OBTAINING SUNFLOWER GENOTYPES, RESISTANT TO IMIDAZOLINONE OR SULFONYLUREA HERBICIDES, WITH IMPROVED GENETIC RESISTANCE TO *PLASMOPARA HALSTEDII* PATHOGEN AND *OROBANCHE CUMANA* PARASITE

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INTRODUCTION

The primary aim of sunflower breeding programs in the state and private sectors, the increase in market segmentation has had a great impact on breeding goals in the last few years (Dozet and Windsor, 2016). This refers, in particular, to the increase in demand for sunflower varieties with higher oil yields, as well as the introduction of herbicide-tolerant hybrids.

For breeding purposes, without a doubt the most important mutations are the vital ones. Many sunflower mutations have been published to date, but very few have found commercial application. One of the most well-known, and economically most significant mutations affects acetohydroxyacid synthase (Al khatib *et al*., 1998). Sunflower tolerance to imidazolinones and sulfonylurea herbicide chemistries has significantly increased competitivness of sunflower in production and enabled manufacturers easier control of weed growth and, with the use of Clearfield technology, the control of broomrape. The ClearField-Plus mutation (Sala *et al*., 2008) patented by Nidera and BASF as well as the +M7 SU trait (Gabard and Huby, 2004) patented by DuPont are widely used in breeding today.

The introduction in agriculture practice of the sunflower genotypes resistant to herbicides it was a revolution for this crop.

The imidazolinone and sulfonylurea herbicides – advanteges: controle a wide weeds spectrum, have a small application rate and low toxicity.

Broomrape (*Orobanche cumana* Wallr.) is a parasitic angiosperm that has been causing a great deal of damage to sunflower production for more than a century.

Nowadays, broomrape causes great damage to sunflower production and new races of the pathogen appear frequently in Russia, Ukraine, Romania, Bulgaria, Turkey, and Spain (Škorić et al., 2010). Sunflower breeders and geneticists have been successful in developing broomrape resistant cultivars but breeding programs are often based on a reduced number of dominant genes and resistance breakdown caused by the appearance of new virulent races that overcome all known resistance genes occurs (Fernández-Martínez et al., 2012). This situation has forced sunflower breeders to continuously search for new sources of resistance and/or using alternative methods of control (Pacureanu –Joita and Perez-Vick, 2014).

The herbicides – imidazolinone are controlling and the parasite *Orobanche cumana* (broomrape). In the last years, the parasite has became slowly, resistant to herbicides. Technology SUNEO – initiated by the companies Limagrain and BASF (hybrids resistant to herbicides as well as to broomrape) is now developing.

Downy mildew (produced by *Plasmopara halstedii*) occurs in all regions around the world in which sunflower is grown as a major oil crop.

Currently, there are at least more than 36 pathotypes of *Plasmopara halstedii* worldwide (Gulya, 2007) but number is increasing rapidly (Virany and Spring, 2011).

A total of 18 DM resistance genes (R-genes) have been reported (*Pl1 to Pl16, Pl21, and PlARG).*

In Romania, eight pathotypes of the pathogen, have been identified, (Pacureanu, 2006; Anton et al., 2017).

The original variability of the cultivated sunflower is very narrow and deficient in genes applicable in selection for improvement of different agronomic traits.

Sunflower wild species are the most important and used genetic resources for the improvement of resistance to diseases, also to the broomrape parasite.

MATERIAL AND METHODS

Biological materials: sunflower lines resistant to herbicides (imidazolinone or sulfonylurea) and sources for resistance to broomrape as well as sources for resistance to downy mildew.

For releasing the infestation with parasite *Orobanche cumana* (broomrape), it has been collected broomrape seed from the most infested fields in the area cultivated with sunflower.

Method for testing resistance to the parasite *Orobanche cumana*: using the pots of 10 liters capacity, having inside a mixture of soil, sand and broomrape seeds.

For releasing the artificial infection with *Plasmopara halstedii* pathogen, there have been collected infected sunflower plants from different fields placed in all important areas of sunflower cultivation, inRomania. Pathogen inocula were directly recovered from infected leaves by brushing the fungal structures or after infected leaves were incubated in a humid chamber at 18 to 20°C in the dark for 24 to 48 h.. Thirty to forty pregerminated seeds for each genotype are inoculated by the whole-seedling immersion technique . After 12 days, plants are maintained at 20°C and 100% relative humidity for 24 to 48 h to enhance pathogen sporulation and evaluate for susceptible (sporulation on cotyledons and/or first true leaves) or resistance (absence of sporulation or weak sporulation only on cotyledons) reactions

All generations of selection are made under treatment with herbicides (Pulsar 40, Pulsar Plus or Express 50 SG) to be sure that we did not lose the genes for resistance.

RESULTS AND DISCUSSION

In our breeding program at NARDI Fundulea, there have been obtained some inbred lines with good resistance to herbicides (IMI or sulfonylurea), lines used as mother (CMS) in the hybrids, also, lines used as father (RF). In table 1 we are presenting these lines.

Table 1. Sunflower lines resistant to herbicides, obtained at NARDI Fundulea

No.	Lines	Genes	Type	Parental line in hybrid
1	CMS 274	IMI; SURES	A+B	X
2	CMS 312	IMI; SURES	A + B	-
3	CMS 425	IMI	A + B	X
4	CMS 482	IMI; SURES	A+B	X
5	RF 572	IMI; SURES	C	-
6	RF 627	IMI; SURES	C	X
7	RF 745	IMI	C	X
8	RF 789	SURES	C	X
9	RF 862	CLHA Plus	C	-
10	RF 943	CLHA Plus	C	-
11	RF 987	CLHA Plus	C	-

In figure 1 it is presented the evolution of the races of broomrape parasite, in sunflower crop in Romania. Already, in 2017 year we have identified that there are more than 8 races, in Braila area.

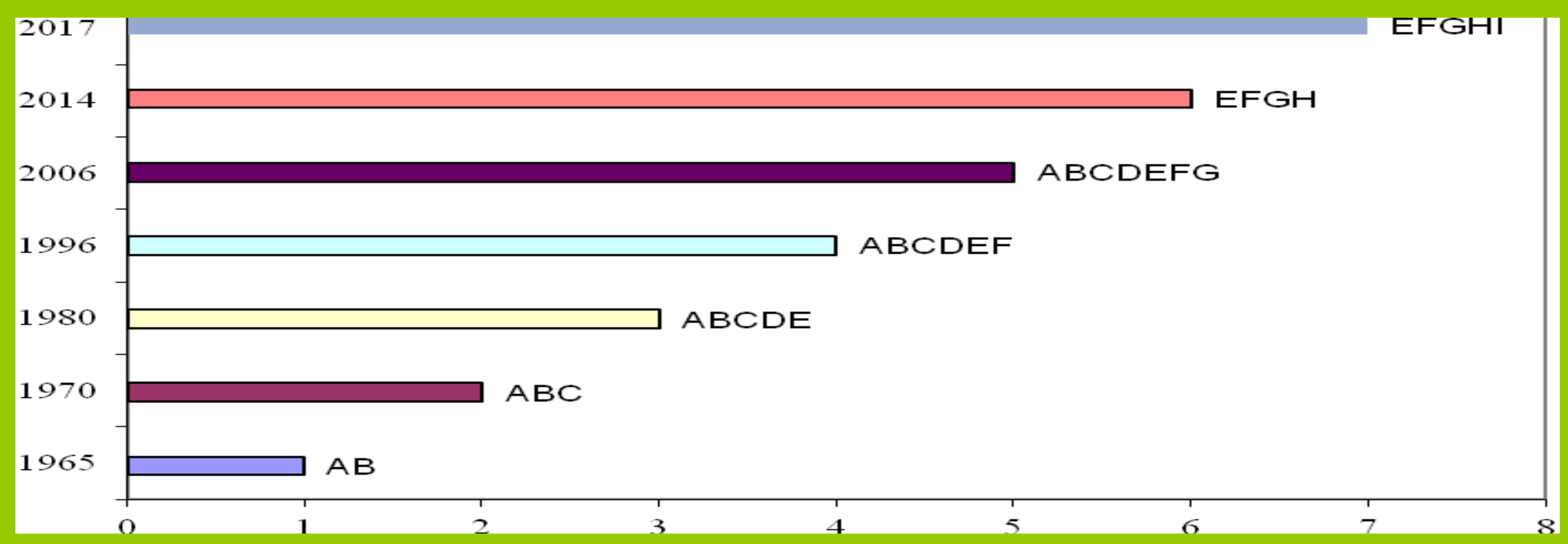

Fig 1. The evolution of the broomrape (*Orobanche cumana)* races in sunflower crop in Romania

For realising the genotypes resistant to both, *Plasmopara* pathogen and *Orobanche* parasite, we were looking for sources of resistance. Testing different wild species from our collection, we have identified some of them high resistant (table 2). By crossing wild species with cultivated sunflower we obtained interspecific hybrids. After generations of selections there have been released some lines, used as sources of resistance for genes transferring into valuable inbred lines resistant to herbicides.

For improving resistance (durable) to both pathogen and parasite in some inbred lines, we used the method presented in figure 2. Crossing a line with genes for resistance to *Plasmopara* with a line having genes for resistance *to Orobanche*, after many generations of selection, made under infection/ infestation with these two factors, finally will receive a valuable line having good and durable resistance.

Table 2. Results regarding the attack of the main sunflower diseases on some sunflower wild species (Fundulea, artificial infection/infestation)

No.	Species	Resistance to downy mildew (*Plasmopara halstedii*) (%)	Resistance to broomrape (*Orobanche cumana*) (%
1	**H. argophyllus**	**0.0**	**4.6**
2	H. tuberosus	14.6	1.2
3	H. divaricatus	0.0	11.9
4	H. maximiliani	18.5	0.0
5	H. hirsutus	21.0	1.2
6	H. mollis	23.3	0.8
7	H. salicifolius	0.0	17.4
8	H. niveus	19.3	15.6
9	H. neglectus	25.5	21.7
10	**H. debilis**	**0.0**	**0.0**
11	H. petiolaris	0.0	17.5
12	H. praecox	29.2	0.5
13	H. resinousus	31.3	22.4
14	H. decapetalus	0.0	19.8
15	H. nuttallii	30.8	31.6
16	H. pauciflorus	0.0	0.7
17	H. grosseserratus	24.6	14.6
18	H. eggertii	0.0	20.3
19	H. giganteus	0.0	12.9
20	Check sensitive	56,4	35.2

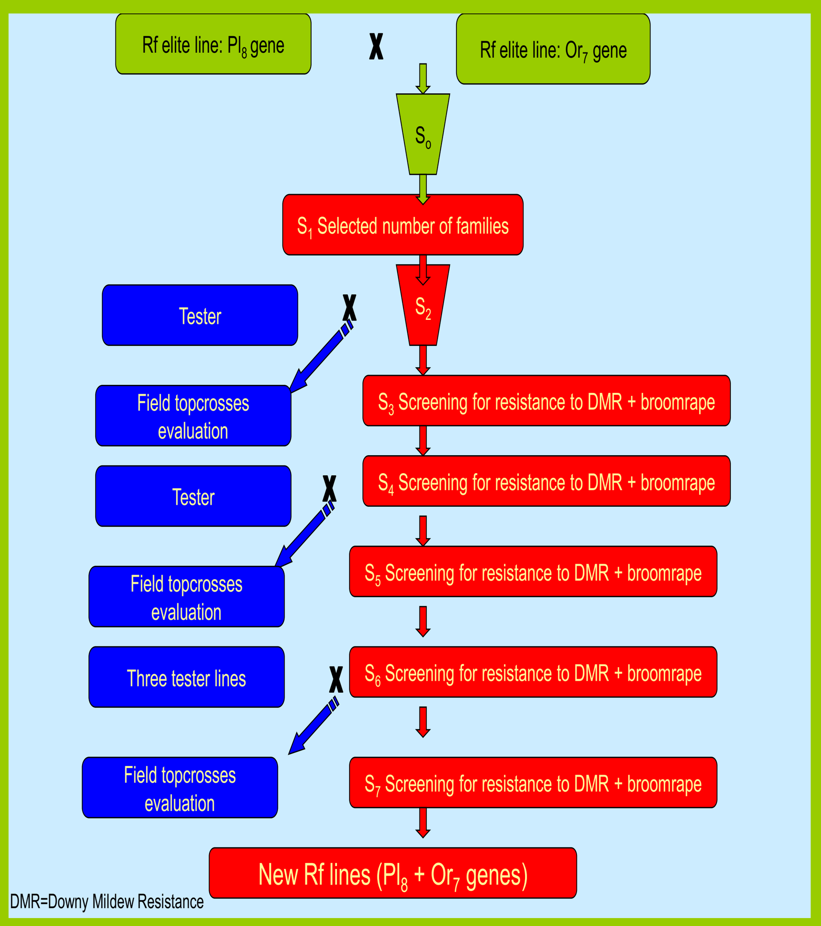

Fig 2. Scheme of selection for durable resistance to broomrape and downy mildew

In table 3 we are presenting the results obtained until now, in our work for introducing resistance to the pathogen *Plasmopara halstedii* and parasite *Orobanche cumana*, in the best lines having resistance to imidazolinone or sulfonylurea herbicides.

Table 3. Sunflower lines resistant to herbicides, in different generations of selection for resistance to downy mildew and broomrape

No.	Lines	Genes	Generation
1.	CMS 274	Pl6; Or 7	BC5F3
2.	CMS 312	Pl8; Or 7	BC6F1
3.	CMS 425	Pl 8; Or 6	BC6F3
4.	CMS 482	Pl6; Or7	BC6F1
5.	RF 572	Pl8; Or7	BC5F2
6.	RF 627	Pl6; Or8	BC6F3
7.	RF 745	Pl8; Or6	BC6F3
8.	RF 789	Pl6; Or8	BC6F1
9.	RF 862	Pl8; Or8	BC6F1
10	RF 943	Pl2;Pl6	BC5F1
11	RF 987	Pl6;Or7	BC6F2

CONCLUSIONS

IMI and sulfonylurea herbicides resistant are commonly use in sunflower production. Additionally, some genetically resistant broomrape plus downy mildew are needed for the seed market request. Novel genes were discovered in some wild type populations and could be transferred to cultural ones as develop more stable resistant genes in the future.

Sunflower will get advantage to other oil transgenic crops because, many important characteristics are developed through non-transgenic way.

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