Scientific Papers. Series A. Agronomy, Vol. LV-2012
ISSN Online 2285-5793; ISSN-L 2285-5785

THE USES OF WILD SPECIES HELIANTHUS ARGOPHYLLUS FOR OBTAINING SUNFLOWER GERMPLASMS WITH IMPROVED RESISTANCE TO DROUGHT AND BROOMRAPE INFESTATION

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Abstract

In this paper we present the results from the study on new sunflower forms obtained through hybridization between cultivated sunflower (Helianthus annuus) and wild species Helianthus argophyllus. The aim was to improve drought and broomrape resistance of some Romanian sunflower inbreed lines. The investigation encompassed the period 2008-2011. Interspecific F1 plants were obtained by embryo rescue techniques, and than as a results of and back-crossing and selfpollination with cultivated sunflower, BC4F2 was obtained. The heritability in first generation was intermediate but the plants strongly resembled the wild species in their most morphological traits. For drought resistance we selecting the plant with pubescent leaves. The BC2, BC3 and BC4 were assayed in vegetation house for broomrape resistance with tests performed with artificial inoculation using broomrape seeds collected from two infested areas with broomrape (F and G races). Concerning drought and broomrape resistance the results indicated good resistance, suggesting successful of gene introgression. Resistant lines will be selfpollinated and retested in the next year and some of the obtained hybrid forms will be included in a sunflower breeding program as genetic sources for drought and broomrape resistance.

Key words: *wild sunflower, interspecific hybridization, embryo rescue, drought and broomrape resistance.*

INTRODUCTION

Sunflower is a plant of the American continent where *Helianthus annuus*, together with *Helianthus tuberosus*, has been used since antiquity. The first attempt at using interspecific hybridization in sunflower was made in Russia in 1916 [4]. After many years, due to climatic changes, the interest in interspecific hybridization is renewed, as a tool to achieved stress and diseases resistance, CMS sources, oil quality or modified biochemical composition.

The usefulness of many species of wild sunflower is limited by their poor crossability and high degree of F1 sterility in interspecific hybrids. These impediments can be overcome by using embryo rescue techniques, chromosome doubling of the F1 and the creation of amphiploids [5].

The wild *Helianthus argophyllus* species possess considerable variability for resistance to drought, diseases and parasitic plant which can be utilized for the improvement of cultivated sunflower [5].

This paper aims to present a part of the results of interspecific hybridization between cultivated sunflower (*Helianthus annuus*) and wild species (*Helianthus argophyllus*) and present their potential useful for breeding and selection.

MATERIAL AND METHOD

Six cultivated sunflower (Romanian sunflower inbreed lines created by NARDI Fundulea, 2n = 34) and wild species (*Helianthus agrophyllus*, 2n=34), were grown under field conditions and vegetation house at NARDI Fundulea during 2008-2011.

Methods included: interspecific hibridization, embryo rescue, selfpollination and back-crossing, field and biochemical evaluation.

For hibridization, starting from the beginning of anthesis, daily the female plants were hand emasculed and fresh pollen was applied to the inflorescences. Pollination was performed with a flannel applicator every two days. The wild species were used both as mother and father parent, in order to make a comparative study and finally to choose what results to be promoted (Table 1).

Table 1. Working scheme

2008	***Helianthus argophyllus***	**x**	***Helianthus annuus* (L 1029 B, L 991B, L 1093B, L 1095C, L 1085 C, L 1088 C**
		↓	Embryo rescue
	F1	x	*Helianthus annuus*
		↓	(Screening for pubescence)
	BC_1F_1	x	BC_1F_1 (Selfpollination)
		↓	
2009	BC_1F_2	x	*Helianthus annuus* (Screening for phomopsis)
		↓	
	BC_2F_2	x	*Helianthus annuus* (Screening for broomrape)
		↓	
2010	BC_3F_2	x	*Helianthus annuus* (Screening for broomrape)
		↓	
2011	BC_4F_2	x	*Helianthus annuus* (Screening for broomrape)
		↓	
	13 lines BC_5F_2 for breeding program		

A method for breaking the dormancy and retrieving seedlings from sunflower embryos 20 days post-pollination was used in case of interspeciphic hybrids with *H. argophyllus*. Embryos allowed to develop in planta for 20 days were excised, dehulled and incubated under lights (12 h photoperiod) in Petri dishes on filter paper moinstened with 10 ml of a solution containing 0.025 ppm GA3, 1 ppm IAA and 2.5 ppm KNO3.
The descendants were investigated for some characteristics important in sunflower breeding. Biometric studies and biochemical characterization of seeds were carried out in F2 generation. For drought resistance were selected the plants with pubescent leaves. The BC2 – BC4 plants were also investigated for broomrape resistance with tests performed under artificial inoculation using broomrape seeds from two Romanian infested areas.

RESULTS AND DISCUSSIONS

The BC1F1 hybrids presented a large variability concerning morphological traits, such as leaf length, leaf weight, height of plants, branches, pubescences and size of head and seeds, (Photo 1 and 2).

Photo 1. *H. Agrophyllus* x LC 1029 B, (BC_1F_2), heights superior to the parents

Photo 2. *H. argophyllus* x LC 1088C, BC1F2, pubescent leaves

Leaf lengths of sunflower inbred ranged from 174 to 258 mm and average of 70 mm for wild species (*H. argophyllus*). In hybrid plants, high value was obtained in combination *H. argophyllus* x LC 1095 C (262 mm). The leaf width presented genotypic variability and ranged from 150 mm (LC 1095 C) to 238 (LC 1093 B) mm for sunflower inbreeds, 37 mm for *H. argophyllus*, and from 150 mm (LC 1095 C x *H. argophyllus*) to 246 mm (LC 1093B x *H. maximiliani*) for hybrid plants (Table 2). This last exemple shows a high hybrid value. This trait is determined by four dominant genes action [9].

Leaf area, like the above characters was variable according to the genotype (Table 2). In fact recent studies have detected common QTL for leaf area at flowering (LAF-P-12-1, LAF-W-12-1) in linkage group 12. Genomic regions on the linkage groups 9 and 12 are specific for QTLs of leaf-related traits in sunflower [3].

Table 2. Variability of parental lines and descendants (BC_1) for morphological aspect of leaves

Biological material	Leaf lenght (mm)	Leaf width (mm)	Leaf area (mm^2)
LC 1029 B	203	206	290
LC 991 B	210	232	335
LC 1093 B	258	238	422
LC 1085 C	246	207	355
LC 1095 C	174	150	179
LC 1088 C	195	156	208
L 1029 B x *H. argophyllus*	168	159	183
L 991 B x *H. argophyllus*	228	226	239
L 1093 B x *H. argophyllus*	224	207	318
L 1095 C x *H. argophyllus*	155	135	143
H. argophyllus x L 1029 B	225	193	131
H. argophyllus x L 991 B	198	150	205
H. argophyllus x L 1093 B	200	150	205
H. argophyllus x L 1085 C	216	177	264
H. argophyllus x L 1095 C	262	187	111
H. argophyllus x L 1088 C	226	192	198
H. argophyllus	70	78	37

Height of plants is an agronomic trait involved in plant productivity. It is polygenically controlled and low stem is controlled by recesive dwarfing genes, but all modes of inheritance for plant height were present in the F1 generation. Heterosis was most frequent, followed in decreasing order by partial dominance, dominance and intermediacy [8].

Our results show that sometimes the height of hybrids was superior to parental lines (Fig. 1). This suggests that wild parent dominated in genetic control of that trait over the cultivated one.

The hybrid plants (BC1, F1) had an intermediate value of weight of 1000 seeds (Fig. 2). This trait is inherited by incomplete dominance [3].

Head diameter is an important yield component. Several workers have suggested significant positive correlation between head diameter and seed yield and thus concluded that increased head diameter could lead to higher seed yield. One the other hand diameter of head is strongly influenced by environmental conditions [7]. Head diameter study releaved a wide range of values of this character. The cultivated sunflower parental lines had a head diameter between 100-120 mm. In comparison with those parental lines some descendants presented high values of this trait (higher 140 mm), (Fig. 3). The size of diameter is a polygenic character with strong additive effect [9].

The oil content is presented in Fig. 4. The average oil content of F2 seeds was very close to that of the maternal parent, indicating almost complete dominance of the maternal parent. This result is in agreement with those obtained in rape [1, 2].

The screening for broomrape resistance was performed in vegetation house with F and G broomrape race (Table 3).

Table 3. The broomrape resistance of descendants (BC4, F2, 2011)

	Frequency [(no. of infested sunflower plants/total no. of plants) x 100]	Intensity (no. of *Orobanche* plants/no. of infested sunflower plants)	Attack degree [(F x I) / 100] (%)
Control	100	23.0	23.0
Arg x 4C (NR)	66.7	5.7	3.8
Arg x 4C (DR)	100	10.0	10.0
Arg x 4C (D,I)	100	4.0	4.0
Arg x 6C (N)	100	4.0	4.0
Arg x 6C (D)	66.7	1.7	1.1
Arg x 5C (N)	100	36.0	36.0
Arg x 5C (D)	100	3.5	3.5
1B x Argo.	100	6	6
2 B x Argo.	100	13.5	13.5
4C x Argo.	66	3.3	2.2
3B x Argo.	33	0.3	0.1
5 C x Argo.	100	24	24
6C x Argo.	100	9	9

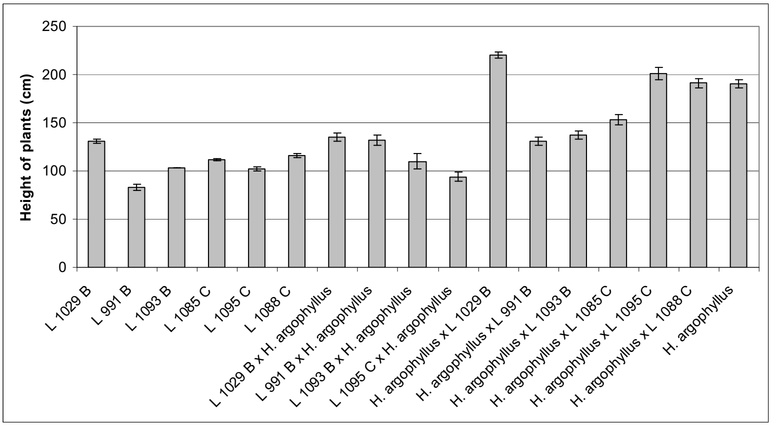

Fig. 1. Height of parental lines and descendants (BC1)

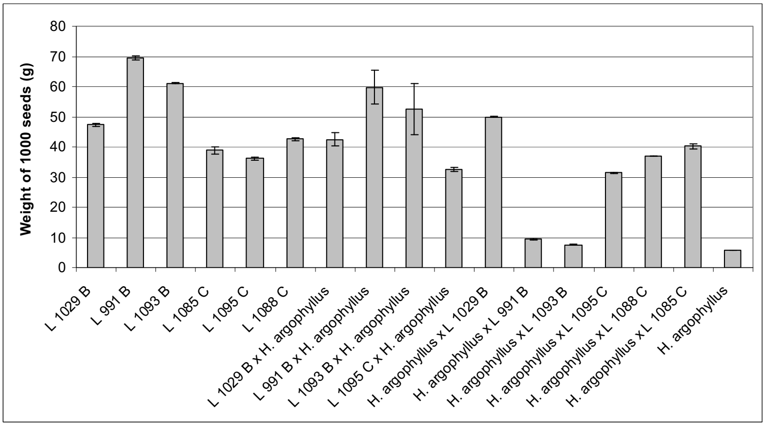

Fig. 2. Weight of 1000 seeds for parental line and obtained descendants (BC_1)

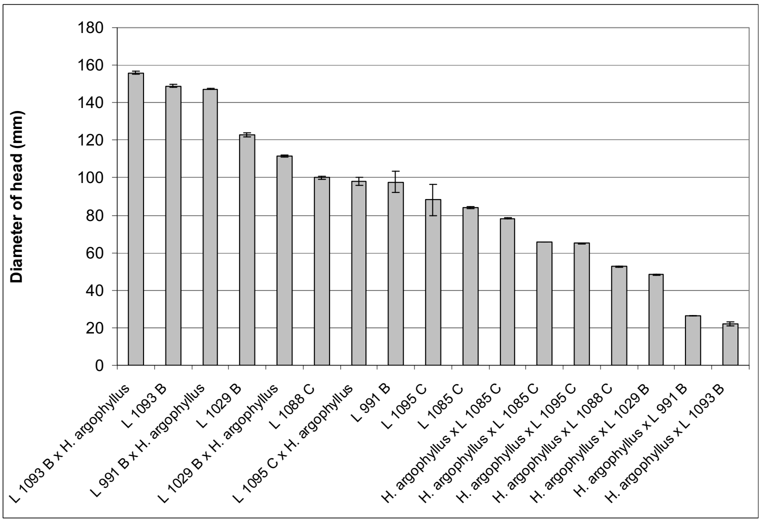

Fig. 3. Diameter of head for parental line and obtained descendants (BC_1)

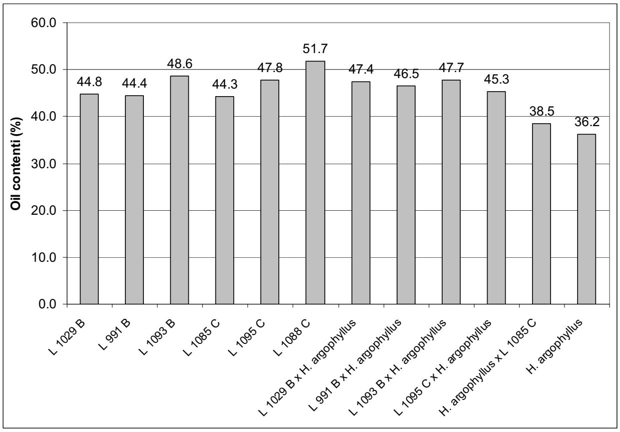

Fig. 4. The oil content in parental line and obtained descendants (BC_2, F_2)

The descendants which were presented the broomrape infestation smaller than control were self pollinated and those with high broomrape infestation (5C x Argo, Argo. X 5C (N) were pollinated with *Helianthus maximiliani*, other sunflower species known as resistant to broomrape infestation. Broomrape presents serious problems to sunflower production in Romania, as well. It is constantly expanding its distribution area, forming new more virulent races [6]. Although some authors indicate possibilities of chemical control of broomrape, though most studies show that genetic resistance is the most important method for controlling the parasite.

CONCLUSIONS

The wild specie *Helianthus argophyllus*, can be crossed as a female parent with *Helianthus annuus* and F1 hybrids were obtained by embryo rescue technique. When cultivated sunflower (*H. annuus*), which was crossed as a female parent with both wild species, the F1 hybrids obtained in this study were fertile and had a combination of morphological traits from both parents. The screening of descendants for pubescences and broomrape resistance was done and results indicated a good drought resistance and broomrape resistance for some of them. The descendants with good performances will be self pollinated in next generation because interspecific hybrids are important as donors for introgressing new favourable alleles into parental inbred lines.

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