NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 36, 2019
Print ISSN 1222–4227; Online ISSN 2067–5720

GENETIC RESOURCES FOR IMPROVING RESISTANCE TO THE MAIN DISEASES IN SUNFLOWER

Luxița Rîşnoveanu[1,2*], Maria Joiţa-Păcureanu[3], Florin-Gabriel Anton[3,4], Mihaela Popa[3,4], Alexandru Bran[4,5], Elisabeta Sava[5]

[1]”Dunărea de Jos” University of Galaţi, Engineering and Agronomy Faculty of Brăila-Agronomy, Centre for Research and Consultancy and Environment „Lunca”, 29 Călăraşi st., Brăila, Brăila County, 810017, Romania

[2]Agricultural Research Development Station Brăila, Viziru km. 9 street, Brăila, Brăila County, Romania

[3]National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Călăraşi County, Romania

[4]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd., District 1, 011464, Bucharest, Romania.

[5]The State Institute for Variety Testing and Registration, Bucharest, Romania, 61, Mărăşti Blvd., District 1, Bucharest, Romania

[*]Corresponding author. E-mail: luxita_risnoveanu@yahoo.co.uk

ABSTRACT

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. For improving sunflower resistance to the main diseases all these genetic resources can be used. In the sunflower breeding program of the National Agricultural Research & Development Institute (NARDI) Fundulea, Romania, we have used especially varieties, hybrids, inbred lines and sunflower wild species. The varieties and inbred lines were used for creating synthetic populations, which formed the base of obtaining new inbred lines resistant to *Plasmopara halstedii* (genes for resistance to different races of this pathogen). Sunflower wild species have been used for crossing with cultivated sunflower, obtaining interspecific hybrids, which allowed creating different donor sources of genes for resistance to *Plasmopara halstedii,* as well as to *Sclerotinia sclerotiorum*. We obtained some populations which are still in the breeding process for creating inbred lines with high resistance to *Phoma macdonaldii* and *Phomopsis helianthi* pathogens. We improved the resistance of some lines with good agronomic traits, to different diseases. The new inbred lines obtained by using different genetic resources were studied as general and specific combining ability for the seed yield and for oil content. Most of them were significantly superior as general combining ability for the seed yield or for oil content, or for both of them.

Keywords: sunflower, genetic resources, diseases, resistance, combining ability.

INTRODUCTION

Sunflower crop has an important place in the word agriculture, due to many advantages, as the capacity to produce high seed yield and good oil content. Sunflower oil has a very good quality, with high percent of the unsaturated acids and capacity to maintain stability and long time conservation.

Diseases are limiting factors in the production of sunflower on all continents where it is grown. Different diseases are dominant in different growing regions, depending on the prevailing environmental conditions. To diminish the effect of diseases on the sunflower seed yield, crop rotation and the farmers’ attitude in this field are of a great importance. The climate changes have an important role in influencing the sunflower yield and production, including the diseases development. More than 30 pathogens that attack sunflower and cause economic loss in production have been identified so far.

For obtaining good commercial sunflower hybrids, the breeding work must be accelerated. In the breeding work, the genetic variability in sunflower germplasm is very important. The original variability of the cultivated sunflower is very narrow and

Received 31 January 2019; accepted 4 March 2019. First Online: March, 2019. DII 2067-5720 RAR 2019-26

deficient in genes applicable in selection for improvement of different agronomic traits.

There have been changes in the racial composition of a number of pathogens. There are several reasons for this, one of the most important being the introduction of hybrids in commercial production, more homogenous with respect to the previous period, when genetically heterogeneous open-pollinating varieties were grown.

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 36 pathotypes of *Plasmopara halstedii* worldwide, but the number is increasing rapidly. In Romania, five pathotypes of the pathogen, were identified, before 2006, and in the last years other three were identified.

There is a rich germplasm possessing genes for resistance to the attack of this pathogen, transferred from wild sunflower species.

White rot (produced by *Sclerotinia sclerotiorum*) is a major problem in countries with a humid climate or in years with a wet summer. The fungus itself is polyphagous, attacking over 360 plant species, this making the selection for resistance difficult. Many of published papers about *Sclerotinia sclerotiorum* deal with the effect of the environmental factors on the occurrence of this pathogen in sunflower.

Stem canker (*Phomopsis/Diaporthe helianthi*) was first registered in Voivodina – Serbia and in Romania in 1980, when it caused large economic damage to sunflower production. Soon afterwards, it was registered in most sunflower growing countries in Europe. In the past three decades, *Phomopsis helianthi* become a most destructive disease on the global scale. Different methods for sunflower genotypes evaluation for resistance to *Phomopsis* have been experimented.

Sunflower breeders have achieved results in finding genes for resistance or high tolerance to certain diseases, in different sources: open-pollinating varieties, inbred lines, hybrids, as well as the wild sunflower species.

Our paper presents the results obtained in identifying some sources of genes for resistance to the main pathogens which attack sunflower crop, as well as in selection of resistant inbred lines.

MATERIAL AND METHODS

Several sunflower wild species have been tested for resistance to the pathogens which reduce downy mildew, white rot and brown spot in the cultivated sunflower.

The wild sunflower *H. petiolaris* and cultivated variety Start were used, for obtaining interspecific hybrids. The purpose was to transfer by crossing, genes for resistance to diseases (downy mildew and white rot) from the wild to the cultivated sunflower.

Pathogen inoculum of *Plasmopara halstedii* 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 hours. Thirty to forty pre-germinated seeds for each differential line (three replications/line) were inoculated by the whole-seedling immersion technique. After 12 days, plants were 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.

Screening germplasm for resistance to *Sclerotinia sclerotiorum* was made in the special nursery in field, containing naturally and artificially incorporated sclerotia in the soil. For *Phomopsis helianthi* a special field was used, where sunflower stem pieces with the pathogen pycnidia were incorporated.

RESULTS AND DISCUSSION

Some accessions of sunflower wild species from our collection in Fundulea were tested for resistance to the attack of *Plasmopara halstedii*, *Sclerotinia sclerotiorum* and *Phomopsis helianthi*. The results presented in Table 1 show that there are some species having resistance to all the three pathogens (*H. argophyllus*), some species resistant to *Plasmopara h.* and *Sclerotinia s.* (*H. petiolaris*), some others resistant to only one pathogen – *Plasmopara halstedii* (*H. eggertii* and *H. giganteus*), some others being resistant to *Sclerotinia* head or stalk attack and/or to *Phomopsis*.

Table 1. Results regarding the attack of the main sunflower diseases on some sunflower wild species (Fundulea, 2010 year)

No.	Species	Resistance to downy mildew (*Plasmopara halstedii*) (%)	Resistance to *Sclerotinia sclerotiorum*				Resistance to *Phomopsis helianthi*	
			Stalk rot		Head rot		Stem	
			%	i	%	i	%	i
1	*H. argophyllus*	0.0	0.7	R	12.7	S	0.8	R
2	*H. tuberosus*	14.6	16.2	S		R	1.2	R
3	*H. divaricatus*	0.0	11.6	S	15.4	S	11.9	S
4	*H. maximiliani*	18.5	0.9	R	14.3	S	0.9	R
5	*H. hirsutus*	21.0	18.0	S	18.6	S	1.2	R
6	*H. mollis*	23.3	25.1	S	15.7	S	0.8	R
7	*H. salicifolius*	0.0	22.4	S	15.2	S	17.4	S
8	*H. niveus*	19.3	0.5	R	19.8	S	15.6	S
9	*H. neglectus*	25.5	1.0	R	17.9	S	21.7	S
10	*H. debilis*	0.0	0.8	R	23.5	S	29.4	S
11	*H. petiolaris*	0.0	1.2	R	21.9	R	17.5	S
12	*H. praecox*	29.2	19.8	S	0.9	R	13.6	S
13	*H. resinousus*	31.3	19.1	S	0.7	R	22.4	S
14	*H. decapetalus*	0.0	17.8	S	0.5	R	19.8	S
15	*H. nuttallii*	30.8	18.6	S	0.8	R	31.6	S
16	*H. pauciflorus*	0.0	20	S	12	R	0.7	R
17	*H. grosseserratus*	24.6	15.9	S	0.9	R	14.6	S
18	*H. eggertii*	0.0	30.4	S	28.6	S	20.3	S
19	*H. giganteus*	0.0	27.3	S	19.4	S	12.9	S
20	Check sensitive	56.4	36.3	S	29.5	S	35.2	S

Crossing sunflower wild species with cultivated sunflower we obtained interspecific hybrids, which are used in selection of sunflower inbred lines with genes for resistance to the pathogens for which breeding work is done.

Results regarding some important characteristics of the interspecific hybrids released by crossing the wild *H. petiolaris* with cultivated variety Start are presented in Tables 2, 3 and 4.

The characteristics which are the most important in the breeding programs were measured, and results regarding some morphological characteristics, as well as the oil content and thousand kernel weight are presented. The data show that in most of cases, differences from the cultivated genotype are statistically important. A positive significance was determined for characteristics like number of branches, branches head diameter, length of branches. These characteristics are very important for the restorer inbred lines, in sunflower breeding work. Due to these data we can to say that the hybrid populations are closed to the cultivated sunflower parent.

Table 2. Characteristics of some interspecific hybrids obtained by crossing cultivated sunflower *H. annuus* (Start variety) with *H. petiolaris* (average 2011-2013)

Genotype	Plant height (cm)	No. of leaves	Leaf width (cm)	Leaf length (cm)	Petiole length (cm)
1. Cultivated sunflower:					
Start	165.4	32.0	20.3	21.0	14.5
2. Wild species:					
H. petiolaris	86.5	191.0	0.6	18.7	0
3. Interspecific hybrids:					
HI 23	138.4**	28.0**	16.7***	16.5***	14.4***
HI 28	132.4**	22.0**	15.1***	16.0***	14.2***
HI 33	108.5**	20.0**	16.7***	15.9	10.2***
LSD: 0.05	12.7	31.4	36.3	9.7	43.5

*Table 3***.** Characteristics of some interspecific hybrids obtained by crossing cultivated sunflower *H. annuus* (Start variety) with *H. petiolaris* (average 2011-2013)

Genotype	Distance between knots (cm)	Stem diameter (mm)	Head diameter (cm)	Number of branches (n)	Length of branches (cm)	Number of flowers ranges (n)
1. Cultivated sunflower:						
Start	6.2	28.2	23.5	0	0	53.0
2. Wild species:						
H. petiolaris	14.9	12.3	1.4	12.0	12.8	15.0
3. Interspecific hybrids:						
HI 23	5.8*	22.5***	12.8**	24.0***	28.6***	48.0*
HI 28	5.5*	24.5***	12.6**	23.5***	21.5***	51.0*
HI 33	4.7***	22.0***	15.0**	n.a.	n.a.	45.0**
LSD: 0.05	5.9	11.2	29.6	36.7	43.9	16.3

Table 4. Characteristics for some interspecific hybrids obtained by crossing cultivated sunflower *H. annuus* (Start variety) with *H. petiolaris* (average 2011-2013)

Genotype	Lateral heads diameter (cm)	Kernel wide (mm)	Kernel length (mm)	Kernel diameter (mm)	Oil content (%)	TKW (g)
1. Cultivated sunflower:						
Start	0	5.9	11.2	4.1	48.0	78.3
2. Wild specie:						
H. petiolaris	14	2.6	4.0	1,3	29,2	6.3
3. Interspecific hybrids:						
HI 23	7.1***	5.0***	11.2	3.6*	45.2**	35.4***
HI 28	8.6***	5.3**	11.0	3.3*	45.0**	37.3***
HI 33	0	5.0***	9.1	3.7*	44.1***	40.3***
LSD: 0.05	23.6	3.8	11.5	5.2	3.1	24.3

Recurrent phenotypic selection was implemented on these three hybrid populations, under infection with the two pathogens which produce downy mildew and white rot. Based on number of plants not infected, compared within the population and with the checks, the selection was used to identify the lines with highest resistance. Selected plants were planted in the greenhouse, in winter time, and a new generation was obtained. The resultants were planted next year in the field, under infection with the pathogen. Lines with highest level of resistance were included in creating the next cycle of selection. Three selection cycles have been completed so far.

The obtained lines were crossed and the best hybrid combinations after studying combining ability for seed yield and oil content were identified. The parental inbred lines and their hybrids were tested for resistance to the pathogens *Plasmopara halstedi* and *Sclerotinia sclerotiorum*. In Table 5, the results regarding the resistance to the different inoculums of the pathogen *Plasmopara halstedii* are presented. They show that in case of inoculums from Brăila and Ialomiţa, all lines, except R 558, are fully resistant, in all other cases only the line B 112 being fully resistant. All the hybrids having as mother this line were fully resistant, taking into consideration that the inheritance of sunflower resistance to this pathogen is of vertical type (one dominant gene).

Table 5. Screening of sunflower inbred lines and hybrids for resistance to downy mildew (artificial infection, Fundulea, 2016)

Genotype	*Plasmopara halstedii* inoculum/attack degree					
	Brăila (%)	Craiova (%)	Ialomiţa (%)	Constanţa (%)	Fundulea (%)	Tulcea (%)
R 241	0.0	1.40	0.0	0.52	1.0	1.24
R 334	0.0	1.56	0.0	1.60	2.70	1.31
R 542	1.46	3.72	1.0	0.73	1.84	3.44
R 558	0.60	1.36	0.40	1.64	2.30	2.52
B 112	0.0	0.71	0.0	0.51	1.55	1.45
B 184	0.0	1.94	0.0	1.72	1.34	2.70
112 x 241	0.0	5.18	0.0	3.55	1.0	2.87
112 x 334	0.0	2.69	0.0	0.89	1.37	1.43
112 x 558	0.0	4.63	0.0	3.86	2.42	3.17
184 x 241	0.0	3.25	0.0	3.63	1.22	4.25
184 x 334	0.0	2.41	0.0	1.70	2.63	3.30
184 x 542	0.0	2.36	0.0	2.89	3.23	1.40
Check	69.34	67.32	73.31	54.56	61.61	65.74

The results of screening for resistance to the pathogen *Sclerotinia sclerotiorum* (lines and hybrids) are presented in Tables 6 and 7. Taking into consideration that the inheritance of sunflower resistance to this pathogen is of horizontal type (minor genes) the genotypes were not fully resistant, but they had a high level of resistance (83-98 percent). The level of resistance was higher in the inbred lines comparing with hybrids. The hybrids had a lower level of resistance comparing with check.

Table 6. Screening of inbred lines for *Sclerotinia* tolerance, over two years and three locations (Brăila, Fundulea, Mircea Vodă)

Inbred line	2014	2015	Mean
	Resistant (%)	Resistant (%)	
R 241	95	93	94
R 334	96	100	98
R 542	83	83	83
R 558	94	92	93
B 112	98	96	97
B 184	95	89	92
Resistant check	86	78	82
Mean			91
LSD 0.05			15

Table 7. The performance of sunflower hybrids grown in *Sclerotinia* nursery in Fundulea, during 2015-2016

Hybrid	2015	2016	Mean
	Resistant (%)	Resistant (%)	
CMS 112 x R241	93	89	91
CMS 112 x R334	89	95	92
CMS 112 x R558	88	78	83
CMS 184 x R241	81	93	87
CMS 184 x R334	80	88	84
CMS 184 x R542	81	83	82
Check resistant	98	95	93
Mean			84
LSD 0.05			13

The results regarding some important characteristics of the obtained hybrids are presented in Table 8. They produced a seed yield higher, or close to the check, in conditions of infection with *Sclerotinia sclerotiorum*, in natural infection condition. The oil content was close to the check hybrid, but not higher. The same was true for thousand kernels weight, except for the hybrid CMS 184 x R 542.

Table 8. Some characteristics of the sunflower hybrids, in trial planted in Brăila, in 2016 year

Hybrid	Yield (kg/ha)	Oil content (%)	Days to flowering	Height (m)	1000 kernel weight (g)
CMS 112 x R241	3178	50.2	68	1.7	65
CMS 112 x R334	3386	47.9	69	1.9	61
CMS 112 x R558	2980	49.4	71	1.7	66
CMS 184 x R241	3042	46.7	68	1.8	63
CMS 184 x R334	2856	51.3	67	2.0	60
CMS 184 x R542	2774	49.6	69	1.7	71
Resistant check	2890	51.8	69	1.8	70
Mean	3015	49.5	68.7	1.8	65
LSD 0.05	216	3.2	3.0	1.0	2.4

CONCLUSIONS

The sunflower wild species are very good genetic resources for several positive characteristics in the cultivated sunflower, including disease resistance. The interspecific hybrids obtained by crossing the wild with cultivated sunflower had important characteristics close to the cultivated sunflower.

Inbred lines selected from these hybrid populations were identified with high level of resistance to the pathogen *Sclerotinia sclerotiorum*. Total immunity was not found.

The obtained hybrids had the seed yield and oil content, close to the check having high resistance to this pathogen.

Some inbred lines had good resistance to the pathogen *Plasmopara halstedii*, the hybrids obtained with one fully resistant parental line, being fully resistant.

REFERENCES

Anton, F.G., Joiţa-Păcureanu, M., Rîşnoveanu, L., Cornea, C.P., Popa, M., 2017. *Downy mildew in sunflower-the management of Plasmopara halstedii pathogen*. Scientific Bulletin, Series F, Biotechnologies, XXI.

Gulya, T.J., 2007. *Distribution of Plasmopara halstedii races from sunflower around the world*. In: Proccedings of the 2nd Int. Downy Mildew Symposium. Palcky University in Olomouc, Czech Republic, 3: 121-134.

Keskin, G., Dellal, I., 2011. *Economic sustainability of sunflower production in Thrace region of Turkey.* Journal of Environmental Protection and Ecology, 12, 1: 245-250.

Konyali, S., Kiper, T., 2012. *Effect of climate changes on agricultural product quantity and yield and its reflection on sustainable development. The case of the Thrace region*. Journal of Environmental Protection and Ecology, 13, 1: 392-397.

Masirevic, S., 1995. *Proposed methodologies for inoculation of sunflower with Phomopsis spp./Diaporthe spp. and for disease assessment*. FAO (Rome) - European Research Network on Sunflower. Proposed Methodologies for inoculation of sunflower with different pathogens and for disease assessment, Bucharest: 11-13.

Masirevic, S., 2000. *Evolution of sunflower germplasm for resistance to Phomopsis stem canker.* Proc. 15th Intl. Sunflower Conf. Toulouse, France, June 12-15, Intl. Sunflower Assoc. Paris, France, 2: 84-89.

Joiţa-Păcureanu, M., 2006. *Evolution of the pathogen-host plant relationship, into Plasmopara halstedii (helianthi) - Helianthus annuus L. system, in Romania*. SUNBIO Conference, Gengenbach, Germany, September 2006. Abstracts, pp. 7.

Skoric, D., Jocic, S., Jovanovic, D., Hladni, N., Marinkovik, R., Atlagic, J., Pankovic, D., Vasic, D., Mladinovic, F., Gvozdenovic, S., Terzic, S., Sakac, Z., 2006. *Achievements of sunflower breeding*. (In Serbian). Periodical of Institute of Field and Vegetable Crops, Novi Sad, 42: 131-173.

Skoric, D., Jocic, S., 2004. *Achievements of sunflower breeding at the IFVC in Novi Sad*. In: Seiler, G.J, Proc. 16th Intl. Sunflower Conf., Fargo, ND, USA, 29 August - 4 September 2004. Intl. Sunflower Assoc., Paris, France, 2: 451-458.

Skoric, D., Seiler, G.J., Liu, Z.C., Jan, C., Miller, J.F., Charlet, L.D., 2012. *Sunflower genetics and breeding*. Serbian Academy of Sciences and Arts, Novi Sad, Serbia: 1-496.

Viranyi, F., Spring, O., 2011. *Advances in sunflower downy mildew research*. European Journal of Plant Pathology, 129(2): 207-220.

Vrânceanu, A.V., 2000. *Floarea-soarelui hibridă*. Edit. Ceres, Bucureşti: 1-1147. (In Romanian)

.