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Evaluation of inbred lines developed in infested fields of *Orobanche cumana* from interspecific sunflower populations

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Abstract Parasitic plants of *Orobanche cumana* cause losses of over 70% of the seed production in the sunflower culture, these effects of the parasite are visible even in the early stages of development. Research programs for sunflowers are struggling to keep up with the superior adaptability of the parasite *Orobanche cumana*. Thus, over time breeders have oriented towards the multitude of wild species available by developing populations with elite lines and stabilization of new resistance genes through repeated exposure to the stress factor in the process of self-pollination. For de development of new inbred lines we included in our research wild species of *H. giganteus*, *H. divaricatus*, *H.hrisutus and H. salicifolius. H. maximiliani and H. ocidentalis* The interspecific population was developed using the public inbred lines, all the offspring's where self-pollinated and selected for their agronomical qualities. In the F_6 generation, a total of 50 offspring were evaluated in the field infested in 3 repeats with *Orobanche cumana* of the E breed according to the breeds check lines that were used in the evaluation process. The new developed lines AH1102 and AH1104 developed from *H. divaricatus*, AH1538 and AH1539 developed from *H. giganteus*, manifest homozygote genetic resistance according to our experiments of evaluation perform in greenhouse and infested field. However, the descending genotypes resulting from the interspecific populations of *H. salicifolius*, *H. hristus, H. maximiliani and H. ocidentalis* were susceptible in our field evaluation. During our research project we propose to continue developing new inbred lines from wild species of sunflower and to develop feasible research depending on the goals of studies to *Orobanche cumana*.

Key words

Orobanche cumana;
wild species; sunflower; infested fields; resistance.

The cultivation of sunflower for the extraction of vegetable oil has known a number of stages in history. Although introduced as an ornamental plant in Europe after the Spanish expeditions from New Mexico, it records the first significant quantities of commercial production at the beginning of the 18^{th} century in Russia. The effect of the *Orobanche cumana* parasite is present today in the most aggressive forms, starting with the initial breeds A, B, C, D and E according to the classification of the inbred differentiating lines to the newer breeds F, G and H causes significant damage in areas where the largest crops are reported of sunflower among which we list: Ukraine, Russia, Romania, Bulgaria, Spain, France, Turkey and many others [25].

Due to the increased adaptability of the parasite to attached to the roots of sunflower plants and use it's nutrient sources [18] new genetic sources of resistance have been developed to combat the *Orobanche cumana* attack together with the resistance genotypes to imidazolinone herbicides [16, 7].

An important and beneficial aspect practiced by farmers in the cultivation of sunflower in the control of diseases is the rotation of the crop, unfortunately, research indicate a germination capacity of up to 10 years of the *Orobanche cumana* seeds leaving this method not efficient enough [21]. On the other hand, several innovative methods of combating the parasite have been used in Russia. By using a number of 1000 adults / ha of Phytomyza orobanchia, a pathogen that manages to reduce the production of *Orobanche cumana* seeds. [17]. Also, research has shown genotypes of maize [19] and soybeans [29] that can be used in the crop rotation. process by inducing germination for parasite control purposes.Important sources for improving resistance to *Orobanche cumana* is the resistance gene Orodeb2 [9] which confers resistance to race G known to be a single dominant gene [27] and inbred lines P-96 and R-96 with resistance to race F [1] with recessive nature of resistance, due to this cause the trait must be included in both parental lines in the development of hybrids.

Other sources of resistance registered are inbred lines BR1 developed from H. grosseserratus, BR2 developed from Helianthus maximilian, BR3 developed from H. divaricatus and BR4 developed from and H. divaricatus and H. grosseserratus with resistance controlled of one single dominant gene [3, 13].

In addition to the pre-haustorial systems that cut off any preliminary connection of the parasite with the sunflower [8] the post-haustorial resistance will not let the development of *Orobanche cumana* plants reach maturity even if they manage to attach to the roots of sunflower plants [20].

The post-haustorial system can effectively prevent the development of new more aggressive nomenclatures of *Orobanche cumana* by preventing the development and multiplication of new mutant genotypes of the parasite.

Numerous researches on obtaining genetic resistance from wild species [4, 5, 11, 12, 22] have led us to start a research program and integrate new sources of wild species that can provide resistance to both *Orobanche cumana* and other important diseases in the development of sunflower hybrids [6]. Our project of research includes wild species of *H. divaricatus*, *H. giganteus*, *H. salicifolius* and *H. hirsutus* in particularly with the objective of developing inbred lines with resistance for both *Orobanche cumana* and *Plasmopara Halstedii* due to the numerous diseases that sunflower wild species can overcome [2, 15, 24, 28].

Material and Method

Plant material and Orobanche cumana seed origin.

Sunflower genotypes were ordered in 2017 from public research bank from USDA, the inbred lines were developed from the use of interspecific populations by crossing public inbred lines with pollen from wild sunflower species. The seeds from *Orobanche cumana* were collected in 2018 and 2019 from the infected areas of Hungary and were identified on the level of aggressiveness using different control lines and hybrids.

Orobanche cumana field evaluation.

A number of 50 new genotypes in F_6 generation, developed from interspecific populations, were tested in natural infested areas of *Orobanche cumana* in 3 repetition with 4 rows/repetition in a randomize block. All 3 repetition contained differential lines that were susceptible and tolerant to breed "E" and breeds "F" for evaluation and determining the level of infestation din each repetition. The plot for each variant contained between 50-80 plants in a 5 m block with 75 cm distance between row and 25 cm between plants.

After flowering the *Orobanche cumana* emerged from the soil and we waited until full maturation of the sunflower plants for an more effective evaluation.

The report of evaluation took place at full maturity of sunflower plants avoiding the plants in the front and back ends. We recorded the number of plants evaluated in the experiment, the number of resistant and susceptible plants and the *Orobanche cumana* aggression factor which is the total number of sensitive plants divided by the total number of *Orobanche cumana* plants present.

Results and Discussions

Following the selections, the developed genotypes in the F_6 generation were integrated into the evaluation system at *Orobanche cumana*. The first stage of the evaluation was carried out in the field in three experimental repetitions, in locations with natural infestation with *Orobanche cumana*, together with the control inbred lines, to detect the presence of the attack on the susceptible material. According to the results in table 1.1, genotypes resistant to the *Orobanche cumana* parasite developed from interspecific populations were observed such as: inbred lines AH1102 and AH1104 originating from the species *H. divaricatus* and the inbred lines AH1538 and AH1539 originating from the species *H. giganteus*.

In the *Orobanche cumana* tests, according to the results of the AH1102 genotype, we obtained an average of the number of plants on the three repetitions of 57.6 ± 3.51, of which no sensitive plants were identified following the evaluations, indicating a homozygous resistance. For the AH1104 genotype, we recorded an average number of plants of 56.6 ± 1.15, from which an average of resistant plants of 55.33 ± 3.05 and sensitive plants of 1.33 ± 2.30 resulted. Also, the inbred lines developed from the wild species of *H. giganteus* were highlighted, where we obtained an average of 55.66 ± 2.51 evaluated plants for genotype AH1538 and 54.33 ± 3.51 for genotype AH1539 of which no sensitive plants were identified. Thus, these results indicate that these genotypes are resistant to *Orobanche cumana*.

The resistant controls included in the experiment did not register sensitive plants according to the results, both for the control of resistance to race E (AHOROE) and for the control of race F (AHOROF) of *Orobanche cumana*. On the other hand, the sensitive control (AHOROS, Hybrid) recorded an average number of plants of 57.66 ± 1.52, resulting in an average number of sensitive plants of 20.6 ± 6.11. The average number of *Orobanche cumana* plants of 33.33 ± 11.06 was identified on the sensitive plants resulting in an aggression factor of 1,612903.

Table 1

Results of the evaluation of the resistance to *Orobanche cumana* of the 3 repetitions in the field of the inbred lines developed from the weak sunflower species.

Nr.	CODE	Pedigree	Generation	Number of Plants	Resistant plants – Average	Susceptible plants - Average	*Orobanche cumana* aggression factor
1	AH1101	*HA89 X H.divaricatus*	F6	57±3,46	34,33±3,51	22,66±1,52	1,411765
2	AH1102	*HA89 X H.divaricatus*	F6	57,66±3,51	57,66±3,51	0	0
3	AH1103	*HA89 X H.divaricatus*	F6	62±3,6	24±7	38±9,84	1,333333
4	AH1104	*HA89 X H.divaricatus*	F6	56,66±1,15	55,33±3,05	1,33±2,3	2
5	AH1105	*HA89 X H.divaricatus*	F6	60±3,6	19,33±2,51	40,66±3,51	1,286885
6	AH1538	*HA89 X H. giganteus*	F6	55,66±2,51	55,66±2,51	0	0
7	AH1539	*HA89 X H. giganteus*	F6	54,33±3,51	54,33±3,51	0	0
8	AH1540	*HA89 X H. giganteus*	F6	57±6,08	14,66±6,02	42,33±2,08	1,645669
9	AH1541	*HA89 X H. giganteus*	F6	55±6,92	21±8,54	34±3,6	1,5
10	AH1542	*HA89 X H. giganteus*	F6	58,33±7,02	30,66±11,59	27,66±4,93	1,421687
11	AH1592	*HA89 X H. hrisutus*	F6	60,33±4,04	27,33±4,04	33±0	1,787879
12	AH1593	*HA89 X H. hrisutus*	F6	60±3,6	34,33±9,01	25,66±5,5	1,792208
13	AH1594	*HA89 X H. hrisutus*	F6	60±5,56	27,33±15,14	32,66±9,81	1,408163
14	AH1595	*HA89 X H. hrisutus*	F6	55,66±5,13	25,66±10,01	30±12,28	1,511111
15	AH1596	*HA89 X H. hrisutus*	F6	62,33±2,08	25,33±7,5	37±9,53	1,675676
16	AH4037	*HA89 X H. salicifolius*	F6	58,66±5,68	22±16,37	36,66±10,69	1,563636
17	AH4038	*HA89 X H. salicifolius*	F6	56±6,08	26,33±4,5	29,66±7,5	1,404494
18	AH4039	*HA89 X H. Salicifolius*	F6	55,66±2,08	30,33±0,57	25,33±2,3	1,776316
19	AH4040	*HA89 X H. salicifolius*	F6	59,33±3,78	23,66±8,02	35,66±11,37	1,121495
20	AH4041	*HA89 X H. salicifolius*	F6	58±4,35	29±9	29±5,29	1,597701
21	AH1201	*HA89 X H. maximiliani*	F6	48±11,53	25,33±10,06	22,66±17,61	1,720588
22	AH1202	*HA89 X H. maximiliani*	F6	52,33±16,56	26,33±12,66	26±9,84	1,551282
23	AH1203	*HA89 X H. maximiliani*	F6	55,66±3,05	22,33±15,04	33,33±14,57	1,59
24	AH1204	*HA89 X H. maximiliani*	F6	54,33±1,52	15,33±8,5	39±8,66	1,205128
25	AH1205	*HA89 X H. maximiliani*	F6	52±14,17	32,66±16,44	19,33±10,01	1,706897
26	AH1206	*HA89 X H. maximiliani*	F6	45,66±7,02	20,66±9,07	25±8,54	1,973333
27	AH1207	*HA89 X H. maximiliani*	F6	50,66±12,01	32,33±6,5	18,33±5,5	2,072727
28	AH1208	*HA89 X H. maximiliani*	F6	49,33±6,11	30,33±2,88	19±6,24	2,421053
29	AH1209	*HA89 X H. maximiliani*	F6	39,66±5,68	15±10,14	24,66±5,5	1,662162
30	AH1210	*HA89 X H. maximiliani*	F6	49±8,88	34,66±11,23	14,33±5,85	2,534884
31	AH1211	*HA89 X H. maximiliani*	F6	46±6,92	17,66±8,5	28,33±4,5	1,588235
32	AH1212	*HA89 X H. maximiliani*	F6	44,33±5,68	18,33±8,38	26±6,24	1,487179
33	AH1213	*HA89 X H. maximiliani*	F6	60,33±10,01	38,33±15,88	22±6,55	1,863636
34	AH1214	*HA89 X H. maximiliani*	F6	53,66±8,14	34,66±12,58	19±6,92	1,859649
35	AH1215	*HA89 X H. maximiliani*	F6	46,66±12,89	22,33±8,62	24,33±10,69	1,506849
36	AH1216	*HA89 X H. maximiliani*	F6	49±14,73	21,66±16,16	27,33±1,52	1,621951
37	AH1217	*HA89 X H. maximiliani*	F6	52,33±15,94	20,33±5,85	32±13,52	1,385417
38	AH1218	*HA89 X H. maximiliani*	F6	45,66±12,01	24,66±15,04	21±3,46	1,47619
39	AH1219	*HA89 X H. occidentalis*	F6	51,66±9,01	21±5,56	30,66±3,78	1,130435
40	AH1220	*HA89 X H. occidentalis*	F6	51,66±13,31	23±18,08	28,66±7,23	1,290698
41	AH1221	*HA89 X H. occidentalis*	F6	55±12,48	22±8,18	33±4,35	1,343434
42	AH1222	*HA89 X H. occidentalis*	F6	54,66±16,65	25,33±21,96	29,33±6,5	1,306818
43	AH1223	*HA89 X H. occidentalis*	F6	50,66±9,86	20±9,64	30,66±2,51	1,195652
44	AH1224	*HA89 X H. occidentalis*	F6	48±12,76	11±11,13	37±1,73	1,153153
45	AH1225	*HA89 X H. occidentalis*	F6	51,33±14,57	28,33±14,57	23±0	1,231884
46	AH1226	*HA89 X H. occidentalis*	F6	46,33±8,38	13±9,84	33,33±6,65	1,17
47	AH1227	*HA89 X H. occidentalis*	F6	46,66±14,15	12,33±12,5	34,33±6,35	1,203883
48	AH1228	*HA89 X H. occidentalis*	F6	62±2,64	31,66±7,63	30,33±5,03	1,296703
49	AH1229	*HA89 X H. occidentalis*	F6	53±11,26	22,33±7,23	30,66±4,16	1,130435
50	AH1230	*HA89 X H. occidentalis*	F6	51,33±8,5	24,33±3,05	27±9	1,197531
X	AHOROE	Control resistance E (linie)	Fn	54,33±4,16	54,33±4,16	0	0
X	AHOROF	Control resistance F (linie)	Fn	60,33±2,3	60,33±2,3	0	0
X	AHOROS	Control susceptible (HYBRID)	Fn	57,66±1,52	37±7	20,66±6,11	1,612903
X	AHOROSL	Control susceptibil (LINIE)	Fn	47,66±7,37	7±5,29	40,66±2,08	1,434426

For the sensitive control AHOROSL, an average number of sensitive plants of 40.66 ± 2.08 was recorded, on which an average number of *Orobanche cumana* plants of 58.33 ± 7.08 resulting in an aggression factor of 1,434426.

For the genotypes developed from the wild species of *H. hrisutus*, we recorded the most valuable results for the genotype AH1593, where we obtained values of the number of sensitive plants of 25.66 ± 5,5. In the genotypes developed from the wild species of *H. salicifolius*, we recorded the most valuable results for the genotype AH4039, where we obtained an average number of sensitive plants of 25.33 ± 2.3 parasitized by *Orobanche cumana*. Also, from a total of 18 genotypes analyzed for *H. maximiliani*, values of sensitive plants were recorded between 14.33 ± 5.85 for the AH1210 genotype and 34.66 ± 11.23 of resistant plant, which is also the most important value obtained, and 39 ± 8.66 for genotype AH1204 being the most sensitive genotype according to our results.

Important values of resistance were recorded for the genotype AH1228 of the species *H. occidentalis* where we recorded from a total of 62 ± 2,64 cultivated plants, and an important number of resistant plants of 31,66 ± 7,63.

Based on the results obtained from the comparison of resistant controls and sensitive controls with the developed genotypes, resistant genotypes were identified in the wild species of *H. divaricatus* and *H. giganteus.* These were similar to the values of the resistant controls. Also, values close to the resistance of the controls in the experiment were also recorded for the *H. occidentalis* species.

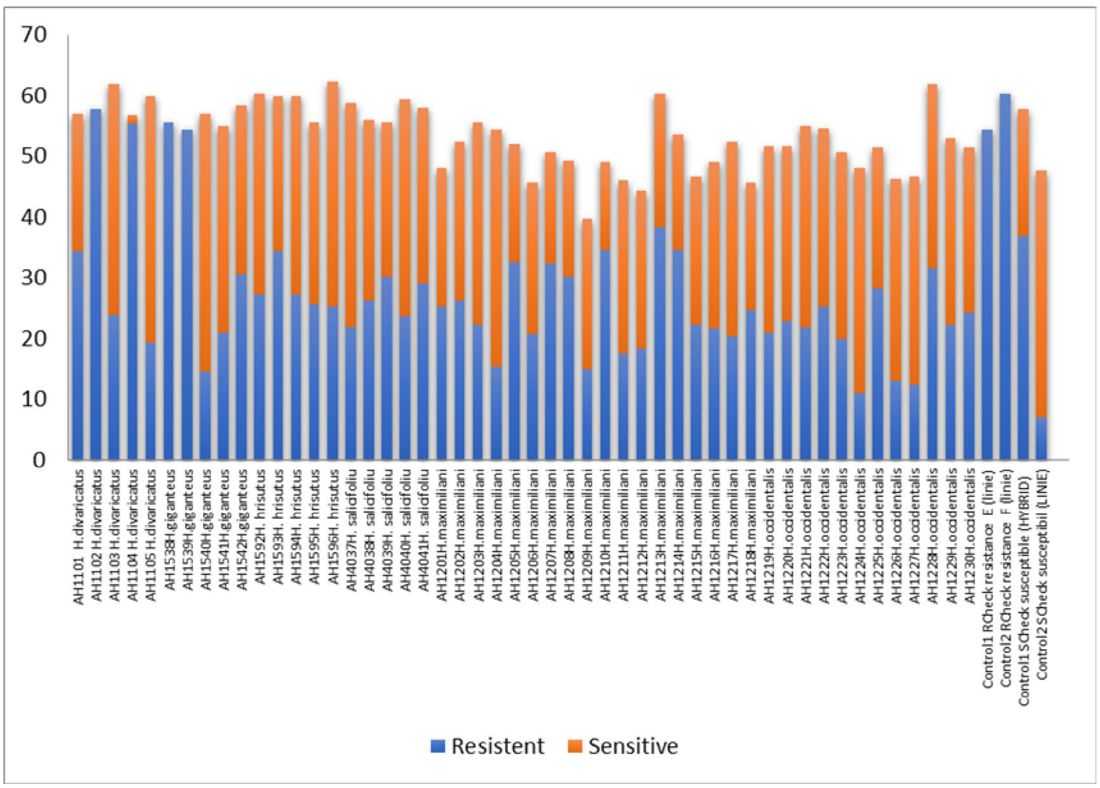

Figure 1. ***Orobanche cumana*** **resistant/sensitive graphic results of sunflower inbred lines developed from wild species.**

Conclusions

Following tests on genetic material developed from wild species, we can say that lines AH1102 and AH1104 from *H. divaricatus* and inbred lines AH1538 and AH1539 derived from *H. giganteus* provide homozygous resistance to race E.

On the other hand, studies show that the lines developed from wild populations with *H. divaricatus* could indicate resistance up to race F [26].

Other scientific research has developed inbred lines resistant to race F in interspecific populations of *H. divaricatus*, namely BR4 and BR3 which can still be found in the germplasm banks of America (USDA).

Genetic resistance was also observed in the germplasm developed by *H. giganteus* and *H. dicaricatus* in race F in the study by Fernández-Martínezet et al [10] with the mention that not all accessions derived from *H. divaricatus* have resistance traits incorporated in their genetics.

Additional tests for race F will be performed on the developed lines AH1102, AH1104, AH1538 and AH1539 resistant to race E to determine the level of resistance of the studied genotypes.

In the process of developing inbred lines from the wild species of *H. hirsutus* and *H. salicifolius*, no materials resistant to *Orobanche cumana* were identified. However, studies by Ruso, J., et al. [23] and Jan, Chao-Chien, et al. [14] indicate resistant genotypes from the wild species of *H. hirsutus* and *H. salicifolius* were). An explanation of the inconsistency of the results could be related to the origins of the germplasm of *H. hrisutus* and *H. Salicifolius*.

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