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DIFFERENTIAL REACTION OF RECOMBINANT INTERSPECIFIC INBRED LINES OF SUNFLOWER TO RED RUST INCITED BY *Puccinia helianthi*

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SUMMARY

Thirty wild *Helianthus* species, several interspecific hybrids and 380 recombinant interspecific inbred lines (RIILs) derived from interspecific crosses between the cultivated sunflower and four diploid annuals (wild *H. annuus, H. argophyllus, H. petiolaris* and *H. debilis*) and three diploid perennials (*H. occidentalis, H. maximiliani* and *H. divaricatus*) were studied for reaction to rust caused by *Puccinia helianthi* under severe natural epiphytotic condition. The disease incidence ranged from 0 to 100% in the stable interspecific derivatives while none of the wild sunflowers and their F_1 hybrids revealed susceptibility to rust. The disease severity in the RIILs varied between 0 and 40%. The mean disease severity was low in lines derived from diploid annuals (6.0-10.4%) as compared with those derived from diploid perennials (23.1-31.1%). One line (PS 1089) derived from *H. argophyllus* × cultivated sunflower and two lines (PS 2011 and PS 2032) derived from *H. petiolaris* × cultivar crosses were found to be immune. Disease severity and disease incidence were correlated with various physiological traits.

Key words: ***Helianthus* species, *Puccinia helianthi*, recombinant interspecific inbred lines, resistance, rust, wild sunflowers**

INTRODUCTION

The major production constraint for sunflower cultivation worldwide is the vulnerability of the high yielding cultivars to diseases resulting in yield losses up to 85% depending on the nature of pathogens and the severity of their attacks. In India, *Alternaria* leaf spot is the major disease of sunflower followed by downy mildew caused by *Plasmopara halstedii* and red rust incited by *Puccinia helianthi* Schw. In recent years, rust has become epiphytotic under favorable conditions of moisture and leaf wetness, particularly during late bloom stage of the crop in south-

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ern parts of the country, resulting in reductions in head diameter, seed size and oil content as well as a deterioration of oil quality. In most of the earlier reports rust was described as occurring only late in the season, at the seed development stage, where the estimated yield loss was 17% with 10% reduction in oil yield. However, with early incidence of the disease, the yield reduction reached up to 68%. Among the various approaches of reducing the severity of rust infection, use of resistant genotypes is economically the most viable option.

Reliable sources of resistance to rust are rather limited in the cultivar germplasm (Siddiqui, 1974; Velazhahan *et al.*, 1991). However, wild *Helianthus* species have been a good source of genes for resistance to the causal agents of economically important diseases. A vast reservoir of rust resistance genes is present in wild annuals *viz.*, wild *H. annuus, H. argophyllus* and *H. petiolaris* (Hennessy and Sackston, 1972; Zimmer and Rehder, 1976; Thompson *et al.*, 1981; Seiler, 1992) and in the sole perennial, *H. hirsutus* (Korell *et al.*, 1996). Quresh *et al.* (1993) observed that resistance to all four rust races (1, 2, 3 and 4) could be found in wild *H. annuus, H. argophyllus* and *H. petiolaris.*

The Directorate of Oilseeds Research, Hyderabad, India, has embarked upon a program on interspecific hybridization of sunflower with the aim of introgressing the desirable traits from wild *Helianthus* species into the cultivated sunflower. As part of this program, 380 recombinant inbred lines were developed using seven diploid species against eight genetic backgrounds. After ascertaining the uniformity, stability and distinctness, the pre-bred material was taken up for evaluation for yield and yield-contributing characters. Based on the *per se* performance, many of the stabilized lines were included in the national sunflower network program. The reaction of these lines to rust under severe epidemic condition was assessed in comparison with wild *Helianthus* species and their interspecific hybrids.

MATERIALS AND METHODS

Plant material

Seeds of wild sunflowers obtained from USDA, USA, and the Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia, were established in the *Helianthus* species garden at the Directorate of Oilseeds Research, Hyderabad, India. All wild species were crossed with the cultivated sunflower in both directions and the F_1 interspecific hybrids obtained with perennial species *viz., H. resinosus, H. tuberosus* and *H. simulans*, were studied for their reaction to rust. Three-hundred-eighty recombinant inbred lines were developed by crossing the cultivated sunflower with four diploid annual species *viz., H. annuus* (wild), *H. argophyllus, H. petiolaris* and *H. debilis*, and three diploid perennial species *viz., H. occidentalis, H. divaricatus* and *H. maximiliani*, following standard procedures of selection and generation advancement till stability of character was attained. These stabilized pre-bred

sunflower lines exhibited wide variability for characters, such as, plant type, stem thickness, plant height, leaf size, shape, angle and texture, days to flowering and maturity, head diameter, leaf area index, seed characters, oil content, etc.

Disease incidence and severity

Each entry was planted in a single row of 5 m length spaced 60 cm apart with a plant-to-plant distance of 20 cm. Disease incidence was scored as the number of plants in a row showing rust pustules and expressed in percentage from the total number of plants. Rust severity was recorded by scoring each plant for presence of rust pustules as per diagrammatic representation of Gulya *et al*. (1990), on the scale 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, 20 and 40%. Disease severity was expressed as weighted mean average of the rust score according to the following formula:

$$\frac{\Sigma\, n\,(0) + n\,(0.1) + n\,(0.5) + \ldots\ldots\ldots\ldots\ldots\ldots\ldots n\,(40)}{\text{Total number of plants}}$$

where n represents the number of plants showing disease symptoms of that particular score. Based on the disease severity score, the lines were categorized as immune (0), highly resistant (< 1), resistant (1-5), susceptible (5-20) and highly susceptible (>20-40). A 40% score on this scale was equivalent to 100% infection.

For each cross combination, disease severity was averaged over the lines in that combination. The leaf area index of the pre-bred sunflower lines was determined using the canopy analyzer (LiCOR). Chlorophyll content was estimated directly using an SPAD meter. The genotypes showing immune reaction were retested for their reaction by spraying with a concentration of 10^6 spores/ml of freshly collected uredospores in water.

RESULTS AND DISCUSSION

Weather and disease occurrence

The weather during the crop growth period was normal for the region, encompassing two distinct seasons - winter and early summer (Figure 1). The mean maximum temperature ranged from 28°C (std wk 42) to 38.5°C (std wk 13). The minimum temperature ranged from 9.6°C (std wk 49) to 21.8°C (std wk 40). The relative humidity ranged from 53% (std wk 12) to 93% (std wk 40) in the morning and from 15.5% (std wk 13) to 76% (std wk 40) in the afternoon. The higher relative humidity values in general corresponded with rainfall events. Irrespective of rainfall, the crop was grown under adequate soil moisture provided through irrigation. However, rainfall events directly influenced the relative humidity while temperature regimes were not altered.

Conditions favorable for rust infection are free water on the leaves, either from rainfall or dew. Rust uredospores multiply rapidly during moist and warm weather

when sunflower plants reach the maximum size and have formed dense canopy (Gulya *et al.*, 1990). A minimum of only two hours of wet leaves is sufficient for rust infection. Six to eight hours of leaf wetness will produce maximum infection. Siddiqui (1972) tested germination of uredospores at 8-10, 18-20, 28-30 and 35°C and found optimum germination at 18-20°C in 12 hours. In the present study with favorable weather prevailing during the post-bloom stage, the rust disease incidence was fully expressed during std wk 6 (second week of February) with 3.7 mm of rain coinciding with optimum temperature (30°C max; 17°C min) and relative humidity (81-40% RH). Reddish brown pustules appeared first on the lowermost physiologically active leaves then on the upper leaves and they eventually spread to the petioles, flower bracts and heads. Pustules were small and circular in shape. There was no variation in pustule size but the number of pustules per leaf and the number of infected leaves per plant varied depending on the genotype and the physiological status of the plant.

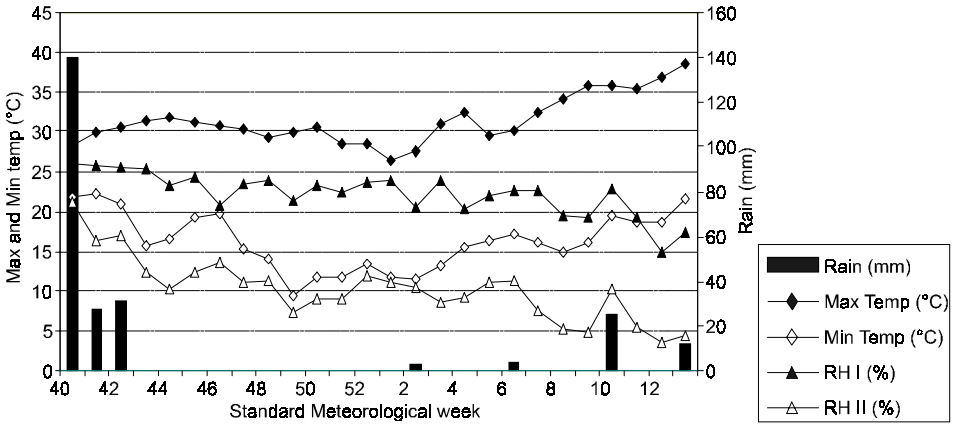

Figure 1: Weather in the period October 2001 to March 2002 at the experimental site

Reaction of wild sunflowers and interspecific hybrids

Observations on the wild sunflowers maintained in the species garden continuously for six years showed no symptoms of rust under field conditions (Table 1). During the six-year period, wide variations were recorded in temperature, rainfall and relative humidity but rust incidence was not observed in any of the species. Similarly, the interspecific hybrids between the cultivated sunflower and *H. resinosus*, *H. tuberosus* and *H. simulans* were found to be immune.

In India, Siddiqui (1971) reported natural incidence of *Puccinia helianthi* Schw. on *Helianthus cucumerifolius* Torrey and Gray. Rust has been reported on seven wild perennial species found in the major sunflower growing area of USA and cross inoculation studies revealed that *P. helianthi* comprised many pathogenic races with considerable specificity to the annual or perennial group and to the species from which it has been collected (Zimmer and Rehder, 1976). Rust evaluations

on perennial *Helianthus* accessions indicated a general immunity to the four prevalent rust races and it is likely that these races are specialized to infect annual *Helianthus* species (Quresh *et al.,* 1993). However, none of the wild sunflowers used in the present investigation showed symptoms of rust under natural conditions. Nevertheless, efforts need to be intensified to identify more reliable sources of resistance in wild sunflowers, particularly to the rust race prevalent in India.

Table 1: Wild sunflowers used in the study

Sl. No	Species	Habit	Ploidy
	H. annuus (wild)	Annual	Diploid
	H. praecox ssp. *praecox*	"	"
	H. praecox ssp. *runyonii*	"	"
	H. praecox ssp. *hirtus*	"	"
	H. argophyllus	"	"
	H. neglectus	"	"
	H. niveus ssp. *canescens*	"	"
	H. petiolaris ssp. *fallax*	"	"
	H. petiolaris ssp. *petiolaris*	"	"
	H. debilis ssp. *cucumerifolius*	"	"
	H. debilis ssp. *debilis*	"	"
	H. debilis ssp. *vestitus*	"	"
	H. debilis ssp. *silvestris*	"	"
	H. debilis ssp. *tardifolius*	"	"
	H. maximiliani	Perennial	"
	H. occidentalis ssp. *plantagineus*	"	"
	H. atrorubens	"	"
	H. giganteus	"	"
	H. nuttallii spp. *rydbergii*	"	"
	H. mollis	"	"
	H. divaricatus	"	"
	H. grosseserratus	"	"
	H. simulans	"	"
	H. decapetalus	"	Tetraploid
	H. hirsutus	"	"
	H. pauciflorus	"	"
	H. resinosus	"	Hexaploid
	H. strumosus	"	"
	H. rigidus	"	"
	H. tuberosus	"	"

Reaction of recombinant interspecific inbred lines

The disease severity ranged from 0 to 40% with the mean for different combinations ranging from 4.9 to 31.1% (Table 2). The severity of rust infection was high in the interspecific derivatives as compared with the cultivar germplasm where a maximum disease severity of 9.4% was scored. The disease incidence in different cross

Table 2: Reaction of recombinant inbred lines to rust

Cross combination	No. of populations/ lines	Disease severity		Disease incidence (%)		Frequency (%) of lines showing different types of reaction*				
		Range	Mean	Range	Mean	Immune	Highly resistant	Resistant	Susceptible	Highly susceptible
Diploid annuals										
H. argophyllus × sunflower	80	0-36.0	6.0	0-100	95.8	1.3	21.2	42.5	23.8	11.3
H. petiolaris × sunflower	56	0-32.9	6.8	0-100	91.3	3.6	14.3	42.9	26.8	12.5
Wild *H. annuus* × sunflower	25	0.11-40.0	10.4	16.7-100	90.2	0	16	20	40	24
H. argophyllus × *H. annuus* (wild) x sunflower (trispecific)	90	0.04-40.0	9.5	42.9-100	98.2	0	12.2	32.2	40	15.5
Sunflower × *H. debilis*	31	0.72-40.0	10.5	80-100	99.4	0	6.5	29	45.1	19.4
Diploid perennials										
Sunflower × *H. occidentalis*	42	0.5-40.0	28.9	100	100	0	2.3	4.8	23.8	69.0
Sunflower × *H. divaricatus*	49	8.1-40.0	31.1	100	100	0	0	0	18.4	81.6
Sunflower × *H. maximiliani*	7	12.5-36.7	23.1	100	100	0	0	0	28.6	71.4
Confectionery type	6	2.0-11.3	4.9	100	100	0	0	50	50	0
Branched type	11	8.3-40.0	19.3	100	100	0	0	0	45.4	54.5
Cultivar	7	3.1-9.4	5.9	100	100	0	0	43	57	-

* Category is based on disease severity score. Immune – 0; highly resistant - <1; resistant – 1-5; susceptible – 5-20; highly susceptible - >20-40%

combinations varied between 0 and 100% with the mean ranging from 90.2 to 100%. The lines derived from diploid annuals recorded lower disease severity and disease incidence values when compared with those derived from diploid perennials. Further, the frequency of lines with immune or highly resistant plants was higher in diploid annuals while the frequency of highly susceptible plants was maximum in lines derived from diploid perennials. One line (PS 1089) derived from *H. argophyllus* x cultivar and two lines (PS 2011, PS 2032) derived from *H. petiolaris* x cultivar crosses were found to be immune. Interestingly, these three lines showed resistant reaction under artificial inoculation with a concentration of 10^6 spores/ml of freshly collected uredospores in water. In susceptible and highly susceptible lines, the reaction to rust was uniform while in the resistant and highly resistant categories disease incidence and severity were low and few of the plants were completely immune. As rust appeared as a severe epidemic during the post-bloom stage and partly at the physiological maturity stage, there was no influence on head diameter but the number of filled seeds was decreased. Yield loss is reported to be only 10% when infection is delayed until two weeks after bloom.

In the present investigation, high level of resistance was found in derivatives of diploid annual species suggesting the usefulness of diploid annuals as valuable sources of resistance to rust. High crossability coupled with high fertility of the F_1 interspecific hybrids facilitates introgression of rust resistance genes into the cultivar germplasm. Reliable sources of resistance in terms of immune reaction were identified in lines derived from *H. argophyllus* and *H. petiolaris* while highly resistant reaction was observed in lines derived from all five cross combinations involving diploid annuals. *H. argophyllus* and *H. petiolaris* are known as sources of resistance to *P. helianthi* and the three resistant sources identified in three different locations in Canada were found to have originated as a result of accidental crossing with wild *H. annuus* (Sackston, 1981).

Interspecific derivatives from diploid perennials showed susceptible reaction to rust although the wild *Helianthus* species showed no symptoms of rust. However, for most other economically important diseases, perennial species proved to be valuable resources (Korell *et al.*, 1996).

In the present study, the disease severity in the cultivar germplasm was low (3.1-9.4%) but none of the tested cultivars were found to be immune or highly resistant to rust. Similarly, Velazhahan *et al.* (1991) screened 196 germplasm lines for resistance to rust and found none to be either immune or resistant. Screening of 94 cultivars revealed that none of the cultivars fell under immune or highly resistant grade while only one of each fell under moderately resistant and moderately susceptible categories (Siddiqui, 1974). Thus, screening of genotypes led to the identification of resistant genotypes but sources in cultivar germplasm that are immune to this disease have not been reported so far in India. The present study constitutes the first report of the availability of sources of immunity to the disease, particularly to the rust race prevalent in the region, and indicates a possible direction for use of the prebred sunflower lines in rust breeding programs. In the resist-

Table 3: Evaluation of pre-bred sunflower lines derived from diploid annual sunflower lines for physiological traits and their correlations with disease parameters

Cross combination*	No. of lines	LAI	Chlorophyll content	Pl. height	Days to flowering	Head diameter	100 seed weight	Yield/pl. (g)
PS line	20	2.1-4.2 (3.37)	30.4-40.1 (34.2)	88-154.2 (117.9)	55-73 (61.6)	8.8-21.0 (14.1)	3.1-6.9 (4.75)	6.5-80.2 (26.5)
H. argophyllus × sunflower	79	2.0-6.6 (3.71)	25.1-38.9 (32.4)	53.6-150.2 (111.7)	53-74 (62.6)	8.2-22.0 (13.2)	2.2-7.4 (4.55)	0.8-43.7 (18.6)
H. petiolaris × sunflower	50	2.1-5.8 (3.77)	28.4-41.5 (34.1)	54.6-181.4 (125.8)	52-74 (61.3)	7.6-19.0 (13.9)	2.7-7.1 (4.7)	1.4-77.3 (27.8)
Wild *H. annuus* × sunflower	22	1.9-4.9 (3.4)	28.7-38.1 (33.1)	56.5-148.4 (106.5)	52-74 (64.2)	9.0-16.0 (12.3)	2.4-7.1 (4.03)	2.1-51.7 (17.2)
H. argophyllus × *H. annuus* (wild) × sunflower (trispecific)	81	1.5-5.0 (3.33)	24.4-39.5 (32.4)	44.8-225.2 (104.2)	52-74 (61.6)	8.2-22.7 (13.9)	2.3-7.1 (4.6)	1.8-73.2 (20.11)
Sunflower × *H. debilis*	28	1.6-3.9 (2.87)	26.8-40.3 (34.4)	95-146.6 (113.2)	55-68 (60.6)	9.6-16.2 (13.4)	2.5-7.0 (4.7)	9.9-42.2 (23.2)
Mean		3.48	33.1	112.2	61.9	13.5	4.57	21.2
Correlation								
Disease incidence		-0.129*	0.018	-0.130*	-0.010	--	--	--
Disease severity		0.227***	0.141*	-0.092	0.053	--	--	--

* PS – physiologically superior plant types; figures in parentheses represent mean values

ant and highly resistant lines, several plants had low frequency of disease incidence coupled with less severe rust infection and such plants based on the agronomic performance *per se* can be intermated and incorporated in breeding programs aimed at broadening the rust resistance base of sunflower cultivars.

Correlation of disease incidence and severity with physiological traits

About 280 promising single plant selections from five cross combinations involving diploid annuals *viz., H. argophyllus, H. petiolaris,* wild *H. annuus, H. debilis* and a trispecific cross involving *H. argophyllus,* wild *H. annuus* and the cultivated sunflower were evaluated for various growth and yield characters which were then correlated with disease incidence and severity (Table 3). Most of the lines exhibited high uniformity and good stability for character expression. Wide variability was observed among the lines for various qualitative traits such as stem thickness, leaf angle and arrangement, leaf color, shape, texture and hairiness, number of leaves, petiole and stem pigmentation, disc color, stigma color and ray floret morphology. Significant variation was recorded for growth and yield characters *viz.*, LAI (1.5-6.62), chlorophyll content as determined by SPAD (24.4-41.5), plant height (44.8-225.2 cm), days to flowering (52-74), head diameter (7.6-22.7 cm), 100-seed weight (2.2-7.4 g) and seed yield/plant (0.8-80.2 g). Among the prebred materials, 15 lines recorded seed yields above 50 g/plant. These were mostly derived from the cross involving H. petiolaris × the cultivated sunflower and the trispecific cross combination. Averaged over the lines, disease incidence showed significant negative correlations with plant height and LAI while disease severity revealed a highly significant positive correlation with LAI and a significant positive correlation with chlorophyll content. Days to flowering had less influence on the spread of rust disease.

Breeding for disease resistance is a major objective of most sunflower improvement programs. Wild sunflowers have been widely exploited as sources of disease resistance genes and in case of rust, two easily transferable dominant genes, R_1 and R_2, have been used extensively by breeders throughout the world to develop resistant cultivars (Putt and Sackston, 1963). In spite of their wide use, continuous evolution of new races and virulence reaction can render these genes useless. Rust resistance is race specific and a genotype rated as totally resistant may possibly be infected either slightly or severely by other races, thus necessitating the work on identification of new sources of resistance to rust. This is a preliminary report to show that rust resistance genes are available in lines derived from crosses involving the cultivated sunflower and diploid annual species, particularly, *H. argophyllus, H. petiolaris* and wild *H. annuus*. Studies need to be undertaken to determine the nature of resistance and the number of genes involved in the resistance reaction. Based on the reaction of differentials available from Canada and USA, the virulence reaction pattern has to be studied to identify the rust race concerned. Molecular markers closely linked to several sunflower rust resistance loci have been identified

which could be used to characterize the pre-bred lines so as to tag other genes of interest.

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REACCIÓN DIFERENCIAL DE LAS INTERESPECIES DE LAS LÍNEAS INBRED DE GIRASOL A ROYA NEGRA CAUSADA POR EL HONGO *Puccinia helianthi*

RESUMEN

Treinta especies salvajes del género de *Helianthus*, varias interespecies del híbrido, y 380 interespecies recombinantes de las líneas inbred, deducidas de las interespecies de cruzamiento entre el girasol cultivado y cuatro especies diploides de la especie anual (*H. Annuus* salvaje, *H. argophyllus, H. petiolaris* y *H. debilis*) y tres especies diploides de perennes (*H. occidentalis, H. maximiliani* y *H. divaricatus*), se estaban investigando a reacción a roya negra, causada por el hongo *Puccinia helianthi*, en las condiciones de la epifitocia natural intensa. La intensidad de la enfermedad, variaba entre 0 y 100% en las líneas de interespecies, mientras que ninguna especie salvaje, ni siquiera sus híbridos F_1, no mostraron sensibilidad a la roya negra. La intensidad de la enfermedad en las interespecies recombinantes de las líneas inbred de interespecies, variaba entre 0 y 40%. La intensidad promedia de la enfermedad era baja en las líneas deducidas de las especies diploides anuales (6.0-10.4%), en comparación con las líneas deducidas de las especies diploides perennes (23.1-31.1%). Una línea (PS 1089), deducida del cruzamiento *H. argophyllus*

× girasol cultivado, y dos líneas (PS 2011 y PS 2032), deducidas del cruzamiento *H. petiolaris* x variedades cruzadas, eran inmunes. Se calcularon las correlaciones entre la intensidad y la aparición de la enfermedad de un lado, y diferentes características fisiológicas por otro lado.

RÉACTION DIFFÉRENTIELLE DES LIGNES RECOMBINANTES INBRED INTERSPECIES DE TOURNESOL À LA ROUILLE ROUGE PROVOQUÉE PAR LE CHAMPIGNON *Puccinia helianthi*

RÉSUMÉ

Une étude a été faite sur trente espèces sauvages d'*Helianthus*, quelques hybrides interspecies et 380 lignes inbred interspecies recombinantes dérivées de croisements interspecies entre le tournesol cultivé et quatre espèces annuelles diploïdes (*H. annuus* sauvage, *H. argophyllus, H. petiolaris* et *H. debilis)* et trois diploïdes vivaces (*H. occidentalis, H. maximiliani* et *H. divaricatus*) pour observer leur réaction à la rouille causée par le *Puccinia helianthi* dans des conditions d'épiphytie naturelle intense. L'intensité de la maladie s'échelonnait de 0 à 100% dans les dérivés interspecies stables tandis qu'aucune espèce sauvage, ni leurs hybrides F_1 ne montraient de sensibilité envers la rouille. L'intensité de la maladie dans les lignes inbred interspecies recombinantes s'échelonnait de 0 à 40%. L'intensité moyenne de la maladie était inférieure dans les lignes dérivées d'espèces diploïdes annuelles (6.0-10.4%) en comparaison aux lignes dérivées d'espèces diploïdes vivaces (23.1-31.1%). Une ligne (PS 1089) dérivée du croisement *H. argophyllus* x tournesol cultivé et deux lignes (PS 2011 et PS 2032) dérivées du croisement *H. Petiolaris* × espèces croisées étaient résistantes. Les corrélations entre l'intensité et l'apparition de la maladie d'un côté et les différentes caractéristiques physiologiques de l'autre ont été calculées.