THE IMPROVEMENT OF OIL QUALITY AND RESISTANCE TO BROOMRAPE IN SUNFLOWER GENOTYPES RESISTANT TO HERBICIDES

Maria JOITA-PACUREANU[1], Luxita RÎŞNOVEANU[2,3], Gabriel Florin ANTON[1, 4], Mihaela POPA[1,4], Alexandru BRAN[5], Elisabeta SAVA[5], Victorita MARIN[5]

e-mail: mariapacureanu2@yahoo.com

Abstract

Sunflower crop has an important place in the word agriculture, due to many advantages, as the capacity to release high seed yield and good oil content.
For improving different characteristics, by breeding methods, we need sources as genes donors.
Genetic resources in sunflower, which could to be used as base of creating new inbred lines or as donor sources for genes controlling different characteristics, in the inbred lines breeding, are made up of old or new varieties, hybrids and inbred lines, induced mutations, synthetic populations, as well as sunflower wild species.
Herbicide resistant crops are becoming increasingly common in agricultural production. A wild population of annual *Helianthus annuus* was the source for developing cultivated sunflower genotypes resistant to imidazolinone and sulfonylurea herbicides. There have been created inbred lines as sources for the genes transferring in the elite lines.
The quality of sunflower oil can be modified by means of induced and spontaneous mutations. The sunflower lines, sources for high oleic acid content, there have been obtained from a Russian variety, created by chemical mutation. Using these sources we transferred genes for improving the oil quality, in our elite lines, resistant to herbicides.
Broomrape, caused by *Orobanche cumana* Wallr. is a parasitic weed which infests sunflower roots, causing severe crop losses. Since broomrape is a highly variable parasite, the breakdown of resistance is a frequent phenomenon and multiple sources of resistance are needed. Genetic resistance to broomrape it was introduced in the sunflower crop from the wild relatives (*H. tuberosus, H. maximiliani, H. debilis*). The inbred lines created by interspecific hybridization have been used for the improvement of resistance to this parasite, of the lines resistant to imidazolinone and sulfonylurea herbicides.

Key words: *sunflower, genetic resources, resistance to herbicides, oleic acid, broomrape parasite.*

Oil crops take up around 10% of the total cultivable area worldwide. On the list of the most important oil crops, sunflowers hold a high position at number four. Romania is situated on the first place in EU as the surface cultivated with sunflower.

The primary aim of 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. In comparison to the situation 15 to 20 years ago, when there were 12 basic segments, there is greater complexity with 24 basic segments present in the contemporary market.

Complexity is further increased with the sub-segmentation of: herbicide-tolerance (HT) between sulphonylurea (SU) and imidazolinone (IMI) resistances and disease races present in specific regions (*e.g.* downy mildew- or broomrape-races).

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.

[1] National Agricultural Research and Development Institute Fundulea, Romania
[2] Engineering and Agronomy Faculty of Braila-Agronomy, Center for Research and Consultancy and Environment
[3] Agricultural Research and Development Station of Braila, Romania
[4] University of Agronomic Sciences and Veterinary Medicine – Bucharest, Faculty of Biotechnologies
[5] Institute for Variety Testing and Registration, Bucharest, Romania

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 (Gebard and Huby, 2004) patented by DuPont are widely used in breeding today.

Fatty acid composition mutations have also found their commercial use. The best known and most widely used is the Pervenets mutation (Soldatov, 1976) obtained by treating the seed of VNIIMK 8931 with 0.5% dimethyl sulfate. It led to the increase in oleic acid to 80-90%. The Pervenets variety is used to obtain gene lines and hybrids of high oleic content.

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 objective of this paper is to present the achievements in sunflower genetics and breeding for resistance to herbicides, the improvement of oil quality and resistance to the parasite *Orobanche cumana* so far and to describe the sources of this resistance as well as the breeding methods.

MATERIALS AND METHODS

Biological materials: sunflower lines resistant to herbicides (imidazolinone or sulfonylurea) and sources for resistance to broomrape as well as sources for high oleic acid content. 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 determination of high oleic acid content: Gaz Chromatograph method which consists in the extraction of sunflower oil from the seeds, using the organic solvents. After obtaining the sunflower oil, this was dried. Each sample of 250 grams of dried oil was treated with 5 ml heptadecanoat methyl, being obtained chromathograme (C14-C24:1).

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.

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 DISCUSSIONS

The oleic type sunflower production reached to almost 90% in US, more than 50% France and reached to very important rates in Spain, Argentina, Australia, etc.. Therefore, oleic type sunflower will is spreading quickly to the Black Sea countries because of presenting very healthy oil to customers and being very suitable for frying oil. Oleic type sunflower hybrids also are suitable for biodiesel, so it has potential for other than human food purposes too.

Using the sources (the fertile maintainer line for CMS lines and the restorer line, obtained from Pervenets variety), coming from North Dakota – Fargo University, USA, we transferred genes for high oleic acid content in the elite lines, resistant to imidazolinone or sulfonylurea herbicides. It has been used the backcross method (Figure 1), crossing the elite line (recurrent parent) with the donor of *Ol* gene (F1 generation), followed by 5-6 generations of backcrossing and 1-2 generations of self-pollination.

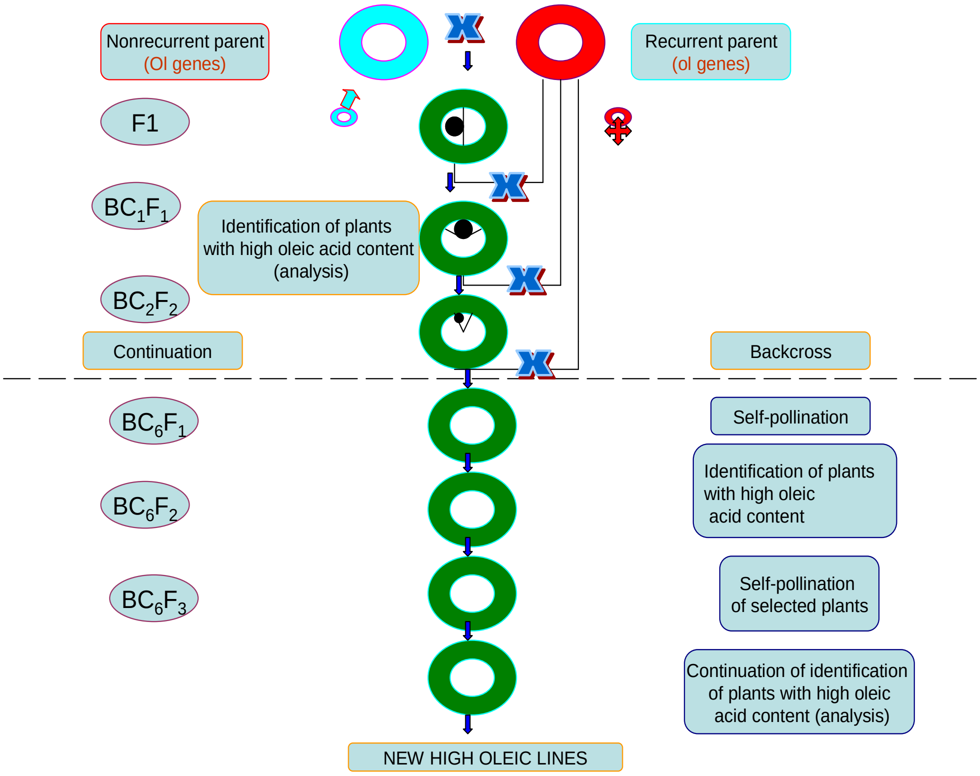

Figure 1 **Scheme of Ol gene transferring in elite lines**

All generations of selections have been done with determination of the oleic acid content and selection of the genotypes with higher one. In this way we obtained inbred lines with very good agronomic traits, resistant to herbicides and improved oil quality.

The oleic acid content it was very high in some lines (around 90%), the variation of this trait being determined by the genotype (lines introduced in this process). The results are presented in table 1.

Table 1.

The oleic acid content of several "high oleic" sunflower lines

No.	Genotype	Oleic acid content
1	LC-1114	84.1
2	LC-1204	86.0
3	LC-1220	88.1
4	LC-1123	88.7
5	LC-1135	89.4
6	LC-1101	87.9
7	LC-1128	85.3
8	LC-1107	84.1
9	LC-1144	84.0
10	LC-1137	90.2

The abundance and diversity of species within the genus *Helianthus* offer numerous and rewarding possibilities, for many characteristics to sunflower breeders. The results indicate that the sunflower gene pool could benefit from introgression of novel alleles from the latent genetic diversity present in the wild species.

Testing resistance of some sunflower wild species, to the newest races of the parasite *Orobanche cumana*, we have identified some of them, being high resistant to the races F,G and H **(**table 2).

Table 2.

Annual and perennial sunflower wild species, full resistant to broomrape (race F- G, H)

Annual species	H. agrestis
	H. anomalus
Diploid perennial species(2n=34)	H. atrorubens
	H. decapetalus
	H. giganteus
	H. grosseserratus
	H. salicifolius
	H. nuttallii
	H. maximiliani
Tetraploid perennial species (2n=68)	H. hirsutus
	H. levigatus
	H. pumilus
	H. strumosus
Hexaploid perennial species (2n=102)	H. californicus
	H. pauciflorus
	H. tuberosus

By interspecific hybridization (Figure 2) we obtained some sunflower lines which can be used as sources for genes transferring into elite lines resistant to herbicides, in order to improve the resistance to the new races of broomrape parasite.

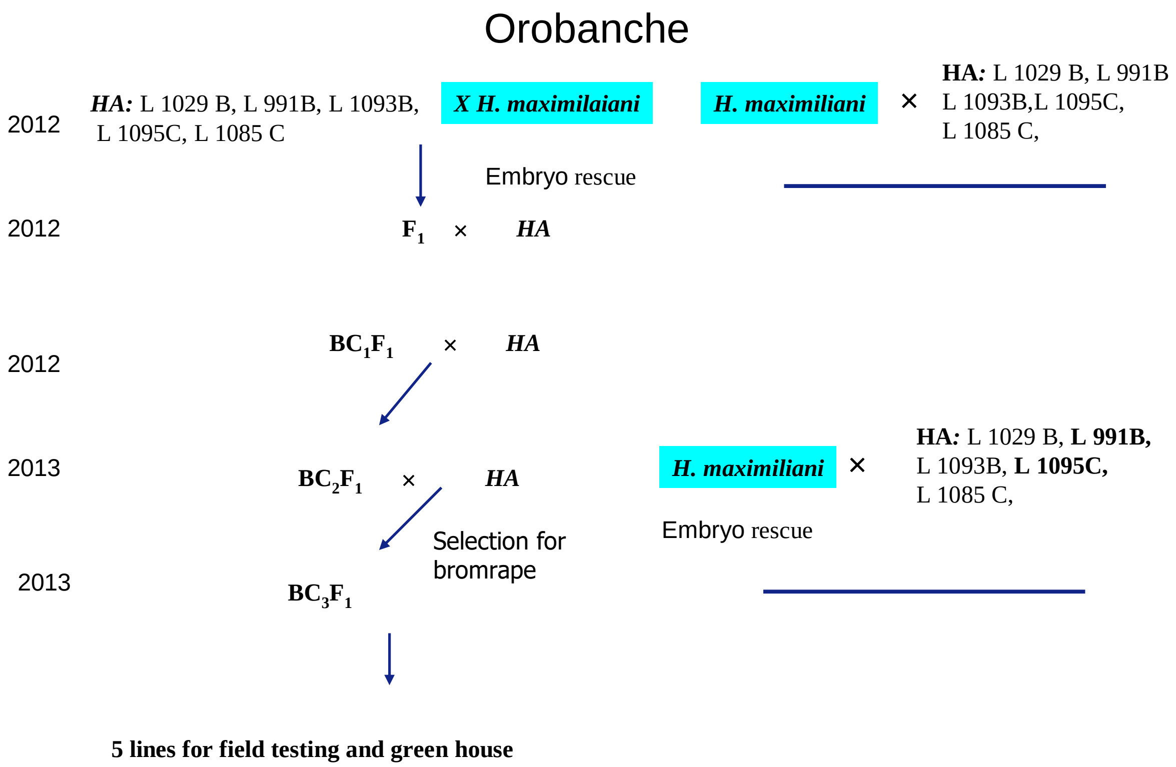

Figure 2. **Interspecific hybridization for resistance to broomrape**

For transferring *Or* genes in our best lines, resistant to herbicides it has been used the backcross method, each generation of selection being under infestation with the most virulent races of the parasite (Figure 3).

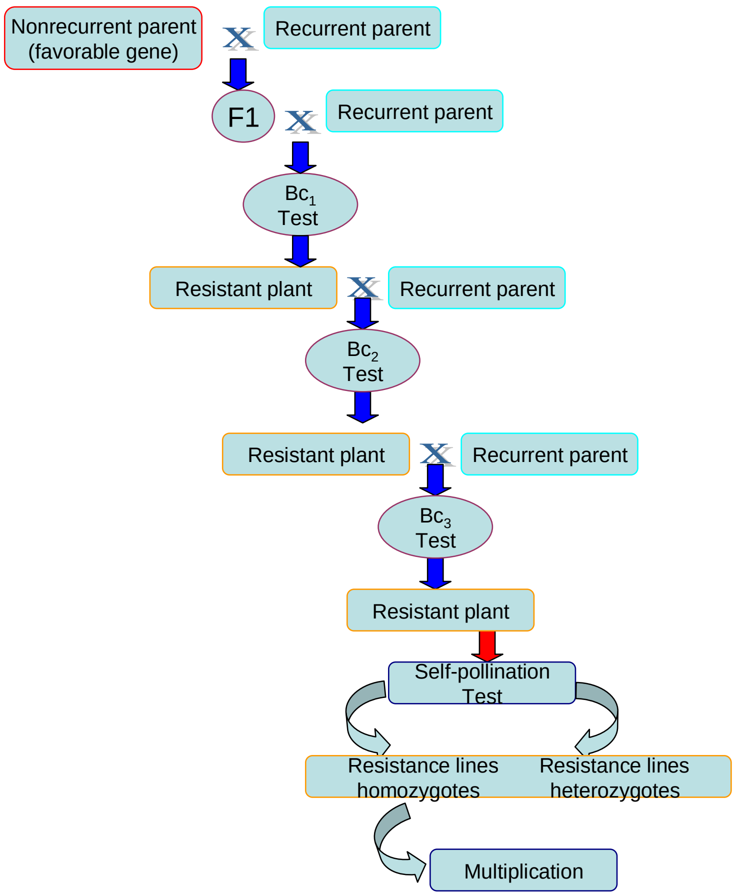

Figure 3. **Scheme of Or genes transferring in elite lines**

For both characteristics (oil quality and resistance to broomrape) it has been used embryo rescue method, in order to accelerate the process of selection (obtaining 3-4 generations/year). We need more generations of selection, specially for CMS lines, used as mother lines in the hybrids.

For these lines, after introducing the genes for high oil quality and for resistance to broomrape, in the maintainer form, we need other generations of selection for obtaining the sterile analogue.

Table 3.

The percent of the mature plant obtained by embryorescue method, in F1 generation, using Or genes for resistance to broomrape

Cross	Number of days from planting to flowering	Number of the saved embryos	Number of mature plants obtained	Percent of the mature plants
B1xSb1	85	105	64	60.1
B2xSb1	83	124	62	50.1
B3xSb1	78	145	102	70.0
B4xSb1	83	174	140	80.6
B5xSb1	81	140	140	100.0
B6xSb1	89	99	64	64.5
B7xSb1	78	95	85	89.7
C1xSc1	81	128	104	81.2
C2xSc1	85	97	83	85.5
C3xSc1	78	138	127	92.0
C4xSc1	83	125	94	75.2
C5xSc1	89	103	92	89.3
C6xSc1	78	84	78	92.8
C7xSc1	83	114	84	73.6

In table 3 are presented the results of using embryo rescue method, in the process of the *Or* genes transferring into elite lines. The percent of the obtained mature plants is enough high.

CONCLUSION

IMI and sulfonylurea herbicides resistance are commonly use in sunflower production. Additionally, some genetically resistant broomrape plus high oil quality 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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