

## THE DIFFICULTYS IN OBTAINING *OROBANCHE CUMANA* SUNFLOWER RESISTANCE HYBRIDS

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### ABSTRACT

One of the biggest issues when developing performant sunflower hybrids is *broomrape*, representing up to 80% of yield losses for the susceptible hybrids

The best way to eliminate the threat of *Orobanche cumana* in the field is to include in the breeding programs the introgression of resistance genes in to the sunflower lines but lately it has become more and more difficult because the interaction of the female line with the genes from the restorer line must fit perfectly in order to have a complete resistance to the parasite, this aspect is caused by the very fast development of this parasite and a rapid appearance of new and more virulent races.

The gene-for-gene model and different other authors agreed with monogenic and dominant inheritance of resistance to sunflower broomrape, but there are also reported two dominant genes, one recessive gene, double dominant epistasis and dominant-recessive epistasis, coming with a conclusive conclusion that resistance must be incorporated into both parental lines for developing resistant hybrid

Since the year 2013 when we began our breeding program where we develop sunflower lines and tested them to *Orobanche.cumana* in 5 different locations in Romania and also 1 location in Bulgaria and 1 in Spain.

The purpose of the scientific paper is to present some strategic conclusions from our testing along this 5 year of our sunflower breeding program.

### INTRODUCTION

There are a large number of objectives when it comes to sunflower breeding programs obtaining resistance to herbicide, resistance to disease and genetic distance between female line and restorer line may be one of the most important keys when it comes to developing performant sunflower hybrids (1, 6).

The objectives for improvement concerning the sunflower may vary a lot dependent on production area, abiotic stress and cultivators' preferences (7, 8). Before developing a program for each character improvement, it is essential to be determined the existent variability and the heritability method. Also, there is

needed some knowledge on the correlations existent between the considered characters (2). The selection of a character may alter the other characters, sometimes reducing the genetic gain (19-23). The determination of the mitotic activity in the sunflower roots could contribute to revealing on the cell division particularities to sunflower genotypes and their correlation with the heterosis (3,4).

It is a substantial threat in Europe, especially in countries around the Black Sea and in Spain (12), it is also a threat outside of Europe, one of the regions of great intensity is Jilin, a province from China with over 100 strains of *Orobanche.cumana* on a single sunflower plant in some cases (5).

In Romania, it was first discovered by Savulescu si colab.(1928-1960) in the

year (1940-1941) quoted by Dumitras and Sesan (1988). The highest intensity and frequency is found in the southern part of Moldova, Dobrogea and the eastern part of Baragan's field.

In order to control this parasite there are several methods, but there still remains one of the most difficult problem in sunflower breeding (9, 14, 15), there is a very high probability of appearing mutation due to the thousands of seeds that are produced by a single broomrape plant, which gives a very high advantage that could lead to different variation and new races of broomrape.

The results with different crosses between different resistant lines and different susceptible parental lines shown that dominance relationships and genetic control of broomrape resistance in sunflower is highly dependent on the race of broomrape, the source of resistance and also, the susceptible parental line used for the cross (16-18). It is very clearly that things started to complicate after *Orobanchecumana* appear more and more aggressive in the fields, requiring a stable and a more complex genetic background of the sunflower lines in order to obtain resistance to the parasite (10-13).

We tested several hundred combinations and evaluated them in Romania, Bulgaria and Spain and the results seems to indicate that genes interaction between different lines seems to lower the possibility in obtaining resistance hybrids if it is not done with a suitable partner.

## **MATERIAL AND METHOD**

Since the breeding programs started we focused especially on developing A-lines and restorer-lines that are resistant to different races of *Orobanchecumana*, we manage until now to develop lines that show resistance and tolerance up to race G.

Our first proces of selecting our new hybrids is the yield performance, after that all the best combination where then tested in fields, with naturally infestation

of broomrape, 4 location in Romania, 1 location in Bulgaria and 1 location in Spain. The hybrids that have good level of resistance were then tested in the green house (artificial infestation conditions) We tested for every location approximately 100 plants from each genotype that sowed very good performance, we evaluated every hybrid with a scale form 1 to 9 where number 1 indicates that there where no plants susceptible on the row and repetition.

In order for us to have a better indication of the level of *Orobanchecumana* we used commercial hybrids in order for us to have a better vision and indication of the broomrape race that is present

### ***Working method***

Plants are grown in soil infested with a known race and concentration of broomrape seeds in plants pots of 5 kg and size 30 X 30 X 7 cm. Mixing soil, sand and broomrape seed – 3 part soil X 1 part sand X 1 gr orobanche seeds. Sand has to be quartz because the particles have a different colour to broomrape and the soil has to be sterilized to be sure that no broomrape seed comes with it. Broomrape populations have always a mix of races with one being dominant. In the mix there may be new races capable to infect any new resistant source. The amount of broomrape seed is calculated according to the volume of soil/sand mix required. The concentration of seed depends on its viability. Fill the pots and label. Make a hole 2 cm deep and place the seeds in the hole, 1 per pot. Close the holes and place the pots in greenhouse the following parameters:

- Day; 14 hours 24°C
- Night; 10 hours 20°C
- Humidity – 50 – 80 %
- Temperature :day 24°C +2°C ; night 22°C +2°C.

The amount of water required will depend on the exact conditions and soil that you have. Regular watering is recommended so that the plants don't wilt but are not standing in water for long

periods. For each test hybrid, there will be 2 replicate trays with 20 plants. In every pots, the 1-st line is susceptible standard. After 45 days the soil is removed from the roots and the number of broomrapes per plant is recorded. The test is suitable for ranking susceptibility of hybrids and lines. Record the number plants/infected.

## RESULTS AND DISCUSSIONS

Our main objective is to create lines that are resistant to different races of *Orobanche.cumana* and to develop an efficient strategy in creating performant hybrids.

Since we started creating sunflower mother lines, we manage to create several resistance lines to *Orobanche.cumana*, we used A-1992 obtained by using Spain germplasm sources which is resistant to race G (Table1). From the 100 new combination with the mother line A1992 and different restorer lines, approximately 5 hybrids were picked according to the phenotypical aspects and to the yield performance from several location in Romania and Hungary.

One of the hybrids that sowed good tolerance is A1992 X Rf 104 as you can see in Table 2 the hybrid A1992 X Rf 105 is significantly different from all the others hybrids, having in Spain all the plants attacked. In the experiment from Spain the origin of the line A-1992 is confirmed, all the material except the hybrid that resulted as susceptible are resistant to races from Spain. However, in other regions from Romania and Bulgaria the hybrids weren't so constant regarding the number of plants that were susceptible.

There are no major differences between hybrid A1992 X Rf 101 and A1992 X Rf 102 both of them showing similar percentages of susceptible plant. Also, between A1992 X 103 and A1992 X 104 there is not a significant difference. Our check hybrid is one of the best on the market for *Orobanche.cumana* sowing resistance to all the races in all location having only 7% plant that are susceptible.

**Table 1. Resistance to race G for A-1992 and Rf lines used to form hybrids**

Genotype	Nr/plants	Green House	
		Nr/susceptible plants	%
A-1992	100	10	90%
Rf-101	100	98	2%
Rf-102	100	89	11%
Rf-103	100	89	11%
Rf-104	100	95	5%
Rf-105	100	90	10%

**Table 2. Orobanche.cumana result for all location tested in 2018**

Nr. crt.	Genotype	Topraisar Rep 1	Sevilia Spania	Radnevo Bulgaria	Drajna	(G) Green-House	% of plants susceptible
1	A1992 X Rf 101	3	1	3	3	8	37%
2	A1992 X Rf 102	5	1	5	3	8	44%
3	A1992 X Rf 103	3	1	1/2	1	6	30%
4	A1992 X Rf104	1	1	1	1	4	21%
5	A1992 X Rf 105	7	7	6	7	9	80%
6	Check HYBRID	1	1	1	1	2	7%

We tested several other combination in this experiment but only

mother A-line 1992 sowed significant difference between restorer lines. As you

can see in Fig.1 it is very clear that the most susceptible hybrid is very distant from other hybrids regarding percentage of plants attacked, indicating that the resistance of the line A-1992 had no effect in combination with the restorer line Rf 105.

The yield performance for this year 2018 in 4 location in Romania for all the 5 hybrids are presented in Fig.2 . We also wanted to see the yield capacity of the hybrids in different location but also in

places where it is a very high intensity of *Orobanche.cumana*. We selected location near Black sea in Cogealac. As you can see in the graphic significant different is only for line A1992 X Rf 105, indicating that the yield capacity was most affected for this hybrid in particular .

Costant results where obtained for the other 4 hybrids, remaning with a clear conclusion that the combination of A1992 X Rf105 is susceptible.

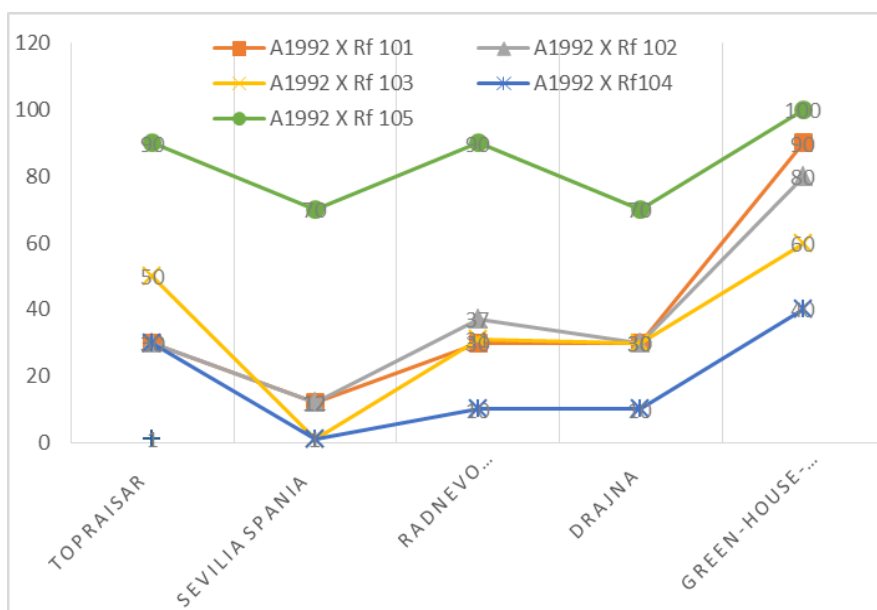


Fig. 1. Percentage of susceptible plants

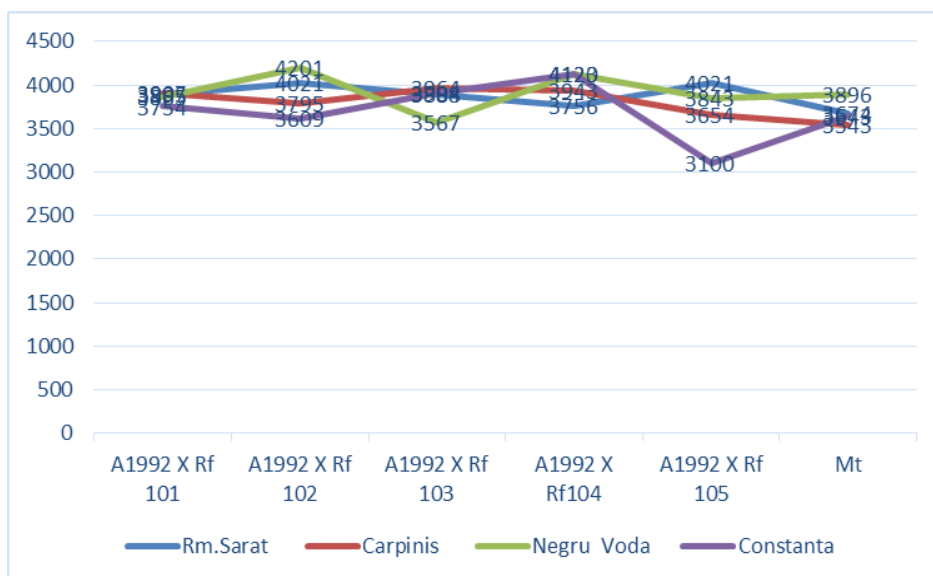


Fig. 2. Yield production in normal condition and *Orobanche.cumana* natural infestation

## CONCLUSIONS

Depending on the combination between the mother lines and restorer lines the result of the genetical interaction can lead to susceptible hybrids or to very tolerant hybrids indicating that resistance can be only obtain with lines that genetically matche.

Nomenclature of the races differs from one region to another , the mother line - A1992 with in all of the 5 hybrids,except the susceptible one, showed resistance in Spain , but for the othere location there was a percent of susceptible plants that needs to noticed.

New strategies and better understanding of how gene interactions between lines work must be develop in order to obtain maximum efficiency in the process of developing resistance hybrids.

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