INTERSPECIFIC HYBRIDIZATION BETWEEN *HELIANTHUS ARGOPHYLLUS* AND *H. ANNUUS*. II. CHARACTERISTICS OF SOME MORPHOLOGICAL AND PHYTOPATHOLOGICAL TRAITS IN SECOND AND FURTHER HYBRID GENERATIONS

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Abstract

A high level of segregation for several traits was observed in the F2 and BC1F1 progenies. Depression traits for plant height and head diameter were established in some hybrid combinations. Almost no significant depression was observed in number of disk florets and seed set of the hybrid populations. This fact is of real importance for the selection process for improving these interspecific sunflower forms. Trait transgression was observed in F2 progenies for plant height, head diameter, number of disk florets and seed set. Interspecific progenies of different generations were evaluated for their resistance to various sunflower pathogens and parasites. Most important of all resistant combinations were the ones which combined resistance to more than one pathogen.

Introduction

The method of interspecific hybridization has been used for almost a century already. With the help of different supporting techniques it is becoming increasingly attractive to breeders because of the opportunities that the method provides to introduce desirable genes from the wild sunflower species into cultivated sunflower. These genes could be either resistance to diseases and stress factors, or cytoplasmic male sterility, or fertility restoration. A species that could be used as a source of all of these desirable genes is *Helianthus argophyllus* T.& G. Christov (1990, 1992, 1996b) reported CMS lines developed from interspecific hybrids with *H. argophyllus*. Tsvetkova and Velkov (1976), Skoric (1988), Christov & Petrov (1988), and Christov (1996a,b) observed the presence of Rf genes derived from *H. argophyllus*.

The wild annual species *H. argophyllus* has been reported to show resistance to stress factors and diseases such as *Phomopsis* (Skoric, 1992; Slyusar and Firaz Alam, 1994), to Mildew (Christov, 1990; Dozet et al., 1990; Seiler, 1991), Sclerotinia (Christov, 1996; Christov et al., 1997), and *Puccinia* (Seiler et al., 1992; Skoric, 1992) and to be successfully used as a donor of resistance to pathogens. One of the aims of this investigation was to determine if resistance to mildew, Broomrape or *Phomopsis* could be obtained in the F2 generation of interspecific hybrids separately or simultaneously as a proof of the ability of the wild parent to successfully transfer genes for resistance to these pathogens.

Investigations conducted so far have focused their attention on the inheritance of traits in the F1 generation. Only short notices have been published about the phenotypic performance of characteristics in F2 progenies. It is also important to follow the diversity of forms in the

F2 generation with the help of genetic parameters such as depression and transgression.

Another aim of this study was to analyze the huge variety of forms in the F2 generation, using depression and transgression traits and to search for initial material suitable for faster cultivation of the hybrid progenies.

Materials and Methods

Helianthus argophyllus is an annual diploid (2n=34) species belonging to the Section Helianthus (Schilling and Heiser, 1981). The populations of accessions E-007, E-008 and E-091 have been maintained in the wild species germplasm collection at the Dobroudja Agricultural Institute, General Toshevo, Bulgaria.

Under the environmental conditions at DAI, *H. argophyllus* began to grow the third week of April. It flowered 90-98 days after plant emergence in the spring, depending upon the accession. Physiological maturity occurred after118 to 24 days. Plants stayed green and vigorous until the first autumn frosts (25 Sept.-10 Oct.). Seed set under open-pollination averaged from 7.41 to 92.59% for the different accessions. When heads of *H. argophyllus* were self-pollinated, their seed set averaged 0-1.19% for E-007, 0-0.66% for E-008 and 0-26.32% for E-091.

After self-pollination of F1 plants and backcrossing with pollen from cultivated sunflower, F2 and BC1 generations were produced.

Morphological and phenological characterization of the wild *H. argophyllus*, the cultivated sunflower inbred lines and the hybrid progenies was done. The observations were made on at least 10 plants grown in field conditions. The following traits were studied: plant height (cm), number of branches, head diameter (cm), head thickness (cm), number of bract leaves, number of ray florets, ratio of length/width of leaves, length of the leaf petiole (cm), length of the longest branch (cm), stem thickness (cm), seed size (length, width and thickness [cm]), color of the stigma; germination, button formation, flowering period, physiological and technical maturity.

Statistical estimation of the experimental data included the following genetic characteristics: depression D=(F1 – F2 / F1) x 100 (%), transgression in degree (Tc) and frequency (Tr) (Voskresenskaja & Shpota, 1967), variation coefficient VC (Mather & Jinks, 1971), mean (x) and mean squared (xq), the differences were compared according to the Student t-test (Mather & Jinks, 1971). Statistical estimations were performed by BIOSTAT, version 5.1., Statistics for Windows 95 and Microsoft Excel, version 7.0.

The evaluation of the material for downy mildew resistance (*Plasmopara helianthi* Novot.) was made in greenhouse conditions according to the standard method (Vear and Tourvieille, 1987). The evaluation of the material for broomrape resistance (*Orobanche cumana* Wallr.) was carried out in greenhouse conditions according to the standard method (Panchenko, 1975). The inoculation for Phomopsis resistance (*Phomopsis helianthi* Munt.-Cvet. et al.) was made according to the method of Tourvieille et al. (1988) in an infection plot with additional irrigation. The testing was performed for six years. Twenty plants were tested from each progeny.

Results and Discussion

A high level of segregation for several traits was observed in the F2 and BC1F1 progenies, as has been stated by Georgieva-Todorova (1976) and Christov and Petrov(1988).

Short and tall plants were obtained with varying head diameters, branching types, coloration of stems, petioles, disk florets, and seed colour. Some plants inherited predominantly wild traits such as long peduncles, anthocyanin coloration of disk florets, seed shattering, and strongly pubescent leaves and stems.

Anthesis of the F2 progenies began as early as 36 to 61 days after germination, i.e., some progenies behaved as very early-flowering ones, other were mid-early. Vegetation period varied from 82 to 110 days depending upon the origin or genetics of the progeny. Seed set in the F2 generation varied from 0.60 to 93.48%, depending on the combinations. This trait had a very high variance coefficient (98.68 to 100.75 (Tables 1 and 2). The depression trait for plant height was established in hybrid combinations *H. argophyllus* (E-007) x L.1234 (Table 1), and L.2607 x *H. argophyllus* (E-091) and L.HA-300 x *H. argophyllus* (E-091) (Table 2), but it was statistically significant in the first one only. Depression in head diameter was found in combinations *H. argophyllus* (E-007) x L.1234 (t=8.76***) and L.HA-300 x *H. argophyllus*_(E-091) (t=7.41***) again. Almost no significant depression was observed in number of disk florets and seed set of the hybrid populations. This fact is of real importance for the selection process for improving these interspecific sunflower forms.

Table 1. Variation, depression and transgression in some traits in F2 plants.

Hybrid combination	VC	Depression		Transgression					
				In degree			In frequency		
		%	t	min.	max.	total	min.	max.	total
Plant height (cm)									
E-007 x 1234	27.42	34.19	3.62 *	-42.98	5.03	48.01	60.00	20.00	80.00
E-091 x 1234	7.44	-186.18	7.90 *	-	106.90	106.90	-	100.00	100.00
E-007 x 3064	22.33	-9.62	0.54 -	-	23.78	23.78	-	50.00	50.00
E-091 x 3064	41.53	-13.38	0.71 -	-	120.69	120.69	-	50.00	50.00
Head diameter (cm)									
E-007 x 1234	22.48	49.57	8.76 ***	-36.81	-	36.81	60.00	-	60.00
E-091 x 1234	6.73	-85.84	9.17 *	-	34.15	34.15	-	100.00	100.00
E-007 x 3064	20.20	-28.52	1.40 -	-	-	-	-	-	-
E-091 x 3064	28.59	-57.81	2.95 **	-	86.57	86.57	-	87.50	87.50
Total number of disk florets									
E-007 x 1234	23.61	-48.88	1.37 -	-	-	-	-	-	-
E-091 x 1234	13.71	-104.72	2.98 -	-	-	-	-	-	-
E-007 x 3064	21.45	-91.03	1.60 -	-	-	-	-	-	-
E-091 x 3064	32.41	-237.80	4.51 **	-	141.09	141.09	-	100.00	100.00
Number of inseminated disk florets / seed set/									
E-007 x 1234	18.27	-462.83	4.30 *	-	-	-	-	-	-
E-091 x 1234	98.68	45.22	0.99 -	-7.33	-	7.33	50.00	-	50.00
E-007 x 3064	91.92	22.33	0.20 -	-36.36	-	36.36	50.00	-	50.00
E-091 x 3064	62.72	-61.85	1.26 -	-	100.52	100.52	-	40.00	40.00

Trait transgression was observed in F2 progenies for plant height, head diameter, number of disk florets and seed set (Tables 1 and 2). Data in Tables 1 and 2 indicate a high transgression both in degree and frequency. Almost all transgressive traits in the F2 progenies of *H. argophyllus* were positive for plant height and head diameter with only two exceptions: plants from combinations *H. argophyllus* (E-007) x L.1234 (Table 1) and L.HA-300 x *H. argophyllus* (E-007) (Table 2).

Table 2. Variation, depression and transgression in some traits in F2 plants.

Hybrid combination	VC	Depression		Transgression					
				In degree			In frequency		
		%	t	min.	max.	total	min.	max.	total
Plant height (cm)									
2607 x E-007	27.18	-14.59	0.83	-	121.31	121.31	-	100.00	100.00
2607 x E-008	20.87	0.0	0.01	-	74.19	74.19	-	72.73	72.73
2607 x E-091	9.05	11.25	1.37	-	62.07	62.07	-	100.00	100.00
HA-300 x E-007	28.82	-5.07	3.24 **	-10.79	102.55	113.34	10.00	70.00	80.00
HA-300 x E-008	18.17	-6.10	0.66	-	70.97	70.97	-	85.71	85.71
HA-300 x E-091	8.53	10.32	1.23	-	-55.86	55.86	-	100.00	100.00
Head diameter (cm)									
2607 x E-007	27.20	-36.84	2.11	-	8.28	8.28	-	20.00	20.00
2607 x E-008	23.83	-45.59	3.24 **	-	65.61	65.61	-	54.55	54.55
2607 x E-091	12.01	-16.11	1.13	-	21.02	21.01	-	66.67	66.67
HA-300 x E-007	39.39	-73.33	1.45	-36.81	-	36.81	10.00	-	10.00
HA-300 x E-008	28.93	4.49	0.28	-	-	-	-	-	-
HA-300 x E-091	17.54	55.84	7.41 ***	-	-	-	-	-	-
Total number of disk florets									
2607 x E-007	9.10	-7.89	0.36	-	-	-	-	-	-
2607 x E-008	35.30	-229.56	3.91 **	-	35.76	35.76	-	33.33	33.33
2607 x E-091	20.57	-45.25	2.16	-	-	-	-	-	-
HA-300 x E-007	45.20	26.59	2.71 *	-	-	-	-	-	-
HA-300 x E-008	38.67	-38.54	0.81	-	5.97	5.97	-	25.00	25.00
HA-300 x E-091	45.40	14.13	0.32	-	-	-	-	-	-
Number of inseminated disk florets / seed set/									
2607 x E-007	82.97	-26.05	0.33	-	-	-	-	-	-
2607 x E-008	100.75	-165.60	1.15	-90.27	19.45	109.72	33.33	33.33	66.66
2607 x E-091	83.06	29.91	0.78	-71.36	-	71.36	33.33	-	33.33
HA-300 x E-007	72.07	-409.67	0.83	-100.00	-	100.00	25.00	-	25.00
HA-300 x E-008	73.95	-57.02	0.61	-91.08	-	91.08	25.00	-	25.00
HA-300 x E-091	65.88	28.79	0.45	-99.16	2.15	101.31	20.00	20.00	40.00

Nevertheless, there were positive transgressions for both traits in these combinations too. The F2 plants of three hybrid combinations showed the presence of transgression in the number of disk florets, but these were positive forms only. The data for the absence of significant depression with regard to head diameter and number of disk florets were confirmed also by the absence of negative transgression forms of these traits in most hybrid combinations included in the research. Sunflower breeding programs are interested in seed set and number of inseminated florets, but unfortunately this trait had a high negative transgressive value (Tables 1 and 2). The fact that plants as early as in the F2 generation cannot overcome the distance between the species, and that the fertility and seed set of these plants remain low, it slows the breeding process. There were a few positive traits, mainly in combinations *H. argophyllus* (E-091) x L.3064, L.HA-300 x *H. argophyllus* (E-091) and L.2607 x *H. argophyllus* (E-008). The first one, *H. argophyllus* (E-091) x L.3064, is of real interest for the breeders, because there was no depression for any of the four traits (two of them significant) and positive transgressive forms were registered for all the studied traits simultaneously (Table 1). The wild species *H. argophyllus* possesses a small number of disk florets and low self-compatibility, but some of the interspecific hybrids of this species overcome these negative characters in the F2 generation, which shortens the breeding process and leads to faster cultivation of the hybrid material. Some other hybrid progenies were observed which showed transgression for two, three, and four traits, simultaneously.

Interspecific progenies of different generations were evaluated for their resistance to various sunflower pathogens and parasites. Second hybrid generation plants were produced after the selection process was performed in the F1 generation according to different traits, one of which was resistance to diseases (where enough seeds were available). Therefore, the resistant F1 plants gave resistant F2 plants, demonstrating that dominant gene(s) controlling resistance were successfully transferred in the next hybrid progeny. Most important of all resistant combinations included in Table 3 were the ones, which combined resistance to more than one pathogen; for example crosses *H. argophyllus* (E-007) x L.2607 (17% resistance to mildew and 57% resistance to Orobanche); *H. argophyllus* (E-008) x L.1607 (55% resistance to mildew and 100% resistance to Phomopsis); *H. argophyllus* (E-091) x L.3064 (100% resistance to *Plasmopara helianthi*, 20% resistance to *Orobanche cumana* and 100% resistance to *Phomopsis helianthi*). These results supported reports made by Saciperov (1916) and Christov and Petrov (1988).

Table 3. Resistance of hybrid material to *Plasmopara helianthi*, *Orobanche cumana*, *Phomopsis helianthi.*

Hybrid	Generation		Resistance (%)		
combination	#	Gener.	*Pl. helianthi*	*O. cumana*	*Ph. helianthi*
E-007 x 2607	79/00	F_2	17	57	-
E-008 x 1607	171/94	F_2	55	-	100
E-008 x 2607	172/94	F_2	60	-	-
E-091 x 1607	173/94	F_2	-	-	100
E-091 x 3064	402/95	F_2	100	20	100
1607 x E-007	243/94	F_2	-	-	100
1607 x E-007	1147/96	F_2	58.4	44.4	-
1607 x E-007	151/01	F_9	90	-	-
2607 x E-007	1160/96	F_2	0	11	-
2607 x E-008	1164/96	F_2	34	-	-
3064 x E-008	954/97	F_3	19	-	-
2607 x E-091	878/99	F_5	-	-	75
E-007-4			100	75	100
E-008-5			75	48	100
E-091-11			100	60	100

Another interesting fact about the maintenance of resistance in advanced generations was that 99 progenies (F8 and F9) from combination E-091 x L.3064 were studied for resistance to mildew and 90 of them were resistant, and only nine lost the trait during selfing. This fact is of great importance for the use of *H. argophyllus* in breeding programs for resistance. Suitable sunflower forms resistant to mildew, Broomrape, Phomopsis or Rust could be easily obtained in a considerably shorter period of time and included in the production of inbred lines as parental forms of commercial hybrids.

Conclusions

A high level of segregation for several traits was observed in the F2 generation. Depression traits for plant height and head diameter were established in some hybrid combinations. Almost no significant depression was observed in number of disk florets and seed set of the hybrid populations. This fact is of real importance for the selection process for improving these interspecific sunflower forms. Trait transgression was observed in F2 progenies for plant height, head diameter, number of disk florets and seed set. Interspecific progenies of different generations were evaluated for their resistance to various sunflower pathogens and parasites. Resistance to *Plasmopara helianthi*, *Orobanche cumana* and *Phomopsis helianthi* from 30 to 100% was discovered in different progenies of the interspecific hybrids of *H. argophyllus*. Most important of all resistant combinations were the ones which combined resistance to more than one pathogen.

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