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Historical Aspects of Sunflower Researches in the Republic of Moldova

Abstract: The first document that attests the cultivation of sunflower on the territory of the Republic of Moldova dates back to 1845. The first evidence of oil production from sunflower seeds has been identified in 1867. Since then, the area cultivated with sunflower has expanded exponentially. Sunflower has now become the third most produced crop, after corn and wheat. In the Republic of Moldova, sunflower research activities have been focused on genetics, breeding and improvement of crop cultivation technologies, with a special emphasis on resistance to disease and pests including broomrape, which can considerably diminish crop productivity. Significant efforts have been invested to identify solutions for fighting parasites, such as the investigation of morpho-physiological, biochemical and genetic aspects of the host–parasite interaction; the development of chemical and agro-technical methods to fight parasites; the evaluation of genetic resistance in artificial and natural conditions; the development of hybrids resistant to a number of *Orobanche* varieties; and the monitoring of broomrape impact on the production indices. The current paper examines the scientific information available, providing a comprehensive view on the management, breeding, resistance and economic impact of sunflower broomrape in the Republic of Moldova. The review aims to provide a perspective for future research strategies to further develop our understanding of the parasite–host interaction.

Keywords: broomrape, race, resistance, screening, sunflower, virulence, yield

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Introduction

Helianthus annuus L. is one of the basic oilseed crops in the Republic of Moldova (RM), having a wide range of industrial uses and a major impact on the economy. In RM there are 76 producers, 19 exporters, 1 large and more than 590 small processors of sunflower. It is the third most produced crop after corn and wheat, having consistent planting areas. According to the Food and Agriculture Organization of the United Nations (FAO), in 2012, RM was ranked the 19th biggest producer of sunflower seeds, contributing to 0.8% of the world production. Annually, the total production is around 320,000 tons.

Considering the economic importance of sunflower, over the last 60 years, government strategies have been focusing on the creation and development of the research infrastructure and the research human capital. Currently, there are 11 research institutions in the RM working on sunflower research. Among these, three universities carry out fundamental research via their Natural Science faculties and six research institutes deal with the full range of agricultural issues under the supervision of the Academy of Sciences of Moldova (ASM) and the Ministry of Agriculture and Food Industry. There are also four private companies operating in the field of breeding and seed production.

Many efforts have been made to develop, attract and retain skilled researchers in this field. Since 1964, more than 35 PhD theses were written on the subject. The majority is focused on Plant Breeding, Genetics, Plant Physiology, Phytotechnology, Agrochemistry and Biochemistry. The most relevant scientific results were included in more than 1,000 outstanding papers and 12 patents. Around 25% of these publications are concentrated on the parasitic plant broomrape (*Orobanche cumana* Wallr.), which is one of the major limitations to sunflower production and profitability.

Over the last decades, considerable efforts have been invested to study the problem of and identify solutions for fighting parasites, including:

- investigation of morpho-physiological, biochemical and genetic aspects of the host–parasite interaction (Moldova State University; University of the Academy of Sciences of Moldova; *The Institute of Genetics, Physiology and Plant Protection of the ASM*);
- development of chemical and agro-technical methods to fighting parasites (Research Institute of Field Crops "Selectia"; Agrarian State University of Moldova; Moldova State University);
- evaluation of genetic resistance in artificial and natural conditions (University of the Academy of Sciences of Moldova; State University of Moldova; Research Institute of Field Crops "Selectia"; AMG – Agroselect, Moldova);

- development of hybrids resistant to a number of *Orobanche* varieties (Research Institute of Field Crops "Selectia"; AMG – Magroselect; AMG – Agroselect);
- monitoring broomrape impact on the production indices (Research Institute of Field Crops "Selectia"; University of the Academy of Sciences of Moldova).

The aim of this brief analysis was to provide a comprehensive view on sunflower broomrape in RM in terms of management, plant breeding, races identification and distribution, mechanisms of resistance, host–pathogen interaction and economic impact.

Historical overview of sunflower cultivation

The first document that confirms the cultivation of sunflower on the territory of Moldova dates back to 1845. The first evidence of oil production from sunflower seeds dates from 1867. Since then, the sunflower cultivated area has expanded exponentially from 287 ha in 1909 to approximately 11,100 ha in 1913 (Lashkov, 1912). In the following years, the dynamics of the cultivated area and the harvested amount have been constantly rising, with occasional drops determined by environmental and cultivation conditions.

It is well documented that agricultural research is one of the key factors that drive economic growth in agriculture. In this regard, the management of *Orobanche* was and is until now a high priority to Moldovan sunflower researchers. The first investigations, focused on studying the productivity in different climate conditions, have been carried out at the Agricultural Station of the Agrarian State University in 1945. The role of crop sequencing, crop rotation, soil adaptability, fertility management, climatic influence, crop water use, disease incidence, etc. was studied at The Research Institute of field crops Selectia, Agrarian State University of Moldova, Moldova State University.

In order to extend the sunflower cultivated area and to increase the harvest it was recommended to cultivate this crop on the same ground not more than once every 6 years and to employ other control strategies such as pest or herbicide treatment. Since the 1950s, research and cultivation strategies for increasing the overall sunflower production and yield have been planned and implemented (Gordienco, 1959). The use of novel technologies and the substitution of the common varieties with high-yielding hybrids during 1976–1990, combined with the extension of the cultivated areas, have led to a steady increase in seed production (Moraru, 1999). After 1991 the total cultivated area was still growing, while the productivity was decreasing (Figure 1).

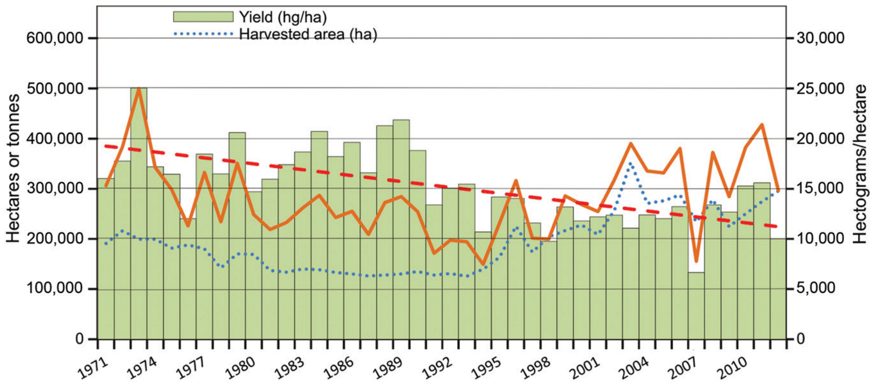

Figure 1: Total sunflower harvested area, production and yield in Moldova during 1971–2012 (Food and Agriculture Organization of the United Nations).

As a result, between 1990 and 2000 the areas covered with sunflower reached the value of 170,000–200,000 ha, with an average yield of 1.2 t/ha and a total production of 227,000 tons. After 2010, according to FAO statistics, the planted area increased to about 263,000 ha, while the average productivity remained constant (320,000 tons of total production). In other words, by the end of this period, although an additional surface of 77,000 ha was cultivated, the same quantity of seeds as in the 1990s was produced (Moraru, 1999; Vronschih *et al.*, 1975, 2002).

In these conditions, the strategy of following the correct crop rotation processes was failing and sunflower crops started to be re-cultured on the same areas after only 3–4 years. Taking into account this fact and the vulnerability of sunflower to various parasites, the necessity for development and cultivation of resistant hybrids as well as for strictly respecting the cultivation rules becomes evident.

Assessment of the sunflower potential for resistance to broomrape

Another major component of the integrated control strategy is **breeding for broomrape resistance**. This is a difficult task, but significant progress in this area has been achieved. The main sunflower breeding program was initiated in

1976 at the Research Institute of field crops Selectia, Balti (Buciuceanu, 1988; Buciuceanu *et al.*, 1994a; Vronschih and Lesnic, 2013). A range of research projects that aim to create highly productive hybrids with complex resistance to different parasites are conducted within the private companies Magroselect and Agroselect from Soroca, and Novosem and Euoceres from Chisinau.

Sunflower breeders have used a wide range of cultivated sunflower varieties to search for valuable agronomic and seed quality traits, as well as for resistance to insects and diseases. Variability of morphological and physiological traits (plant height, flowering period, leaf and achene characteristics, etc.) was used as an indicator for sunflower breeding approaches (Buciuceanu *et al.*, 1987, 1994b; Petcovici, 2008; Petcovici and Lungu, 2008; Buciuceanu *et al.*, 2009; Petcovici and Lungu, 2009). During the last decades, 26 hybrids were registered in the Catalog of Plant Varieties of RM. Currently, this Catalog includes 137 sunflower varieties, with close to 30% resistant to broomrape (Figure 2). The large majority belongs to the oil group and five to the special group used in confectionery.

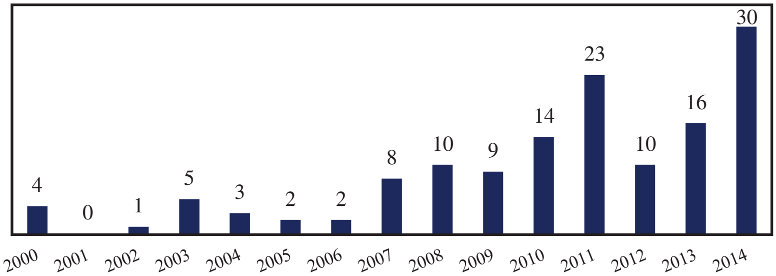

Figure 2: Sunflower varieties registered in the Catalog of Plant Varieties of RM, from 2000 to 2014.

One of the most important steps in breeding for broomrape resistance is the **evaluation of germoplasm** and effective selection of resistant genotypes. There are a number of researches realized practically by all public and private institutions oriented on this topic. Special attention was given to triggering chemical signals exuded by the host (Glijin *et al.*, 2009, 2011a) and to the host–parasite interaction at various stages of development (Duca *et al.*, 2013b) and at different temperatures (28°C and 15°C) of cultivation (Rotarenco, 2010). Following extensive field evaluation and various laboratory techniques, more than 2000 genotypes were classified into three categories: *resistant*, *tolerant* and *susceptible* (Duca *et al.*, 2011b, 2013d; Glijin, 2012b).

Thus, sunflower screening (frequency, intensity and attacking rate) for susceptibility to broomrape has been achieved in controlled (Rotarenco, 2010; Duca *et al.*, 2008; Duca, 2011a) and natural conditions (Duca and Popescu, 2007;

Glijin, 2012b). Additionally to phenotypic screening, a Random Amplified Polymorphic DNA (RAPD) (Duca *et al.*, 2013c; Duca *et al.*, 2014) and Sequenced Characterized Amplified Region (SCAR) analysis (Duca *et al.*, 2009a, 2010; Rotarenco, 2010) was performed (Figure 3).

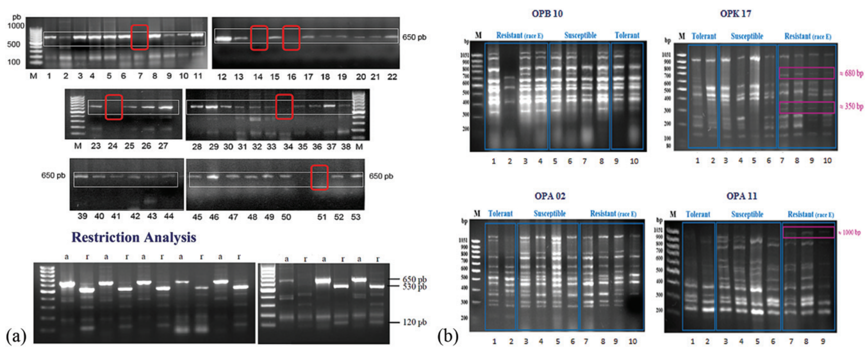

Figure 3: Molecular screening of the resistance to broomrape. (a) SCAR *(race E)*; (b) RAPD profiles.

The RAPD profiles showed a genetic polymorphism between susceptible and resistant sunflower genotypes (unpublished data) and the presence of the *RTS05* locus (the *Or 5* gene conferring resistance to race E of *O. cumana*) in most of the samples (88.7%). No correlation was detected between the presence of this genetic marker in the genome and phenotypic resistance to broomrape in most of the analyzed sunflower genotypes (78%), highlighting that the infection was not due to race E, but rather to more aggressive races (Rotarenco, 2010; Duca *et al.*, 2010).

Understanding the mechanisms of **resistance to the parasite** and of the host–parasite interaction is critical in developing strategies for broomrape control. Phenotypical, biochemical, physiological and molecular approaches have been employed to help understand the specific, nonspecific and systemic acquired resistance (SAR) mechanisms in sunflower to *O. cumana*. These have been carried on susceptible and resistant sunflower genotypes, in roots and leaves and at different stages of phytopathogen interactions.

Recently, aspects of multiple defense strategies, such as the constitutive (Rotarenco, 2010) and inducible defense systems for combating broomrape (Glijin *et al.*, 2011b), have been described. Phenotypical and physiological studies of host–*Orobanche* relationships have revealed several modes of resistance, including no or low stimulation of broomrape-seed germination, low number of parasitic attachments and its necrosis in host tissues. In a complementary approach, bioinformatics investigations were also performed (Duca *et al.*, 2009b; Duca, 2013).

The molecular mechanism of resistance was studied at the level of pathogenesis-related genes, such as genes of antioxidative system (MnSOD1, APX3, AOX1A), genes of cell-wall reinforcement (PAL) and signal transduction, plant defense activation (NPR1), jasmonate (PR5) and salicylate (defensine) metabolic pathways, involved in different defense responses. The presence of *O. cumana* seedlings close or attached to sunflower roots showed altered expression of this genes in both resistant and susceptible genotypes. The highest values of transcript accumulations in response to parasite were detected for PR5 gene (3–120 fold), a marker of SAR. The result of the reverse transcription polymerase chain reaction correlated with enzyme activity.

Current distribution and racial status

Knowledge of the parasite races is vital for any breeding program. Thus far, there have been many studies describing a virulence of different populations of broomrape from RM.

The first documented data of broomrape on Moldovan fields was published in the Annual Report of Agricultural Chamber of Balti on January 31, 1940, based on the evidence of Afanasev and Arhanghelschi from 1937. According to their data, a large area of the left side of Nistru River was designated as low to moderately infected, while the right side of the territory was considered a heavily infected area. Due to the high infection level of resistant sunflower varieties (race A), the authors also concluded the presence of a new race (race B) (cited by Sharova, 1977). In order to extend the area cultivated with sunflower and to increase the harvest it was recommended to plant the Jdanovsk 82–81 varietal type and to cultivate this crop on the same ground no more than once every 6 years on the entire territory of the republic (cited by Duca *et al.*, 2011c).

Over many years, researchers from Moldova investigated broomrape populations from 30 geographical regions, including Romania and Ukraine, establishing a great diversity of physiological races and describing their peculiarities including the morphology of *Orobanche* seeds, degree of attack and distribution. Differences in the level of aggressiveness among populations of *Orobanche* were established (Sharova, 1977).

In the beginning of the 1970s, another broomrape biotype started to spread rapidly over all sunflower growing regions and to infect genotypes that were considered resistant (Antonova *et al.*, 2013). This new race could not be controlled by the genes for resistance to races A and B and was called the Moldovan race (Sharova, 1969), or race C (Burlov, 1976).

For the identification of current races belonging to different geographical populations of *O. cumana*, a standard differential set for broomrape races D, E, F and G was used, provided by NARDI Fundulea, Romania. Artificial infection of differentials in pots allowed identification of the races collected from RM (Balti, Ciadir-Lunga, Stefan-Voda, Soroca, Anenii Noi) and Romania (Tulcea) (Gisca *et al.*, 2013; Glijin *et al.*, 2014). The authors found a high genetic variability and a differentiation by origin and race of aggression of the broomrape populations analyzed (Gisca *et al.*, 2013). It was revealed that broomrape from Balti infected the differential for race E, but did not infect the differential for race F. Broomrape from the south part of RM infected differentials that are resistant to race F. This data suggests that *O. cumana* from Stefan-Voda and Ciadir-Lunga belong to race G or more aggressive races, which is in line with other findings (Pacureanu-Joita *et al.*, 2012).

More recently, RAPD analysis and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clusterization were performed in the University of the Academy of Sciences using *O. cumana* collected from different geographical regions: ten populations from RM, one from Romania (Fundulea) and one from Ukraine (Izmail). A low intra-population variability and a high inter-population variability were detected. At the morphological level, broomrape seeds from Fundulea (Romania), Taraclia (south part of RM) and Donduseni (north part of RM) significantly differ from other populations.

Based on molecular and phenotype screening (Figure 4), broomrape populations from the northern part of Moldova belong to race F and the populations from

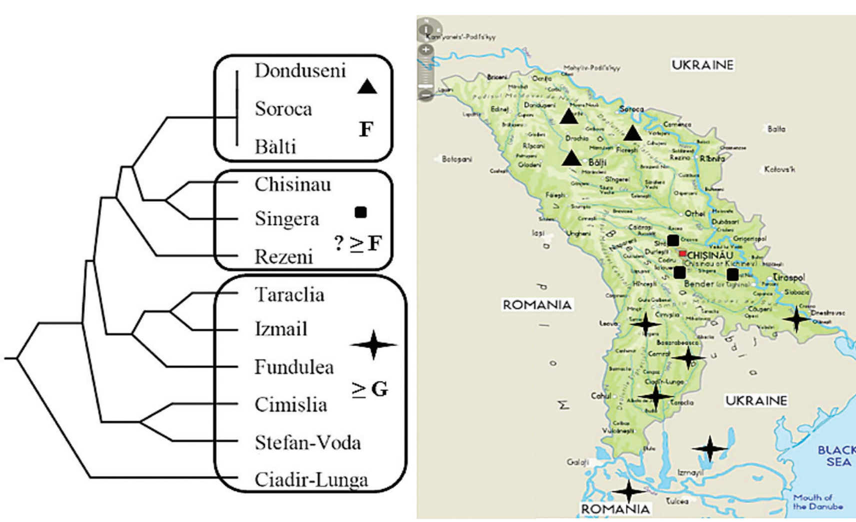

Figure 4: Dendrogram (UPGMA) derived from RAPD analysis of different geographical populations of broomrape and their racial distribution.

the central part of the republic supposedly belong to race ≥F (or more aggressive) and populations from southern part can be attributed to race ≥G (Glijin *et al.*, 2014).

As a result of the analysis of situation, we conclude that all races of *Orobanche* known in the world were detected on the territory of RM and the areas affected by this parasitic plant is spread across all regions of RM and have extended considerably.

Economic impact

The parasitic plant *O. cumana* spreads across all regions of RM, bearing a significant economic, social and environmental impact. An estimation of the economic cost of the sunflower productivity losses is needed for appropriate future prevention and response strategies. Furthermore, the regulatory authorities and individual farming enterprises in order to avoid the spreading of pests must implement adequate monitoring.

Researches carried out for many years (*Moldovan VNIIMK Station, Research Institute of Field Crops "Selectia", Moldova State University, and University of the Academy of Sciences of Moldova*) have revealed that the broomrape races are not only widespread but also very aggressive. Moreover, the losses due to the spread of broomrape on farmers' fields are very poorly documented and it is often quite difficult to obtain accurate data at country or regional level. There is some evidence that this parasitic plant can cause up to 100% losses in yield for infected crops. An attempt to monitor the broomrape epidemics was made in the early 1960s. It has been found that sunflower crops in the districts of Causeni and Dubasari were affected to an extent of about 70–100% in 1963–1964. In 1972, half of the sunflower crops in Slobozia were affected by broomrape that had reached up to 100% infection rate (Sharova, 1977).

Field experiments confirmed a major impact of crop rotation on the productivity of sunflower, process of appearance of new broomrape races and their rapid spread (Sharova, 1977). It was found again that the damage caused by the parasite is devastating, with reported losses up to 50–90%, leading to a significant reduction in the amount and quality of the oil (Siminel, 1998).

An attempt to forecast the economic impact was carried in 2006–2007, in the frame of an American grant provided by the CRDF (Civilian Research and Development Foundation). In a different project aimed to promote the direct use of research and development by farmers, the risk of loss and economic impact has also been estimated (Glijin *et al.*, 2007). It has been found that the damage by the parasite is dependent on the infestation level in the field, ontogenetic stage and duration of infection. The Moldovan areas infested are vast and ever growing and are spread especially in the South and Central parts of the republic.

In the experimental field the significant decreases in the production and quality of seeds were demonstrated. The highest impact was on the total achene weight per head (average value –37.7%), head diameter (–20.4%), in seed fat content. The negative correlation between the number of broomrape per sunflower plant and seed fat/protein content was observed. Several studies aimed to elucidate the sunflower's phenotypic and biochemical modifications induced by *O. cumana* (Rotarenco, 2010; Glijin *et al.*, 2011b; Glijin, 2012a; Duca *et al.*, 2012). Broomrape infestation in natural environments showed direct consequences on the yield parameters and caused a large reduction in the total achene seed weight per head (Glijin, 2014), the most affected being the weight of 1000 grains (20.1%) and the mass of 1000 kernels (20.7%) (Duca and Glijin, 2013a). Also, *O. cumana* infestation had a significant effect on protein (Glijin, 2012a) and fat content (Duca *et al.*, 2012).

Recently, the influence of natural infection with *O. cumana* on different productivity parameters (Duca and Glijin, 2013a) and the quality of seeds (Glijin, 2012a; Duca *et al.*, 2012) was described (Figure 5).

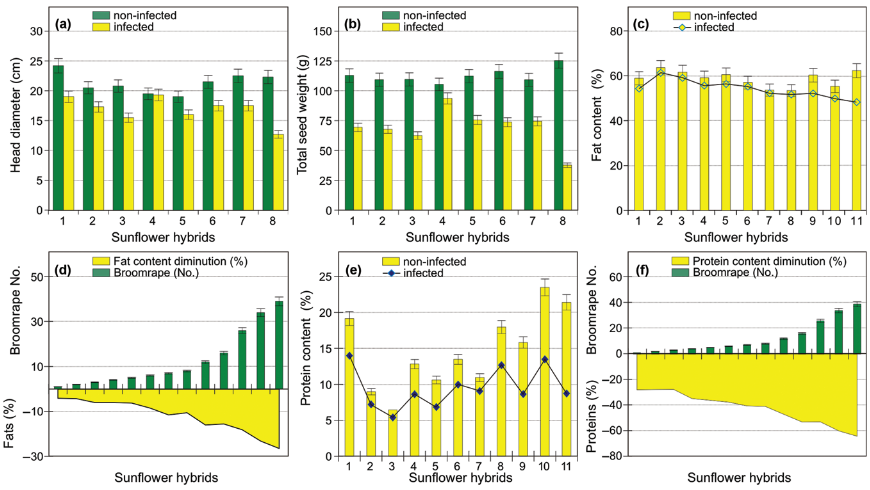

Figure 5: The influence of natural infection with *O. cumana* on different productivity parameters and quality of seeds: (a) *O. cumana* attack influence on sunflower head diameter; (b) *O. cumana* attack influence on total seed weight per head; (c) effect of sunflower broomrape on fat content (% dry weight) in kernels of different sunflower hybrids; (d) correlation between kernel fat content diminution (%) and broomrape number per sunflower plant; (e) effect of *O. cumana* on protein levels (%) in kernels of different sunflower hybrids; (f) correlation between kernel protein content diminution (%) and broomrape number per sunflower plant.

Over the past decades, there have been a limited number of studies attempting to quantify the economic losses caused by the spread of plant pests and to assert the immediate need for appropriate public policies.

Conclusions and future prospects

This paper reviews the history and current status of the sunflower broomrape research in RM, including the morphological, physiological, genetic and molecular aspects of host–parasite interaction, disease management and economic impact. A serious constraint for sunflower productivity is, to a great extent, the failure to respect crop rotation along with increased areas for cultivation, the low quality of agricultural procedures, as well as the protection measures against pests, diseases and weeds.

A future challenge would be to fortify the collaboration between scientific centers and private companies with the aim of exploiting new knowledge and developing more resistant sunflower varieties or alternative broomrape control strategies. Further advances in the identification of the current broomrape racial regional distribution and status could be achieved via international cooperation focusing on the use of race collections, common differential sets and molecular approaches.

In conclusion I would like to mention the positive impact internal and external collaboration has had on the Moldovan sunflower research community. We have developed highly productive and resistant hybrids which are cultivated not only in RM, but also in Ukraine, Belarus and the Russian Federation.

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