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DEVELOPING DROUGHT AND BROOMRAPE RESISTANT SUNFLOWER GERMPLASM UTILIZING WILD *Helianthus* SPECIES

Petcu, E.* and Pâcureanu, J.M.

National Agriculture Research and Development Institute Fundulea, 915200 Fundulea, Câlâraşi, Romania

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SUMMARY

In this paper we present the results of the study of new sunflower forms obtained through hybridization between cultivated sunflower (*Helianthus annuus*) and two wild *Helianthus* species. Wild species *H. argophyllus* and *H. maximiliani* were used in order to improve drought and broomrape resistance of some Romanian sunflower inbreed lines. The investigation encompassed the period 2008-2009. Interspecific F_1 plants were obtained by embryo rescue techniques, and BC_2, F_3 were obtained as a result of self-pollination and back-crossing with cultivated sunflower. The heritability in the first generation was intermediate, but the plants strongly resembled the wild species in most bio-morphological traits. The plant with pubescent leaves was selected for drought resistance. The F_2 plants were also investigated for broomrape resistance using tests performed under artificial inoculation with broomrape seeds from two infested areas in Romania. Concerning drought and broomrape resistance the results indicated good resistance, suggesting successful gene introgression. Resistant lines will be self-pollinated and retested in the next year and some of the obtained hybrid forms will be included in the sunflower breeding program as genetic sources for drought and broomrape resistance.

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

INTRODUCTION

Sunflower is a plant of the American continent where *Helianthus annuus*, together with *H. tuberosus*, has been used for ages. The first attempt at using interspecific hybridization in sunflower was made in Russia in 1916 (Iuoraş *et al.*, 2002). After many years, due to climate change, the interest in interspecific hybridization has been renewed as a tool to obtain stress and diseases resistance, *cms* sources, oil quality or modified biochemical composition.

* Corresponding author: e-mail: petcue@ricic.ro

The usefulness of many species of wild sunflower is limited by their poor crossing ability and high degree of F_1 sterility in interspecific hybrids. These impediments can be overcome by using embryo rescue techniques, chromosome doubling of the F_1 and the creation of amphiploids (Jan *et al.*, 2008).

The wild *Helianthus argophyllus* and *H. maximiliani* species possess considerable variability for resistance to drought, diseases and parasitic plant Jan *et al.* (2008) which can be utilized for the improvement of cultivated sunflower.

This paper aims at presenting a part of the results of interspecific hybridization between cultivated sunflower (*Helianthus annuus*) and two wild *Helianthus* species and presenting their potential use in breeding and selection.

MATERIAL AND METHODS

Four cultivated sunflower (Romanian sunflower hybrids created by NARDI Fundulea, 2n=34) and two wild species, (*Helianthus agrophyllus*, 2n=34 and *Helianthus maximilani*, 2n=34), were grown under field conditions and vegetation house at NARDI Fundulea.

The methods included: interspecific hybridization, embryo rescue, self-pollination and backcrossing, field and biochemical evaluation.

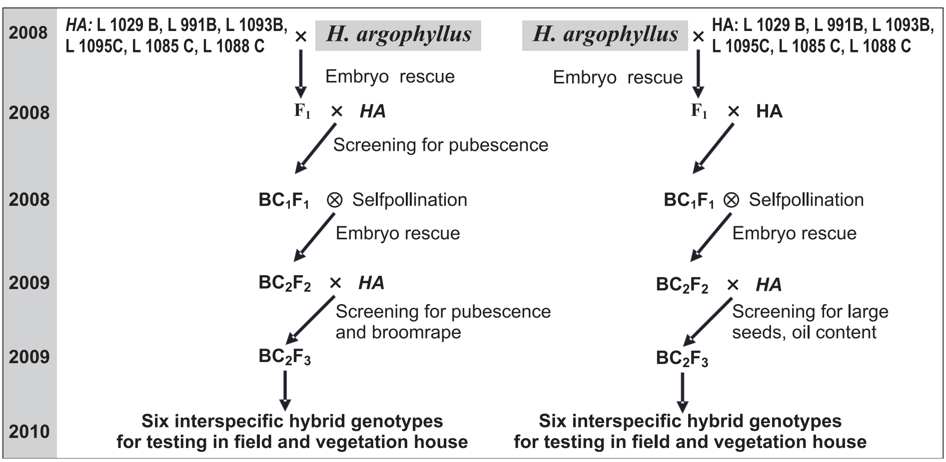

Figure 1: Working scheme for drought resistance (HA=inbred lines of Helianthus annuus from National Agricultural Research and Development Institute Fundulea, Romania)

Interspecific F_1 hybrids were produced between wild species and cultivated sunflower with continued backcrossing with cultivated sunflower and self-pollination of BC_3F_2 plants. For this the female plants were hand emasculated and fresh pollen was applied to the inflorescences. Pollination was performed with a flannel applicator twice at two-day interval, starting from the beginning of anthesis. 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 (Figures 1 and 2).

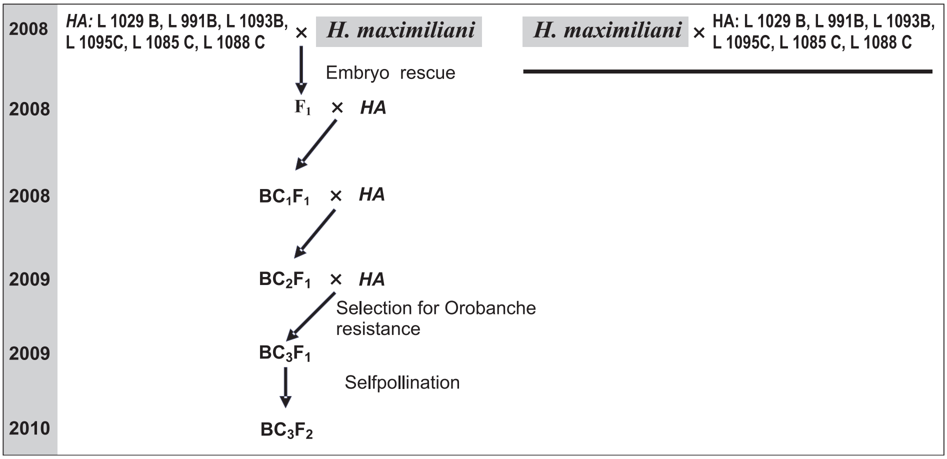

Figure 2: Working scheme for broomrape resistance

A method for breaking the dormancy and retrieving seedlings from sunflower embryos 20 days after pollination was used in case of interspecific hybrids with *H. argophyllus.* In case of hybrids with *H. maximiliani* as mother parent embryo rescue was not possible because of the small size of seeds. Embryos were allowed to develop for 20 days, and were excised, dehulled and incubated under lights (12 h photoperiod) in Petri dishes on filter paper moistened with 10 ml of a solution containing 0.025 ppm GA_3, 1 ppm IAA and 2.5 ppm KNO_3.

The descendants were investigated for some characteristics important in sunflower breeding. Biometric studies and biochemical characterization of the seeds were carried out in F_3 generation. The plants with pubescent leaves were selected for drought resistance. The F_2 plants were also investigated for broomrape resistance with tests performed under artificial inoculation using broomrape seeds from two infested areas from Romania.

RESULTS AND DISCUSSION

The BC_2F_2 presented a large variety concerning morphological traits, such as leaf length, leaf weight, height of plants, branches, pubescences and size of head and seeds.

Leaf lengths of sunflower inbred ranged from 174 to 258 mm, and in wild species from 70 mm *(H. argophyllus*) to 90 mm (*H. maximiliani*). In hybrid plants, high value was obtained in combination *H. argophyllus* × LC 1095 C (262 mm). Also, leaf width presented genotypic variability and ranged from 150 mm (LC 1095C) to 238 (LC 1093 B) mm for sunflower inbred lines, 12 mm for *H. maximil-*

iani, 37 mm for *H. argophyllus*, and from 135 mm (LC 1095 C × *H. argophyllus*) to 246 mm (LC 1093B × *H. maximiliani*) for hybrid plants (Table 1). The last example shows a high hybrid value. It means that this trait is determined by four dominant genes action (Vrânceanu, 2000).

Leaf area, like the above characters, was variable depending on the genotype (Table 1). 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 (Haddadi *et al.*, 2011).

Table 1: Variability of parental lines and descendants for morphological aspect of leaves

Biological material	Leaf lenght	Leaf weight	Leaf area
	(mm)	(mm)	(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
L 1029 B x *H. maximiliani*	170	180	211
L 991 B x *H. maximiliani*	235	195	326
L 1093 B x *H. maximiliani*	258	246	385
L 1095 C x *H. maximiliani*	157	140	150
H. argophyllus x L 1085 C	213	183	269
H. argophyllus	70	78	37
H. maximiliani	90	20	12

Height of plants is an agronomic trait involved in plant productivity. It is polygenically controlled and low stem is controlled by recessive dwarfing genes, but all modes of inheritance for plant height were present in the F_1 generation. Heterosis was the most frequent, followed in decreasing order by partial dominance, dominance and intermediacy (Terzić *et al.*, 2006).

Our results show that sometimes the height of hybrids was superior to parental lines (Figure 3). This suggests that wild parent dominated in genetic control of that trait over the cultivated one.

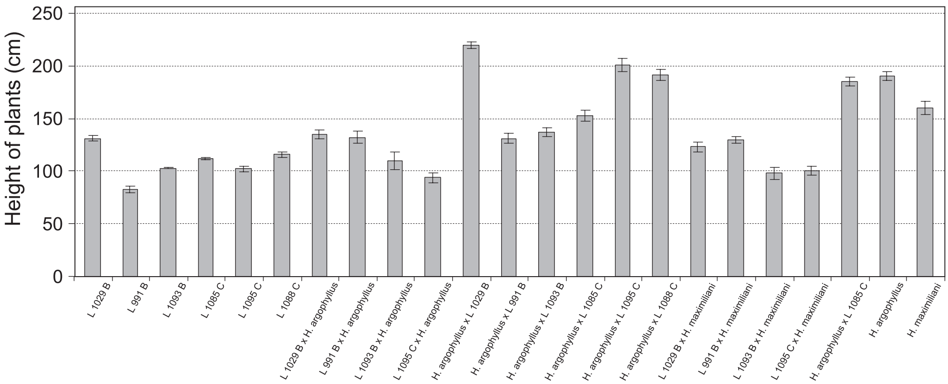

Figure 3: Height of parental lines and descendants

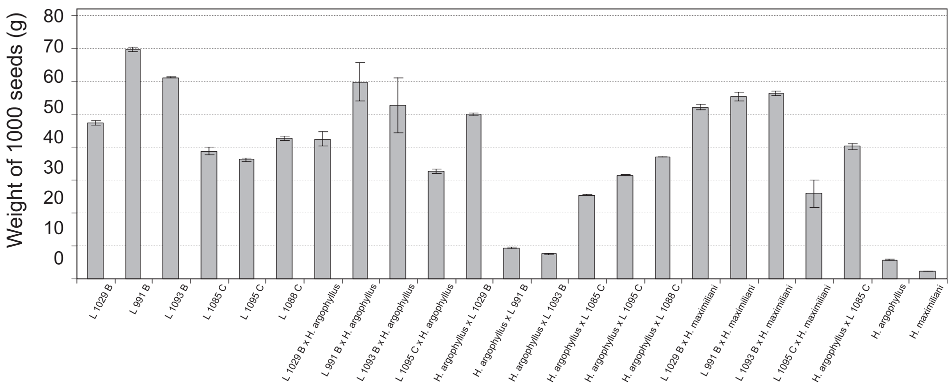

Figure 4: Weight of 1000 seeds for parental line and obtained descendants

The hybrid plants obtained in this study had an intermediate value of weight of 1000 seeds (Figure 4). This trait is inherited by incomplete dominance (Haddadi *et al.*, 2011).

Head diameter is an important yield component. Several researchers 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 (Petcu *et al.*, 2010). Head diameter study revealed 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) (Figure 5). Those results confirm that the size of diameter is a polygenic character with strong additive effect (Vrânceanu, 2000).

The oil content is presented in Figure 6. The average oil content of F_2 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 (Delourme *et al.*, 2006).

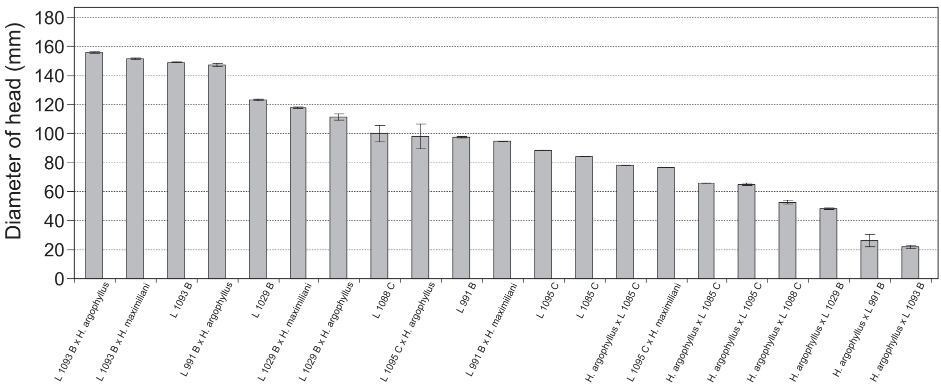

Figure 5: Diameter of head for parental line and obtained descendants

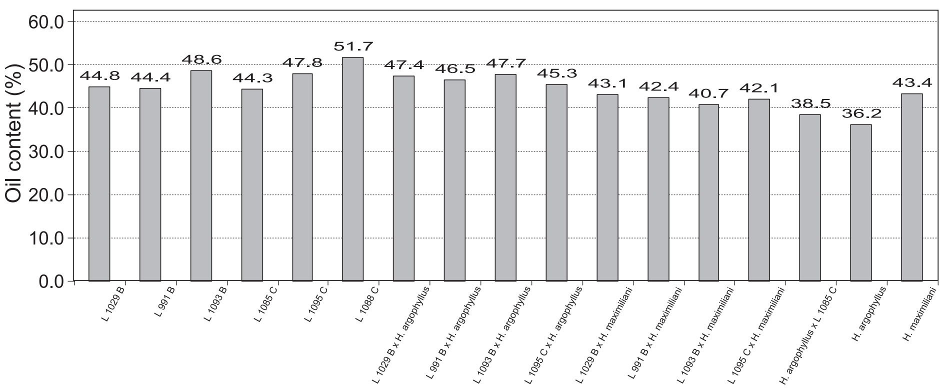

Figure 6: The oil content in parental line and obtained descendants

The screening for broomrape resistance was performed with F and G broomrape race (Table 2).

The sunflower plants which showed the broomrape infestation smaller than control were self pollinated.

Broomrape presents serious problems to sunflower production in Romania, as well. It is constantly expanding its distribution area, forming new more virulent races (Pâcureanu *et al.*, 2008). Although some authors suggest possibilities for chemical control of broomrape, most studies indicate that genetic resistance is the most important method for controlling the parasite.

Table 2: The broomrape resistance of descendants

Combination	Replicates	F race (number of broomrape/plant)	G race (number of broomrape/plant)
	1	5	5
Control	2	4	5
	3	5	6
	1	13	0
LC1029 B x *H. agrophyllus* (BC_2F_2)	2	3	2
	3	5	7
	1	2	16
Lc 1029 B x *H. maximiliani* (BC_3F_1)	2	10	12
	3	5	24
	1	5	4
LC 991 B x *H. maximiliani* (BC_3F_1)	2	3	2
	3	15	5
	1	2	5
LC 1095 C x *H. maximiliani*$(BC3_2F_1)$	2	9	6
	3	10	4
	1	0	2
H. argophyllus x LC 1085 C (BC_2F_2)	2	1	7
	3	0	15

CONCLUSIONS

The wild species *Helianthus argophyllus*, can be crossed as a female parent with *Helianthus annuus* and F_1 hybrids were obtained by embryo rescue technique.

The F_1 hybrids between *H. maximiliani* and *H. annuus* obtained in this study were infertile.

When cultivated sunflower (*H. annuus)* was crossed as a female parent with both wild species, the F_1 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 the results indicated a good drought resistance and broomrape resistance for some of them.

The descendants with good performances will be self pollinated in the next generation because interspecific hybrids are important as donors for introducing new favourable alleles into parental inbred lines.

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