Inheritance of Resistance to *Orobanche cumana* in Sunflower

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ABSTRACT

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The inheritance of resistance to *Orobanche cumana* (sunflower broomrape) in the resistant sunflower lines SW-501 and RW-637 and the hybrid Sunbred-254 was studied in crosses with susceptible sunflower cultivars Aya and DI-1, in the field and in the growth chamber. Resistance in SW-501 and RW-637 was conferred by a single dominant gene. SW-501 was homozygously resistant, and RW-637 was heterozygously resistant, having one resistance allele and one susceptible allele. The testcross progeny of Sunbred-254 (SW-501 × RW-637) to the susceptible Aya segregated 3 resistant : 1 susceptible, indicating that Sunbred-254 is composed of two genotypically different subpopulations in equal parts, representing the genetic differences between RW-637 and SW-501 in the resistance to *Orobanche cumana*.

Additional keywords: genetics, *Helianthus annuus*, parasitic weed, resistance genes.

The broomrapes (*Orobanche* spp.) are holoparasitic flowering plants infecting the roots of dicotyledonous plants of many families in warm and dry regions. The parasitic plants are totally devoid of chlorophyll and consist of a flowering stalk without leaves. Three factors contribute to the buildup of *Orobanche* seeds in the soil. First, the seeds are stimulated to germinate by host root exudates that are active in the rhizosphere. Second, seeds that are outside the rhizosphere can remain dormant yet viable for more than 10 years. Finally, broomrapes are capable of producing many thousands of tiny seeds (0.2–0.3 mm long) per plant. Because of these and other factors, broomrape control is extremely difficult. The limited control measures available include soil fumigation (5) and soil solarization (4), both of which are prohibitively expensive.

Broomrape seeds are spread with soil that is transferred by farm machinery, surface water, and wind and with soil attached to agricultural products such as hay and seeds. Another important route for broomrape spread, particularly in developing countries, is through the digestive system of grazing animals.

World distribution of *Orobanche* spp. includes all the countries surrounding the Mediterranean, eastern Europe, and all the countries located between and including the Middle East and India. The broomrape problem in Israel is severe, because of the wide distribution of several economically important *Orobanche* spp. and to the great diversity of field and vegetable crops, many of which are susceptible to the parasite. The problem of *O. cumana* in sunflowers is of special importance because of the increased production of sunflower (*Helianthus annuus* L.) in infested areas (6,7).

Breeding for resistance is one of the most promising approaches to alleviating yield losses from *Orobanche* parasitism. Resistance to *O. cumana* in sunflower has been studied extensively in eastern Europe, where sunflower is a major oil crop and broomrape a severe problem (3,9,12,18). Genetic studies of sunflower resistance to *O. cumana* have suggested several different hereditary mechanisms for this trait. Some studies (8,10,19) describe a monogenic, dominant inheritance, whereas others (13) suggest a monogenic, recessive inheritance. Pustvoit (11) claims that the resistance is quantitative, whereas others (14) found an inheritance with cytoplasmic factors involved.

The objective of this study was to achieve a better understanding of the inheritance of resistance to *O. cumana* and thereby facilitate the breeding of broomrape-resistant sunflower cultivars.

MATERIALS AND METHODS

Germ plasm. Five cultivars of sunflower, two susceptible to *O. cumana* and three resistant to it, were used in this study. The susceptible cultivars were Aya (received from Y. Shchori, Volcani Center), a male-sterile, ornamental type, and DI-1, a male-fertile confectionary cultivar (Hazera Ltd.). Both Aya and DI-1 are dwarf, open-pollinated, self-incompatible sunflowers. The three resistant cultivars were semidwarfs used for oil production and developed by Northrup King, Fresno, CA. The hybrid Sunbred-254 (from the cross SW-501 × RW-637) and the open-pollinated RW-637 are self-compatible and male-fertile. The third resistant cultivar, SW-501, is male-sterile and self-compatible. We chose these five cultivars in the light of our earlier screening work (*unpublished data*), which showed that the reaction of the cultivars to *O. cumana* was consistent in different locations in Israel over several years (Table 1). In the present work, seeds were sown manually in March and April at a depth of 4 cm, and plants reached physiological maturity 135–145 days after sowing. Plant spacing was 1 m between the rows and within the rows. This wide spacing facilitated accurate broomrape counts as related to the individual sunflower plants.

Crosses. Crosses were made between resistant and susceptible cultivars, and their F_1 progeny were backcrossed to either parent in the manner described in Table 2. Most crosses were made in the field, but a few were done in growth chambers with potted plants. Capitula were covered with muslin cloth bags before anthesis, and flowers were hand pollinated every day for about a week. Because Aya is male-sterile, it was used as the maternal parent only. To determine whether resistance genes in SW-501 and RW-637 were allelic, we crossed the homozygous resistant individuals obtained from the cross RW-637 × SW-501 and testcrossed the F_1 progeny plants with the susceptible Aya. Homozygous resistant individuals were obtained through selfing of RW-637 and selection during five generations.

Evaluation of resistance in the field. Experiments were conducted during 1983–1988 in fields naturally and heavily infested with *O. cumana* at Kibbutz Hasolelim (Lower Galilee, alluvial brown grumusol soil), Kibbutz Dovrat (the Valley of Yizre'el,

alluvial brown grumusol soil), Moshav Metav (Ta'anach region, alluvial brown grumusol soil), and Bet Dagan (coastal region, hamra soil). Because no established method is available for characterizing the level of *Orobanche* infestation in the field, we defined heavily infested fields as those in which complete destruction of the crop (zero yield) was observed. Experiments were performed using a randomized block design with six replications per parent/cross. No escapes from infections were observed among the populations of the susceptible cultivars (DI-1 and Aya) grown in the fields. Resistance to *O. cumana* was assessed when host plants reached physiological maturity (R9 growth stage [16]) by counting the number of emerged *O. cumana* shoots within a radius of 25 cm around each sunflower plant and the number of nonemerged *O. cumana* seedlings attached to the root system of each host plant. The latter counts were taken after the root ball had been removed from the soil, at a depth of about 30 cm, and washed with water. Resistant plants had no emerged broomrape shoots and no more than three nonemerged broomrape seedlings attached to their root ball. Such plants were 120–150 cm tall, with a capitulum 15–25 cm in diameter. Susceptible individuals had many emerged broomrape shoots (15–156 shoots per sunflower plant, $\bar{X} = 48$) and many nonemerged broomrape seedlings (12–68 seedlings attached to a single sunflower plant, $\bar{X} = 25$). Such plants were 20–40 cm tall with a capitulum 0–2 cm in diameter. No intermediate types of resistance were found.

Inoculation and evaluation of resistance in growth chambers. Seeds of *O. cumana* for the growth chamber experiments were collected from field-infected sunflower plants, and their germination percentage was determined before inoculation. Only one strain of *O. cumana* exists in Israel (6); hence all the collected seeds were of the same strain. A soil mixture (clay soil, tuff [ground porous volcanic stone], and sand, 1:1:1) was amended with 15 mg of viable *O. cumana* seeds per liter. Plants were grown in 10-L pots (9 kg of air-dried soil) at 25°C under continuous light of about 130 μE $m^{-2}s^{-1}$. NPK-containing fertilizer was added twice per week. Under these conditions, the plants grew normally, not showing any stress or nutrient deficiency, and reached physiological maturity (R9 growth stage [16]) 115–120 days after sowing. Each experiment included four replicates, with each treatment (cross) consisting of four pots (one plant per pot), and was organized in a completely randomized design. The trays carrying the pots were moved weekly within the chamber to avoid any possible influence of the location in the chamber. Emerged shoots and nonemerged seedlings of *O. cumana* were counted in each pot as was done in the field experiments. Under these conditions, susceptible individuals had 8–50 emerged broomrape shoots ($\bar{X} = 34$) per sunflower plant and 10–47 nonemerged broomrape seedlings ($\bar{X} = 22$) attached to a single sunflower plant. Such plants were 20–40 cm tall with a capitulum 0–2 cm in diameter. No intermediate types of resistance were found in the growth chamber.

Statistical analysis. Segregation ratios of F_1, F_2, and backcrossed populations were tested for goodness of fit to hypothesized ratios by the chi-square test (1). Data collected from different

TABLE 1. Reaction of parental sunflower cultivars used to study the inheritance of resistance to *Orobanche cumana*

Parent	Number of Plants[a]			Number of Experiments	Year	Locations[b]
	Total	Susceptible	Resistant			
Aya	777	777	...	7	1985–1988	1, 2, 4
Dl-1	1,655	1,655	...	11	1983–1988	1–4
RW-637	909	...	909	3	1987–1988	1, 4
SW-501	1,003	...	1,003	3	1987–1988	1, 4
Sunbred-254	1,050	...	1,050	9	1984–1988	1, 2, 4

[a]Number represents pooled data of all experiments.
[b]Locations: 1 = Kibbutz Hasolelim, 2 = Kibbutz Dovrat, 3 = Moshav Metav, 4 = Bet Dagan.

TABLE 2. Segregation for resistance to *Orobanche cumana* in crosses between resistant and susceptible sunflower cultivars

Cross[a]	Generation[b]	Number of Plants[c]			Expected Ratio	χ^2	*P*	Number of Experiments	Year	Locations[d]
		Total	Susceptible	Resistant						
Aya × Sunbred-254	F_1	198	57	141	1:3	1.52	0.5–0.75	4	1984–1985	1, 4, 5
SW-501 × DI-1	F_1	185	...	185	...	...	...	3	1987	1, 5, 5
SW-501 × DI-1	F_2	430	108	322	1:3	0.003	0.97–0.99	3	1987–1988	1, 1, 4
SW-501 × (SW-501 × DI-1)	BC^R	230	...	230	...	...	...	2	1988	1, 4
DI-1 × (SW-501 × DI-1)	BC^S	403	214	189	1:1	1.56	0.5–0.75	4	1987–1988	1, 1, 4, 4
Aya × RW-637	F_1	196	104	92	1:1	0.74	0.5–0.75	3	1987	1, 4, 5
Aya × [RW-637 (resistant individuals)]	F_2	264	68	196	1:3	0.08	0.95–0.97	2	1988	1, 5
Aya × [RW-637 (susceptible individuals)]	F_2	284	284	...	...	...	...	2	1988	1, 5
[Aya × RW-637 (resistant individuals)] × RW-637	BC^R	253	65	188	1:3	0.50	0.3–0.5	2	1988	1, 5
Aya × [Aya × RW-637 (susceptible individuals)]	BC^S	216	216	...	...	...	...	2	1987–1988	1
[Aya × RW-637 (susceptible individuals)] × RW-637	BC^R	215	110	105	1:1	0.12	0.5–0.75	2	1987–1988	1
Aya × [Aya × RW-637 (resistant individuals)]	BC^S	212	111	101	1:1	0.48	0.25–0.5	2	1987–1988	1, 4
RW-637	Self	149	43	106	1:3	1.19	0.5–0.75	3	1987–1988	1, 4
SW-501 × RW-637	F_1	132	...	132	...	...	...	3	1987–1988	1, 4
SW-501 × RW-637	F_2	190	16	174	1:15	0.92	0.5–0.75	3	1987–1988	1, 4
SW-501 × [RW-637 (homozygous resistant individuals)]	F_1	78	...	78	...	...	...	3	1988	1, 1, 4
SW-501 × [RW-637 (homozygous resistant individuals)]	F_2	69	...	69	...	...	...	2	1988	5, 5

[a]Female parent is listed first.
[b]BC = backcross, R = resistant, S = susceptible.
[c]Number represents pooled data of all experiments, plants of each experiment originating from four sunflower heads. Differences between experiments within a cross were not statistically significant ($P < 0.05$).
[d]Locations: 1 = Hasolelim, 2 = Dovrat, 3 = Metav, 4 = Bet Dagan, 5 = growth chamber. Locations that appear twice for the same year hosted two separate experiments in the same year.

experiments, representing different locations and/or different years, were pooled, because they were not statistically different (one-way analysis of variance, $P < 0.05$, [15]).

RESULTS

The F_1 populations of the cross between the resistant Sunbred-254 and the susceptible Aya segregated 3 resistant : 1 susceptible (Table 2). Because populations of Aya were uniformly susceptible and Sunbred-254 uniformly resistant to *O. cumana*, we concluded that Sunbred-254 is composed of equal parts of two genotypically different subpopulations. One half of the population is homozygous resistant and the other half is heterozygous resistant to *O. cumana*. The F_1 progenies of the cross between SW-501 (resistant) and DI-1 (susceptible) were all resistant to *O. cumana*, indicating dominance of resistance in SW-501 (Table 2). The F_2 progeny segregated 3 resistant : 1 susceptible, indicating that resistance in SW-501 is conferred by a single dominant gene. This conclusion was further supported by the 1 resistant : 1 susceptible segregation ratio obtained in the backcross progeny to the susceptible parent and the absence of susceptible individuals in the backcross progenies to the resistant parent (Table 2). Because SW-501 is male-sterile, it was impossible to self it and study possible maternal factors involved in its resistance.

The F_1 progenies of the cross between Aya and RW-637 segregated in a 1 resistant : 1 susceptible ratio. Because of the segregating F_1 generation, the F_2 and backcrosses had to be done separately with resistant and susceptible F_1 individuals (Table 2). The F_2 progenies from resistant individuals segregated 3 resistant : 1 susceptible, whereas F_2 progenies from susceptible individuals were all susceptible. The progeny of the backcross of resistant individuals to the resistant parent segregated 3 resistant : 1 susceptible, whereas the backcross of susceptible individuals to the susceptible parent yielded susceptible offspring only. The backcross of susceptible F_1 individuals to the resistant parent and the backcross of the resistant F_1 individuals to the susceptible parent both segregated 1 susceptible : 1 resistant. The self of RW-637 yielded progeny that segregated 3 resistant : 1 susceptible. These data suggested that resistance to *O. cumana* in RW-637 is controlled by a single dominant gene, which is present in a heterogenous state in the genotype.

To produce the F_1 hybrid cultivar Sunbred-254, its parents were crossed (SW-501 × RW-637). The segregation patterns in F_1, F_2, and BC generations are shown in Table 2. The F_1 progeny were all resistant. The F_2 population segregated 15 resistant : 1 susceptible, reflecting the susceptible allele of RW-637. Homozygous resistant individuals of RW-637 (F_5 of selfing and selection) were crossed with SW-501, and the resulting offspring in F_1 and F_2 populations were all resistant.

DISCUSSION

The present study indicates that resistance to *O. cumana* in the sunflower lines SW-501 and RW-637 is conferred by a single dominant gene. The results indicate that this gene is present in a homozygous state in the genotype SW-501 and in a heterozygous state in RW-637. The cross between these two lines resulted in a resistant F_1 population, of which 50% of the individuals were homozygously resistant. Thus, Sunbred-254 is genetically heterozygous. Sunbred-254 segregated 15 resistant : 1 susceptible in the F_2 generation, as expected.

Our conclusion, that there is single, dominant gene inheritance of resistance to *O. cumana* in sunflower, is in agreement with the conclusions of Longvinko and Longvinko (8), Pogorletskii and Geshele (10), and Vranceanu et al (19), who studied different sunflower genotypes. Single, dominant gene inheritance of resistance to *Orobanche* spp. has also been reported in other crops, e.g., resistance to *O. cumana* and *O. aegyptiaca* in tomato (2) and to *O. cumana* in hemp (17). However, other hereditary mechanisms, such as monogenic recessive (13), quantitative (11), and cytoplasmic (14) inheritance, have been proposed for the resistance of sunflower to *O. cumana*. These differing findings could be caused by different sunflower genotypes, different *O. cumana* strains, or environmental effects on gene expression.

Our results indicate good agreement with the ratio expected for a single dominant gene conferring resistance. Since the distinction between resistant and susceptible individuals was unequivocal, partial dominance or quantitative inheritance was not involved. In this study, we used cultivars that were male-sterile; therefore, reciprocal crosses to test maternal inheritance could not be done. Thus, cytoplasmic inheritance in the resistance to *O. cumana* in sunflower was not studied.

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