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INTROGRESSION OF DROUGHT-RESISTANCE GENE(S) FROM *HELIANTHUS ARGOPHYLLUS* TO *HELIANTHUS ANNUUS* SPECIE, USING EMBRYO RESCUE TECHNIQUES

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ABSTRACT

In order to improve drought resistance of sunflower in the context of the global warming and to accelerate this improvement, introgression of resistance genes from wild species was attempted, using embryo rescue techniques, combined with classical procedures to improve crossing, self-pollination and backcrossing. Thirty four approved or pending approval lines (B and C) were tested for combination capacity with the wild Helianthus argophyllus. After crossing between H. annuus and H. argophyllus, 6 generations in two years (2008-2009) were obtained, as follows: F1 (interspecific crossing - harvest immature hybrid embryos), BC1F1; BC2F1; BC3F1; BC4F1; BC4F2. In case of the crossing between species H. argophyllus and H. annuus only four generations (F1; BC1F1; BC1F2; BC2F1) in 2 years were obtained, due to the long vegetation period of the wild species, the long flowering period, the high degree of branching and not finally higher incompatibility between the two species.

Key words: sunflower, immature embryos, embryo rescue, interspecific crossing.

INTRODUCTION

Climate changes will cause greater damages and much faster than expected. Increased hunger in Africa and Asia, increasing ocean levels, flooding, drought, heat waves, floods, the disappearance of fauna and flora species are just some of the consequences of overheating the atmosphere.

Approximately 20-30% of animal and plant species have been assessed so far as having high risk of extinction if global temperature increases by only 1.5-2.5° Celsius. Although known as a drought resistant plant, sunflower strongly reduces its yields on soils without water or under low atmospheric humidity conditions.

Improving drought resistance is not simple, due to the complex (polygenic) control of the trait. Generally, a whole set of characters that contribute to adaptation to water stress must be improved.

According to data provided by Baldini et al. (1992a; 1992b; 1996), and Cecconi et al. (1996) the wild species *H. argophyllus* can be used in programs to improve resistance to drought, as possessing a certain avoidance of dehydration, probably due to stronger root development and better water absorption. In order to reduce the genetic incompatibility, the embryo rescue can be used to obtain interspecific hybrids (Răducanu, 1998; Răducanu et al., 2000).

NARDI Fundulea has obtained an invaluable genetic basis during over 50 years of research, being considered as main institution in the country producing sunflower parental lines and hybrids (Vrânceanu et al., 2005; Păcureanu et al., 2005). Taking into account the climatic changes and current requirements at European level, the improvement of the drought resistance for sunflower hybrids is one of the main objectives in the sunflower breeding program, and this is especially true for sunflower germplasm to be cultivated under the organic (ecological) agricultural system.

MATERIAL AND METHODS

Thirty four approved or pending approval lines of cultivated sunflower and *Helianthus*

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argophyllus were used in study. Five plants/each genotype were isolated, manually emasculated and pollinated.

The pollen from cultivated sunflower was applied at three-day intervals on emasculated flowers of the wild species and reciprocally, pollen harvested from wild species was used for pollination of the sunflower lines.

In order to accelerate the breeding process, 15 day-old immature embryos were harvested and cultivated on MS media without hormones (Răducanu, 1998). Normal seedlings were transferred to soil: sand mixture (3:1) and grown in vegetation house and greenhouse. The pollen harvested from the experimental field was stored at 4-5°C in order to perform the pollination during winter period. The seeds from BC4 generation were obtained.

To obtain seed in accordance with the requirements of organic agriculture principles, the whole experiment was conducted during 2008-2009 in a field where organic agricultural system has been applied, in vegetation house and green house without chemicals.

The plants for backcrossing generations were selected taking into account the following morphological trials; degree pubescence of leaves, diameter of head and number of seeds/head.

RESULTS AND DISCUSSION

A very good result was obtained for some sunflower genotypes pollinated with the wild species (for example LC 1039B (440 achenes/head); LC1099C (330 achenes/head); LC 1010B (270 achenes/head); LC 1066C (241 achenes/head) (Table 1). Only 5-19 achenes were obtained from the LC0303C; LC18B; LC985B; LC1101C and LC 0925C.

Table 1. Compatibility degree estimated by number of achenes and the *embryo rescue* capacity in crosses between cultivated sunflower lines x *H. argophyllus*

No.	Genotype	Achenes/ head (no.)	Immature embryos inoculated (no.)	Transferred plants (no.)	Plants transferred in green house (no.)
0	*1*	*2*	*3*	*4*	*5*
1.	LC08B x *H. argophyllus*	54	47	22	4
2.	LC18B x *H. argophyllus*	10	8	8	4
3.	LC985B x *H. argophyllus*	13	11	19	5
4.	LC991B x *H. argophyllus*	241	197	17	5
5.	LC1001B x *H. argophyllus*	33	22	17	4
6.	LC1002B x *H. argophyllus*	91	90	18	2
7.	LC1004B x *H. argophyllus*	101	83	19	6
8.	LC1003B x *H. argophyllus*	23	23	12	4
9.	LC1011B x *H. argophyllus*	122	109	23	4
10.	LC1019B x *H. argophyllus*	81	71	20	4
11.	LC1029B x *H. argophyllus*	103	102	23	5
12.	LC1039B x *H. argophyllus*	440	230	24	6
13.	LC1042B x *H. argophyllus*	42	39	12	2
14.	LC1010B x *H. argophyllus*	270	190	30	4
15.	LC1015B x *H. argophyllus*	77	67	11	4
16.	LC1016B x *H. argophyllus*	23	18	9	4
17.	LC1050B x *H. argophyllus*	63	50	25	3
18.	LC1093B x *H. argophyllus*	71	67	17	3
19.	LCEHP15598B x *H. argophyllus*	179	171	21	5
20.	LC1064C PL1 x *H. argophyllus*	205	175	15	6
21.	LC1064C PL2 x *H. argophyllus*	199	171	19	3
22.	LC1054C x *H. argophyllus*	109	87	18	4
23.	LC1066C x *H. argophyllus*	241	198	24	5

0	*1*	*2*	*3*	*4*	*5*
24.	LC1068C x *H. argophyllus*	116	101	14	5
24.	LC1088C x *H. argophyllus*	83	73	17	4
25.	LC1095C x *H. argophyllus*	210	158	23	5
26.	LC1085C x *H. argophyllus*	145	109	18	5
27.	LC1101C x *H. argophyllus*	17	12	6	4
28.	LC0925C x *H. argophyllus*	19	85	12	4
29.	LC0305C x *H. argophyllus*	5	5	4	2
30.	LC1059C x *H. argophyllus*	68	66	12	5
31.	LC1103C x *H. argophyllus*	61	51	12	5
32.	LC1099C x *H. argophyllus*	333	209	20	5
33.	LC1053C x *H. argophyllus*	150	140	12	5

Generally, a correlation between the compatibility degree at hybridization expressed as achenes/head and the response to *in vitro* culture for embryo rescue was not observed. For example, from a genotype with many achenes/head, 230 embryos were inoculated on MS media and only 24 grew normally and the seedling could be transferred into soil. But, from LC18B genotype only 10 achenes were obtained and from these 8 embryos and 8 seedlings were obtained.

In the case of *in vitro* culture, white plants were observed, more obviously in two genotypes (LC1066C-70% and LC1010-50%).

The compatibility degree in crosses between *Helianthus argophyllus* x *Helianthus annuus* was very low and few achenes were obtained (Table 2).

Table 2. Compatibility degree estimated by number of achenes and the *embryo rescue* capacity in crosses *H. argophyllus x H. annuus* (2009)

No.	Genotype	Achenes/ head (no)	Immature embryos inoculated (no)	Transferred plants (no)	Plants transferred in green house (no)
0	*1*	*2*	*3*	*4*	*5*
1.	*H. argophyllus* x LC08B	5	3	1	1
2.	*H. argophyllus* x LC18B	3	3	3	2
3.	*H. argophyllus* x LC985B	14	9	9	9
4.	*H. argophyllus* x LC991B	28	27	7	4
5.	*H. argophyllus* x LC1001B	10	10	8	8
6.	*H. argophyllus* x LC1002B	15	10	6	4
7.	*H. argophyllus* x LC1004B	4	4	3	3
8.	*H. argophyllus* x LC1003B	22	20	20	13
9.	*H. argophyllus* x LC1011B	3	3	1	1
10.	*H. argophyllus* x LC1019B	1	1	-	-
11.	*H. argophyllus* x LC1029B	16	11	2	4
12.	*H. argophyllus* x LC1039B	29	22	13	13
13.	*H. argophyllus* x LC1042B	44	39	16	7
14.	*H. argophyllus* x LC1010B	31	29	21	15
15.	*H. argophyllus* x LC1015B	86	76	56	35
16.	*H. argophyllus* x LC1016B	9	8	8	8
17.	*H. argophyllus* x LC1050B	-	-	-	-
18.	*H. argophyllus* x LC1093B	-	-	-	-
19.	*H argophyllus* xLCEHP15598B	9	6	5	5
20.	*H. argophyllus* x LC1064C, pl1	8	7	3	-
21.	*H. argophyllus* x LC1064C, pl2	6	5	3	1

0	*1*	*2*	*3*	*4*	*5*
22.	*H. argophyllus* x LC1054C	11	10	4	1
23.	*H. argophyllus* x LC1066C	25	21	7	4
24.	*H. argophyllus* x LC1068C	24	22	18	15
25.	*H. argophyllus* x LC1088C	2	1	-	-
26.	*H. argophyllus* x LC1095C	28	28	19	16
27.	*H. argophyllus* x LC1085C	1	1	-	-
28.	*H. argophyllus* x LC1101C	53	45	14	12
29.	*H. argophyllus* x LC0925C	12	11	8	5
30.	*H. argophyllus* x LC0305C	-	-	-	-
31.	*H. argophyllus* x LC1059C	15	14	7	5
32.	*H. argophyllus* x LC1103C	4	4	2	2
33.	*H. argophyllus* x LC1099C	11	11	6	3
34.	*H. argophyllus x* LC1053C	10	9	6	4

For six sunflower genotypes the level of compatibility was zero (*H. argophyllus* x LC1019B; *H. argophyllus* x LC1093B; *H. argophyllus* x LC0305C; *H. argophyllus* x LC1019B; *H. argophyllus* x LC1088C; *H. argophyllus* x LC1085C). If genotype 1058 B was used as female parent and *H. argophyllus* as male parent the compatibility was > 50%, while if *H. argophyllus* was male parent the compatibility was zero (Table 2).

Table 3. Compatibility degree estimated by number of achenes and the embryo rescue capacity (BC4F2)

Genotype	Achenes (no.)	Immature embryos inoculed (no.)	Immature embryos germinated (no.)	Plants transferred in soil (no.)
F 6 [(RPC-46-5412B x *H. argophyllus*)/ RPC-46-5412B]	256	189	9	7
F 7 [(Tard/85-20072B x *H. argophyllus*)/ RPC-46-5412B]	393	164	139	30
F 10 [(dwarf population/79-16439B x *H. argophyllus*)/ dwarf pop./79-16439B]	318	278	278	19
F 12 [(LC1093B x *H. argophyllus*) / LC1093B]	119	94	70	15
F 2 [(Polet-11273B x *H. argophyllus*)/ (Polet-11273B)]	154	144	136	17
F 4 [(O-7493B x *H. argophyllus*)/ O-7493B]	185	153	112	19

Results of crosses (*H. annuus* x *H. argophyllus*)//*H. annuus* in BC4F2 are presented in Table 3.

From the interspecific hybrid F7 [(Tard/85-20072B x *H. argophyllus*) /RPC-46-5412B)], 393 achenes/head were obtained, 139 embryos were transferred on MS and 30 normal seedling were obtained.

Generally, all sunflower lines presented good compatibility with the wild species, but the embryo rescue was difficult for some of them. For example, for F 6 [(RPC-46-5412B x *H. argophyllus*)/RPC-46-5412B] genotype 256 achenes were obtained, 189 embryos were inoculated, only 9 of them germinated and 7 seedlings were transferred into soil.

Concerning the crossing between wild species *Helianthus argophyllus* as female, 17 pairs of genotypes were used, from those six hybrid combinations were obtained and 22 hybrid plants were transferred to greenhouse (F1). After self-pollination (BC1F1 generation) achenes from six genotypes were obtained:

1. *Helianthus argophyllus* x RPC-46-5412B (3 achenes);
2. *Helianthus argophyllus* x Tard./85-20072B (1 achene);
3. *Helianthus argophyllus* x O-10401B (2 achenes);
4. *Helianthus argophyllus* x Tard./85-19982B (22 achenes);
5. *Helianthus argophyllus* x LC991B (1 achene);
6. *Helianthus argophyllus* x Krasnodarski-885-5 (15 achenes).

In the spring of 2009, these achenes were sown in vegetation house, BC1F2 and BC2F1 generations were obtained by *embryo culture* but, only two hybrid combinations germinated (*Helianthus argophyllus* x Krasnodarski-885-5 and *Helianthus argophyllus* x Tard./85-19982B) and 5 plants from first and 2 plants from the second were obtained (Table 4).

Table 4. Results obtained from *Helianthus argophyllus* x inbred lines crossing, BC2F1 generation

Genotype	Embryos old (days)	Immature embryos (no.)	Achenes (no.)	Immature embryos (no.)	Seedling (no.)	Plants transferred in greenhouse (no.)
[*(Helianthus argophyllus* x Tard./85-19982B) / Tard./85-19982B]	26	56	58	45	32	5
[*(Helianthus argophyllus* x Krasnodarski-*885-5)/* Krasnodarski-*885-5*]	26	212	422	200	31	2

CONCLUSIONS

Four cultivated sunflower lines (LC 1039B; LC1099C; LC 1010B; LC 1066C) presented a high compatibility with the wild species when *Helianthus annuus* was used as female parent and *Helianthus argophyllus* as male parent.

In crosses between *Helianthus argophyllus* and *Helianthus annuus*, the absence of compatibility was registered for: *H. argophyllus* x LC1050B; *H. argophyllus* x LC1093B and *H. argophyllus* x LC0305C.

Generally, a correlation between the compatibility degree at hybridization expressed as achenes/head and the response to embryo culture was not observed.

The results represent a promise for the acceleration of sunflower breeding for drought resistance and for increasing biodiversity in the global warming conditions.

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