakya region. Sunflower hybrids were evaluated existed in the 2010 registration trials in the most infested areas of Trakya region in the study. PR63F73, XF-4223 PR64H37 and LG5400 hybrids showed resistance to new broomrape races in all locations but PR64H34, NX 53938, 08-TR-003 and Tunca exhibited generally resistance to new races also but in some location they are found as tolerant with staying at less than 20% frequency level in the trials in 2010. While, the highest oil yields were obtained from LG5580, 08-TR-003 and Tunca hybrids; in the seed yield; LG5580, PR63F73, TUNCA and PR64H34 hybrids were ranked respectively in the trials. In the oil content of sunflower hybrids, 08-TR-003 hybrids from Trakya ARE existed in first rank as 52% and DKF-2525, 08-TR-004, NX 53938, 05-TR-198, LG 5400, and TUNCA hybrids followed it respectively. Both broomrape resistant hybrid and also higher seed and oil yielding hybrids also developed by TARI and will start to sell in 2011.

Keywords: Sunflower, Resistance, Broomrape, New Races

CHEMICAL CONTROL OF BROOMRAPE AND KEY WEEDS WITH IMIDAZOLINONE HERBICIDE AND RESISTANT HYBRIDS IN SUNFLOWER PRODUCTION IN TURKEY

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Imidazolinone (IMI) herbicides applied post emergence controls efficiently both broomrape and key weeds such as Xanthium strumarium Wallr. Chenopodium album L., Echinochloa cruss-galli, Solanum nigrum L., Datura stramonium L. etc. in sunflower production in Turkey. The system is calling Clearfield System as the use of IMI herbicide with using IMI resistant hybrids increase year by year and reached about 50% of Trakya Region which is the main sunflower growing area in Turkey. The study covered seed and oil yield performances and herbicide tolerance observations of IMI resistant hybrids in registration trials conducted in Trakya region and also yield performances of experimental hybrids developed in National Sunflower Project conducting by Trakya Agricultural Research Institute (TARI) in 2010. All IMI hybrids in the trials exhibited non herbicide toxicity. However, the main problem of IMI type hybrids in sunflower production in Turkey, higher yielding hybrids have less oil content and yield and in reverse, higher oil content hybrids have lower seed yield. The highest seed yield was obtained from NX63728 and SANAY hybrids from Syngenta Seed Co but they had lower oil content (42-44%). On the other hand, EFE, ARMADA and OLIVA hybrids had higher oil content (48-50%) but lower seed yield. There was a difference of at least 10% in both oil content and also in seed yield between two groups. Therefore, National Sunflower Program conducted by TARI focused mainly on development of higher oil content and oil yielding IMI hybrids, some promising hybrids were obtained in 2010 trials and if they keep high performance this year, they will commercialize in 2011.

Keywords: Sunflower, Herbicide Resistance, IMI resistant Hybrids, Key weeds, Broomrape

EFFECT OF ROOT EXUDATION FROM DIFFERENT SUNFLOWER GENOTYPES ON BROOMRAPE SEEDS GERMINATION

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Growth and development of *Orobanche* occurs at the expense of the host plant (Morozov et al., 2000). Broomrape plant lives directly on their hosts by attaching strong haustoria to their roots, penetrating the tissues, and absorbing the food gathered by the host plants for their own development (Alkhateeb et al., 2003).

After germination, the parasites have a long underground phase, and by the time they emerge, much of the damage has already been produced. Upon germination, stimulated by host root-exuded chemical signals, broomrape seed develops a small seedling that attaches to the host root and differentiates in the attachment organ (appressorium). After host tissue penetration and connection to the vascular system through the haustorium, the parasite becomes a major sink for plant photosynthates, gradually forming a tubercle from which a shoot arises to emerge from the soil to flower and produces seeds (Parker, Riches, 1993; Westwood, 2000; Sira Echevarria-Zomeno et. al., 2006). Appressorium and haustorium development in these plants can be monitored *in vitro* by applying host exudates or purified haustorial inducing factors to aseptic parasite seedlings (Riopel, Musselman, 1979; Baird, Riopel, 1984; Chang, Lynn, 1986).

In the present work, we studied seed germination of five different broomrape populations induced by the root exudates of five different sunflower genotypes. Root exudates of each genotype were assayed separately in each broomrape sample in order to identify a potential recognition pattern in broomrape populations. This may help to discriminate between the specificity requirements of broomrape geographical populations and open a route to understanding the complex interactions between the signal and receptor that trigger germination in each broomrape population. *Orobanche cumana* seeds were collected from five regions of the Republic of Moldova (Balti, Rezeni, Stefan Voda, Cimislia and Ciadir-Lunga). The seed germinability was evaluated using aqueous solution as a control and root exudates obtained from five differentials for the broomrape pathogen races (D, E, F and G). Differentials were provided by NARDI Fundulea, Romania.

Application of root exudates had a significant effect on broomrape germination. Broomrape seeds collected from Cimislia area were highly (40%) stimulated by *Helianthus annuus* L. exudates. In particular, seeds from the South part of the country recorded 70% of germinated seeds on exudate of two differentials for the broomrape pathogen race G – one of the most aggressive races. Interestingly, broomrape seeds from Cimislia did not germinate on exudate from differential to the race D. The comparative study demonstrated that seed germination ability is strictly dependent of the differentials exudate. Thus, on exudate from differential to the race D, germinated seeds of *O. cumana* were only 11%, to race E - 22%, F - 26% and to G race - 44%. *O. cumana* seeds germinated only in presence of the sunflower root exudates and did not germinated in the control. This again confirms the obligatory presence of germination stimulations produced by host-plant. Germinated seeds were viable on exudate 15-20 days, then started to dehydrate and then to necrotizing. It was conditioned by the lack of host plant roots near the broomrape germinated seeds. Thus, seeds of parasitic plants *O. cumana* germinate only if stimulated by host root exudates and start producing a tubercle only if they are near enough to the host roots.

EVALUATION OF RESISTANCE OF SUNFLOWER HYBRIDS TO BROOMRAPE IN NS BREEDING PROGRAMS

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Broomrape (*Orobanche cumana* Wallr.) presents a serious problem in sunflower production in a number of countries, it reduces sunflower seed yield and negatively affects other sunflower traits in sunflower production. Sunflower breeders and geneticists have been successful in responding to the rapid changes in the race composition of broomrape. They found genes for resistance to this pathogen and incorporated them into elite lines of cultivated sunflower, making it possible to develop Orobanche - resistant hybrids (Škorić et al. 2010). The population of broomrape has been stable in Serbia for the longest period of time, but the racial composition has changed in recent years, with race E being predominant in the regions of North Bačka and Banat, (Hladni et al. 2010).

Continued work on creating new sunflower hybrids resistant to broomrape demands testing of breeding materials in both field conditions and in controlled conditions of a greenhouse. The best solution is to do the testing simultaneously in order to verify the congruence of the evaluation of the broomrape resistance achieved by these two methods. The method of testing breeding materials in field conditions is performed in a field infected with broomrape. The reliability of results acquired by this method depends on agro-ecological conditions and the amount of broomrape seeds in the soil. Much more accurate results can be obtained by putting broomrape seeds into in pots containing compost and perlit which are then placed in the controlled environment of a greenhouse.

At the Institute of Field and Vegetable Crops, Novi Sad, with the application of the method of testing lines and hybrids on broomrape in field conditions and controlled greenhouse conditions new hybrids are being created with good production characteristics resistant to broomrape. Based on the achieved seed and oil yield in production conditions in 2008 and 2009 on two locations in comparison to standard chosen were 15 new high productive sunflower (NS-H-6284, NS-H-6287, NS-H-6385, NS-H-6289, NS-H-6721, NS-H-6396, NS-H-6255, NS-H-6249, NS-H-6382, NS-H-6395, NS-H-6242, NS-H-6253, NS-H-6397, NS-H-6250, NS-H-6506) for testing on broomrape on infected field conditions and controlled greenhouse conditions. The screening of the new chosen 15 sunflower hybrids for broomrape resistance was done in 2009 and 2010 in infected field on three locations in Vojvodina Province and in 2010 in glasshouse. Both the field and the greenhouse test rated the hybrids exclusively on the basis of presence or absence of the parasite.

The resistance of new 15 hybrids to broomrape was checked by counting broomrape shoots on three locations in Vojvodina with three controls: hybrid Bačvanin, resistant to race E, hybrid NS-H-111 resistant to race (A, B, C, D) susceptible to E race and line AD-66 susceptible to all broomrape races.

The greenhouse test was done in pots containing 10:1 compost, send and perlit mixture (1:1:1 v:v:v) with addition of broomrape seed. Sunflower plants were grown for 7 weeks using 16 h photoperiod. Broomrape attack was assessed by uprooting and careful observation of root system for broomrape nodules and stalks.

The contamination of hybrids with broomrape has shown that from 15 hybrids tested, with 9 hybrids, the presence of broomrape was not spotted, these hybrids were fully resistant in both years in both field conditions and in controlled greenhouse conditions.

Keywords: breeding, broomrape, hybrids, resistance, sunflower.

MOLECULAR PROFILES OF SUNFLOWER LINES RESISTANT TO BROOMRAPE (*Orobanche cumana* Wallr.)

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Orobanche cumana Wallr. is a parasitic plant that attaches itself to sunflower roots, draws nutrients, weakens the host and therefore causes severe yield loss. Seeds of this holoparasitic angiosperm are very small, and can be transported by wind, insects, animals, or even attached to the sunflower pericarp. They can stay viable in the ground for a long period of time, and germinate successively, in the presence of a host plant.

Broomrape is a world-wide problem. In addition to Russia, Ukraine, Romania, Bulgaria, Turkey, and Spain, it is also present in Serbia, Hungary, Moldova, Greece, Israel, Iran, Kazakhstan, China, Mongolia, and Australia.

Breeding for resistance started early in the 20th century, and resulted in a range of sunflower cultivars. Genetic resistance to broomrape was introduced to sunflower from wild Helianthus species, such as *Helianthus tuberosus*, *Heliantus maximiliani* and *Helianthus debilis*. The resistance of sunflower to broomrape races A-E is conferred by five dominant genes: Or1, Or2, Or3, Or4 and Or5, which are either allelic or tightly linked. Races can be determined by using five sunflower differential lines that were developed by Vranceanu et al. (1980), and presence of Or6 can be determined by differential line that was developed by Pacureanu et al. (1998). The difference in sources of the resistance has lead to development of lines that are resistant to the same race of broomrape but vary in their genetic constitution. As a result, different inheritance patterns have been reported by different authors, especially when it comes to Or6 gene.

In our study we did SSR analysis of 20 sunflower lines, which differ in their resistance to *O. cumana* as well as in their genetic background. The aim of this study was to determine if the markers previously reported to be closely linked to Or locus could be used for MAS. The obtained results did not match the ones reported by other authors. In order to elucidate these differences, we pursued screening the part of the genome of interest, namely LG3 that supposedly carries Or5 locus. Eleven additional SSR markers located on the LG3 (ORS1040, ORS545, ORS1112, ORS683, ORS665, ORS820, ORS718,ORS555, ORS1021, ORS1222, ORS1114) were selected, and lines with the resistance to races E and F but with different genetic background, as well as the differential lines for broomrape races B-D were screened. Different molecular patterns were obtained, proving that the lines differ significantly in molecular profile of the screened region.

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BROOMRAPE (*Orobanche cumana* Wallr.) CONTROL, BY BREEDING IN SUNFLOWER

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The pathogenic composition of broomrape populations has changed over the years, slowly at first, then rapidly in Eastern Europe, Turkey and Spain. In the last years, in Europe, new and aggressive populations of the parasite, have attacked sunflower crop, specially in Turkey, Spain, Romania, Russia, Ucraina and Bulgaria. Sunflower selection for broomrape resistance makes use of different methods for testing breeding materials (in field, greenhouse or at molecular level), looks for resistance sources in certain wild species of genus *Helianthus*. Dominant genes for resistance to races A,B,C,D,E and F, have been found and incorporated into cultivated sunflower.

Greenhouse testing conducted at Fundulea institute in 2009 and 2010 years, has managed to identify two inbred lines that are resistant to these new populations of broomrape.

Since these lines could be used directly as a parent to produce commercial hybrids, as well as a source of resistance to broomrape in sunflower breeding program, it was studied the inheritance of resistance to the new population of *Orobanche cumana,* in Romania. The resistance in these two lines was found to be under the control of two dominant genes.

The rapid changes in broomrape races composition have forced sunflower breeders to not only search for genes for resistance to the new races of the parasite, but, to also look for alternative solutions to the problem of broomrape control. The use of imidazolinone herbicides resistance in sunflower breeding through the introduction of IMI- resistance into cultivated sunflower provides a broad spectrum of weeds control and is specially effective in controlling *Orobanche* parasite.

Keywords: breeding, broomrape, races, resistance , inheritance, sunflower

THE PARASITE *Orobanche cumana* Wallr., IN SUNFLOWER CROP IN ROMANIA

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Broomrape (*Orobanche cumana* Wallr.) is the most important problem in sunflower crop in southearn and southeastern Romania. This leads to considerable losses expressed in sunflower seed yield decrease and in a low quality of the obtained produce.

In the last years, the parasite has developed new and virulent populations which overcame the resistant hybrids

The different sunflower genotypes (hybrids, lines and populations) have been tested for resistance to broomrape attack, with a view to identify new sources of resistance to the most virulent populations of this parasite and to establish a new differential set for the parasite races.

The testing has been performed in natural infested areas with this parasite as well as under artificial inoculation using broomrape seeds proceeded from three areas of infection in Romania.

There are three important areas infested with broomrape (*Orobanche cumana*) in Romania, different as presence of different populations of the parasite and infestation degree.

The behaviour of some commercial hybrids, in the natural infestation conditions, in 2009 and 2010 years, show an increasing of the virulence of the parasite in Constanta area. The parasite is more virulent in Tulcea and in Constanta areas, comparing with Braila-Ialomita. Some hybrids resistant to the race F, have been high infested (89.1 and 47.9%) in Tulcea and in Constanta area. In Braila-Ialomita area, the infestation degree was not so high, some hybrids being full resistant.

The testing of resistance under artificial inoculation conditions, with different broomrape sources from Romania has emphasized the different behaviour of some sunflower genotypes (lines, populations and hybrids) to the attack of different broomrape sources.

Keywords: sunflower; broomrape; virulence; races; resistance

GENES INVOLVED INTO NONSPECIFIC RESISTANCE OF SUNFLOWER TO *Orobanche cumana* Wallr.

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The interaction between the sunflower and *Orobanche cumana* Wallr. is an interconnection that occurred during the evolution of these two species, which represents an important model of study for host-parasite specific interaction. So far various sources of sunflower resistance to broomrape have been described, most important and frequently used being consistent dominant genes. Elucidating the molecular mechanisms that determine the relationship between host plant and parasite contributes considerably to facilitate the selection of resistant forms, which is currently one of the main concerns of researchers in improving sunflower. As a result of plant infection by biotrophic pathogen a series of defense reactions carried out that increase plant resistance to current infection or later. Identifying potential genes involved in various stages of adaptability and resistance of sunflower to broomrape was achieved by using DNA sequence data resources, RNA or protein, in application of bioinformatics methods. Investigation into the database revealed the first keyword references to 20 different nucleotide sequences (DNA sequences 10 and 10 mRNA sequences) involved in the mechanism of resistance of sunflower to broomrape (Tab. 1).

Table 1. Results of the selection of the sequences involved in resistance to broomrape

Access code	*Description*	*Source*
	Secvențele de DNA	
GM719388	Sequence 10 from Patent W02008083198	construct sintetic
GM719387	Sequence 9 from Patent W02008083198	construct sintetic
GM719386	Sequence 8 from Patent W02008083198	construct sintetic
GM719385	Sequence 7 from Patent W02008083198	construct sintetic
GM719384	Sequence 6 from Patent W02008083198	construct sintetic
GM719383	Sequence 5 from Patent W02008083198	construct sintetic
GM719382	Sequence 4 from Patent W02008083198	construct sintetic
GM719381	Sequence 3 from Patent W02008083198	construct sintetic
GM719380	Sequence 2 from Patent W02008083198	*Helianthus annuus*
GM719379	Sequence 1 from Patent W02008083198	*Helianthus annuus*
	Secvențele de ARNm	
DQ837214	glucan synthase-like 4	*Helianthus annuus* x *Helianthus debilis*
DQ837213	glucan synthase-like 3	*Helianthus annuus* x *Helianthus debilis*
DQ837212	glucan synthase-like 2	*Helianthus annuus* x *Helianthus debilis*
DQ837211	glucan synthase-like 1	*Helianthus annuus* x *Helianthus debilis*
AY899921	methionine synthase	*Helianthus annuus* x *Helianthus debilis*
AY899920	glucantion S-transferase (gst)	*Helianthus annuus* x *Helianthus debilis*
AY899919	quinone-oxidoreductase	*Helianthus annuus* x *Helianthus debilis*
AY094064	elongation factor 1 alpha	*Helianthus annuus* x *Helianthus debilis*
AF071887	chalcone synthase (CHS)	*Helianthus annuus* L. x *Helianthus debilis*
AF074401	chalcone synthase (CHS)	*Orobanhe cumana* Wallr.

Subsequently the results were processed to determine properties towards any polypeptides involved in the mechanism of resistance to *O. cumana*. This study allowed the establishment of six full or partial mRNA sequences, five of which relate to sunflower. The results of search in databases and scientific publications on the issue allowed determination of components possibly involved in the mechanisms of sunflower resistance to *O. cumana*. Both resistance and tolerance to *O. cumana* is determined by the ability to oppose the process of attachment of the plant parasite to the host plant's root system and ability to penetrate the plant tissue haustorium.

SOME ASPECTS OF SUNFLOWER BREEDING TO BROOMRAPE IN REPUBLIC OF MOLDOVA

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Introduction

Sunflower (Helianthus annuus L.) is the main oil crop grown in Republic of Moldova (FAO, 2010). But, sunflower breeding is affected by several parasites. Broomrape (Orobanche cumana Wallr.), downy mildew (Plasmopara halstedii) and white blister rust are main parasites that greatly reduce production and represent a permanent threat to sunflower breeding. The broomrape is a root holoparasitic angiosperm considered as one of the major constraints for sunflower production in Mediterranean areas and Eastern Europe, including Republic of Moldova. Breeding for resistance is regarded as the most effective, feasible, and environmentally friendly solution to control of this parasite. Pathogens as broomrape greatly reduce production and represent a permanent threat to sunflower breeding. Therefore, selection of genotypes of sunflower resistant to broomrape will be useful to mitigate the current pathological problems that affect this crop.

Material and methods

Different inbreed sunflower lines A and Rf and hybrids F1 have been taken as research materials. The testing of sunflower lines and hybrids to broomrape was performed by growing in spots, in greenhouse, according to Pancenco A. (1975). For research was taken different populations of broomrape, collected from South (Cimislia), Ceneter (Rezeni) and Balti.

Different methods of breeding have been used for obtaining sunflower forms resistant to Moldavian populations of broomrape. The inbreeding method is most effective. Resistance in the self-pollinated lines, grown among broomrape, began to appear in the F2 and was strengthened in subsequent generations by selection among interline and variety-line crosses to give some lines completely resistant to broomrape.

Results and discussions

New broomrape races spread out quickly year by year and reached 70 % of sunflower production in Republic of Moldova. Investigation carried out in Research Institute of Field Crop "Selectia" during 2006-2011 years elucidated the broomrape natural population from South and Central parts of Republic of Moldova have five races (A, B, C, D and E). Appearance of new F race was registered since 2006. The parasite population from South region was more aggressive in comparison with North part. Thus, in South regions as Vulcanesti and Ciadir-Lunga have been find the races A to E, and further F. The composition of different races in Republic of Moldova where as following: A 30,6-50,1%, B 20,8-26,7%, C 18,2-22,0%, D 8,0-19,3%, E 0-5,4% and F 0-1,0% respectively.

The investigations in Institute of Field Crops "Selectia" related to sunflower breeding to disease have been stared since 1985. In this period several lines and hybrids have been studied that where resistant to different races of broomrape. Hybrids created in the Institute, that are resistant to the new aggressive races of broomrape, were found to be HS-0428 and Ortac. These hybrids have a yield potential in the range of 3.0-4.0 tons /ha of seeds and 1.5-1.6 tons /ha of oil. Thus, the biotest realized in 2011 of resistance to broomrape (race from Balti) of HS-0428 and Ortac showed the affecting rate of 4 and 5% respectively.

BROOMRAPE SEEDS GERMINATION ON NUTRITIVE MEDIA AND POSSIBILITY OF ITS BIOLOGICAL CONTROL

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Broomrape (*Orobanche cumana*) is one of the most important devastating parasites of sunflower. It has been appearing with varying intensity almost every year in the agroecological conditions of Serbia and can cause significant damage. Resistant or tolerant sunflower hybrids are the most efficient and the most economical measures in the suppression of this parasitic plant. In the tests of sunflower hybrids susceptibility to broomrape under artificial infestation, high germination rate of broomrape seed used for infestation is an obligatory condition. On the other hand the key to improved sunflower production is a reduction in the enormous amount of seeds in the soil. One of the ways to reduce such great potential of seeds is biological control.

The aim of this paper is to evaluate influence of sunflower roots and biological agent Trifender on broomrape seed germination. Trifender is a biological pesticide from Trichoderma asperellum acting as plant growth promoter with beneficial side effect to control of soil borne pathogens when incorporated in soil. Because of mean of its application Trifender is suitable for broomrape control.

Seed germination was evaluated on two different media. The first medium which was confirmed in previous investigations as a good medium for provoking broomrape seed germination (Masirevic et al, 2011) was used as a check and includes water agar, giberellic acid (GA conc. 25 ppm) and presence of roots of the susceptible sunflower hybrid NS-H-111. Medium for the evaluation of influence of biological agent to seed germination was the same with Trifender added in concentration of 1%. Seeds of Orobanche cumana were collected in the sunflower fields in Vojvodina at five localities: Bačka Topola, Vršac, Svetozar Miletić, Vatin and Zrenjanin during 2009. Seed samples were kept in the fridge on +4°C during 10 months due to break dormancy. Surface sterilized seeds (25 seeds in 4 replicates for each treatment) were put in Petri dishes with nutritive media and incubated at 25°C in the dark. Broomrape seeds were put on different distances from sunflower root due to evaluation of influence of sunflower root on germination. Germination rate, germ length and distance of germinated broomrape seed from sunflower root was determined every 7 days under dissecting microscope. Data were analyzed by ANOVA and Duncan test using software Statistica 10.

After 28 days on basic media broomrape seed germinated in the range of 44-68% depending on location. Seeds on nutritive media with Trifender had significantly lower germination (4-41%) compared to seeds on basic media. Orobanche seed which was closer to the sunflower root had better germination on both media.

It is also significant that germ lengths of seeds on basic media were significantly longer than those on medium with Trifender. Average broomrape length on basic media was 0.96 mm while on media with Trifender it was 0.36 mm.

These results indicate that roots of susceptible sunflower genotypes could be used in the field experiments for provoking broomrape seed germination. The first preliminary results confirm that there is some kind of inhibition of broomrape seed germination by the bio-agent based on T. asperellum. Therefore the effect of this bio-pesticide on broomrape seed germination should be further investigated.

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EXPLORING THE ENVIRONMENTAL DETERMINANTS OF BROOMRAPE (*Orobanche cumana* Wallr.) GEOGRAPHIC DISTRIBUTION

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Broomrape is one of the most serious biotic constrains to sunflower crop production. In the more extended sunflower crop area of the world, distributed across Eastern Europe, the parasitic weed shows a high frequency of occurrence. The weed, native of Caucasus region, has progressively migrated to the South of the Eurasia continent. The actual invaded area reaches Spain and Israel territories. Due the usual seed exchange between macro-regions it is not clear why this weed problem is absent in other extended sunflower crop area located in South America. It is possible that the invasive process could be limited by abiotic constrains. With the aim to estimate the influence of environmental factors on the actual orobanche geographic distribution we evaluated fourteen sunflower habitats of Serbia with different natural infections levels and nine natural habitats of wild sunflower in Argentina. The altitude, latitude, longitude, mean hottest month temperature, mean coolest month temperature and average rainfall of ten habitats from Serbia showed no differences between infected and non infected soils. The natural initial infection of four soils from Serbia did not influence the orobanche attack intensity in a greenhouse experiment with artificial inoculation. Soil texture, total N, humus content, calcareous and pH have no effect on orobanche attack intensity. In this glasshouse experiment, Principal Components Analysis showed that orobanche attack intensity (parasitic tassel number per plant) was inversely associated only with P availability (AL-P_2O_5), but the linear relationship between both variables was not significant. When included in the database the Argentine habitats, strong differences between invaded and not invaded areas were found with respect to geographic localization, as expected. The invaded habitats differe by the lower cool month mean temperature, but no significant differences were found for the others climatic parameters. The soil fertility and texture of eleven soils with different natural orobanche infection showed no differences between invaded and not invaded habitats. A better understanding of the abiotic determinants of orobanche geographic distribution could help to design management's tools to prevent the continuous increase of the invaded area and to limit the damage on sunflower in the regions actually affected by the weed.

SUNFLOWER PROTEIN CONTENT MODIFICATION INDUCED BY BROOMRAPE (*Orobanche cumana* Wallr.) INFECTION

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Induced defensive response is the one of the most common and important types of the plant defensive responses to pathogen attack. These include oxidative stress related enzymes, synthesis of PR proteins, hydroxyproline-rich glycoproteins thus induces some modifications in qualitative and quantitave protein content of affected plant cells.

Investigation of protein content modifications during sunflower broomrape infection was performed using *Protein LabChip* quantification studies in Agilent 2100 Bioanalyzer. Assays were included analysis of leaf and root proteins extracted from 6 sunflower genotypes (Xenia and Valentino F_1 hybrids and their parental forms) in different stages of infection development (15, 20, 30, 40, 50, 60 days after parasite inoculation).

It was demonstrated that broomrape infection induces insignificant modification of polypeptide bands number but substantial alteration of fraction quantities. Thus increase of synthesis of 16,5; 26,8; 28,5 and 36,2 kDa fractions was observed in roots of Xenia F_1 hybrid and their parental forms in stage of 15 and 20 days from inoculation. Valentino group of genotypes sensibility reaction was lower than such of the Xenia group of genotypes, for which were mentioned essential modifications in polypeptide content from the beginning of infection. It was a weak impact of broomrape infection on content of leaf protein fractions in comparison with root proteins.

Furthermore, the parasite attack at certain stages caused early biosynthesis (in comparison with control variant) of polypeptide bands (68.5, 70.3 and 111.1 kDa), which were distinguished in more advanced stages in both the control and infected plants. These modifications reflect the intensity of the defensive response of investigated genotypes and show temporal effect of the stress-proteins synthesis, which have an increased rate at early stages of infection, followed by reorganization of biochemical metabolism in function of the level of infected plants resistance.

Also, quantitative protein content estimation using *Protein LabChip* technology confirmed significant changes in polypeptide fractions content observed in SDS-PAGE. Thereby it was shown the decrease of 28,2 kDa fraction of Xenia paternal line from 2327,8 ng/µl (leaves of control non-infected plant) to 12,61 ng/µl (infected plant) and quantity 58,5 kDa fraction of maternal line of the same hybrid was reduced from 389,79 ng/µl to 111,9 ng/µl.

In general, polypeptide profiles of F_1 hybrids revealed higher physiological homeostasis grade than appropriate of the parental lines.

So, we concluded that more significant effects of broomrape infection were demonstrated in modification of quantitative protein content and weak qualitative changes were observed.

SUNFLOWER SESQUITERPENE LACTONES AS GERMINATION STIMULANTS FOR *Orobanche cumana*

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Numerous compounds with germination inducing capacity for seeds of parasitic weeds of *Striga, Orobanche* and *Phelipanche* have been identified in recent years (Bouwmeester et al. 2003). In general their chemical structure followed the principal skeleton of strigol, a four-ring terpenoid from cotton, which is derived from carotenoids. Only few other metabolite types have been reported to exaggerate similar bioactivity. Among them, some sesquiterpene lactones (STL) became known as germination stimulants for *Striga asiatica* (Fischer et al. 1989) and *Orobanche cumana* (Galindo et al. 2002). However, no specific STL could be shown to act in root exudates of sunflower as a chemical signal for the germination of *O. cumana* until very recently, when Joel et al. (2011) found dehydrocostus lactone (DCL) as a natural STL in sunflower roots being released into hydroponic solutions.

Studies on the host / parasite interaction of *Helianthus annuus* and *O. cumana* clearly showed that more than one inducing compound is present in the root exudate. We have identified costunolide as a second STL in sunflower roots and in the exudate. It induced germination at an ED_{50} of ca. 5 nM. This is in the same range as for DCL. In addition, at least three more yet unidentified compounds with spectroscopic traits characteristic for STL were found in the exudate. The experiments showed that the germination of *O. cumana* highly depends on the concentration of the STL. Above 1 µM, costunolide and DCL seemingly lost their activity, but in fact, overdose application of STL inhibited germination permanently and irreversible. This is most likely a consequence of the nucleophilic reaction of the exocyclic methylene moiety in the compounds which is known from many cytotoxic effects of STL. This hampers the identification of the additional active compounds in sunflower root exudates, because bioassay-guided purification is extremely complicated when depending on the small range of suitable concentration between under- and overdose. In the course of our experiments, we also found that the STL located in glandular trichomes of aerial parts of the sunflower (Spring et al. 1989) cause similar stimulating effects on *O. cumana* seeds. Germination could be achieved with the STL amount present in a single trichome. Simple leave washings with water, simulating natural rain events, strongly induced germination of *O. cumana*. Therefore, we suppose that germination stimulants for sunflower broomrape are not just released from the roots, but may also reach the soil from leaching of leaves and decaying plant parts.

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ASSESSMENT OF INFECTION IN SOME GENOTYPES OF SUNFLOWER BY THREE POPULATION OF *Orobanche Cumana* Wallr.

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Selection of resistant forms of sunflower to *O. cumana* was and remains o basic concern of researchers. Use of resistant forms is followed by the appearance of new races, more virulent, overcoming existing sources of resistance.

The main purpose of the present work is to reveal the infection ability of tree population of *Orobanhia* collected from from different geographical regions - Republic of Moldova, Rostov and Volgograd (Russia) in some moldavian genotypes of sunflower.

Plants were grown in plant pots in which was introduced to the soil mixture (1sand: 1peat) uniform *O. cumana* infected seeds (1 kg of mixture 0.8 g of seeds). Vessels were exposed in culture room at a temperature of 23-25 °C, photoperiodicity 14-16 hours, humidity 60%.

The material was collected and analyzed after 8-9 weeks after sowing. Sunflower plant roots were washed and analyzed with MBS-9 binocular microscop. Parasite attachments on host roots LANL were counted and classified.

The researchers have revealed the interdependence of the infection ability from both genotype and the origin of parasites. It has been revealed that a population of broomrape, which had been received from Volgograd, had the most lanced capability for infection in comparison with populations from Rostov and Republic of Moldova. Some genotipes showed a fence malt resistance at all three investigated *O. cumana* population.

REDOX STATUS OF DIFFERENT GENOTYPES OF SUNFLOWER (*Helianthus annuus* L.) INFECTED BY *Orobanche cumana* Wallr.

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Growth and development of crop plants is permanently influenced by biotic and abiotic stress, affecting morphology, physiology, and ultimately, productivity, every year causing considerable losses of harvest crops. Plant exposure to stress conditions induce formation of reactive oxygen species (ROS), such as H_2O_2, O_2^- and OH^- radicals, changing cellular redox status.

Oxido-reducing enzymes are involved in plant cell response to the stress of the environment. This group of enzymes determines the level and nature of energy source in the cell and serves as a criterion for their viability. The most studied oxido-reductase enzyme in plant cells is peroxidase and catalase, which have the function to protect against the pathogen.

There were analyzed two sunflower hybrids and their parental lines (Xenia and Valentino) infected with *Orobanche cumana* Wallr. infection of sunflower plants with broomrape, penetration of the parasite in root system causing a stress effect.

Peroxidase activity was determined by spectrophotometric and gazometric method. Because *Orobanche cumana* is a rizoparazit, perception and signal transmission is initiated as a result of infection in the root system. Therefore we studied the activity of enzymes in leaves and roots of sunflower plants in normal growth and infection *Orobanche cumana* background.

Our results showed that the activity of peroxidase is increased in the root system of sunflower genotypes attacked by the parasite, as a consequence of the increased accumulation of peroxide and its conversion into non toxic cell compounds as well as the possible involvement of peroxidase in lignifiction cell wall. Peroxidase activity in leaves under normal conditions was higher by1,2-4,2 times, but broomrape infection caused its reduction, especially in the parental lines. Probably this is caused by intense involvement at the site of infection. Also, it was observed that hybrid genotypes have an increased lability under *Orobanche cumana* infection. Catalase activity decreases upon the background of the infection leaves of Xenia family as a result of ROS accumulation, while the genotypes of Valentino family manifested a reverse reaction, which showed greater susceptibility to the parasite. Peroxide amount correlates with enzyme activity, both in normal conditions and after the *Orobanche cumana* attack. Thus, the results of investigations allow us to conclude that enzyme activity can be used as an indicator of response reaction of plants to *Orobanche cumana* infection.

RESULTS REGARDING THE TESTING FOR RESISTANCE TO BROOMRAPE (*Orobanche cumana* Wallr.) IN SUNFLOWER CROP IN ROMANIA

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In the last years, in sunflower crop in Romania, broomrape (*Orobanche cumana* Wallr.) becomes one of the most serious factor for the seed yield decreasing. The aggressivity of this parasite increased significantly, the new races being identified in the area situated near Black Sea. The study carried out during 2008, 2009 and 2010 by Biotech Foundation, in 3 locations in south-eastern Romania identified 2 different virulent populations of the parasite. Each year there have been tested around 300 sunflower hybrids, in 2 replications, the results showing differences in them behavior, as resistance to the parasite. The susceptible check and the resistant one were planted after each 20 plots with hybrids, for a better tracking of field infestation. As resistant check has been used Favorit hybrid in Braila and Ialomita area and PR64A71 hybrid, in Constanta area. It was calculated the seed yield in the infestation conditions for each hybrids, the results showing the high influence of the parasite attack, on this.

Keywords: broomrape, sunflower, seed yield, parasite attack.

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