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REACTION OF WILD SUNFLOWERS AND CERTAIN INTERSPECIFIC HYBRIDS TO *Alternaria helianthi*

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SUMMARY

Twenty-two wild *Helianthus* species and eight interspecific hybrids were evaluated for resistance to the leaf spot disease coused by *Alternaria helianthi*. Cultural characteristics of the fungal pathogen at temperature range of 20-30°C and on medium with different carbohydrates were also studied. Colony growth of *Alternaria helianthi* was maximum on sunflower leaf extract medium (SLEM) devoid of carbohydrates while the maximum spore concentration with the largest conidiophore length (154.9 µm) was obtained on SLEM with 2% sucrose. Temperature had significant effect on the colony growth but had no influence on the spore concentration and spore size. A simple, rapid and reliable laboratory technique using detached leaves for screening germplasm against *Alternaria* leaf spot has been developed to assist the field-based selection for resistant types. Significant differences were detected between various species for their reaction to *Alternaria* as measured by the percentage of leaf area infected. All diploid annuals and their hybrids were found highly susceptible. Maximum resistance was conferred by *H. mollis*, *H. maximiliani*, *H. divaricatus*, *H. pauciflorus*, *H. tuberosus*, *H. resinosus* and *H. simulans*. Field evaluation under artificial epiphytotic conditions revealed a close agreement in the reaction of the wild sunflowers to *A. helianthi* under both laboratory and field conditions.

Key words: ***Alternaria helianthi*, *Helianthus* spp., resistance, screening, wild sunflowers.**

INTRODUCTION

Sunflower (*Helianthus annuus* L.), is an important oil crop in India with an area and production of 2.2 mil. ha and 1.3 mil. tonnes, respectively. The average productivity of sunflower is 614 kg/ha (DES, 1995-96, India). The increase in area and production is concomitant with an increased incidence and severity of foliar diseases. Among the major foliar diseases, leaf spot caused by *Alternaria helianthi* (Hansf.) Tubaki et Nishihara is economically important and appears in most grow-

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ing seasons and locations in India. The disease is reported to reduce seed and oil yields by 27 to 80% and 17 to 33%, respectively, besides leading to germination losses varying from 23 to 32% (Reddy and Gupta, 1977; Balasubrahmanyam and Kolte, 1980). The disease can assume serious proportions and causes premature senescence and is eventually responsible for lodging and poor seed filling. Several control measures, chemical, biological and phytosanitary methods are being adopted but genetic resistance would be the most economic means of reducing losses in sunflower due to this disease. Breeding for resistance to *Alternaria* leaf spot faces the challenge of a gene pool containing only modest levels of resistance. Furthermore, earlier studies on identification of sources for *Alternaria* resistance were confined to screening of germplasm under natural infection (Velazhahan *et al.*, 1991; Nagaraju *et al.*, 1992). Wild sunflowers are potential sources for several desirable characteristics including disease resistance (Seiler, 1992). Results of previous studies indicated that significant genetic variation exists in wild sunflowers with regard to the sensitivity to *Alternaria helianthi* (Morris *et al.*, 1983; Lipps and Herr, 1986; Seiler, 1991). There is a strong need to screen the *Helianthus* species against *Alternaria* isolates of this geographical region and identify the potential donor species with genes for resistance to *Alternaria helianthi* to be incorporated in the cultivated sunflower. The present study was undertaken with the objectives of standardizing culture conditions for optimal growth of *A. helianthi* and identifying sources of resistance in *Helianthus* species under favorable controlled environment.

MATERIALS AND METHODS

Culture of *Alternaria helianthi*

The pathogen was initially isolated from infected sunflower plants and maintained on sunflower leaf extract medium (SLEM). To prepare 1.0 l of SLEM, 200 g of sunflower leaf were taken and cut into small pieces. The leaf pieces were boiled in minimal amount of tap water for 15 min and filtered through two layers of muslin cloth. The filtrate was made up to a final volume of 1.0 l with tap water. The medium was gelled with 2.0% (w/v) agar (Himedia, India). To study the effect of carbon sources on fungal growth, the carbohydrates, sucrose, maltose and glucose (2%), were supplemented individually to the SLEM medium. To assess the effect of temperature on the cultural characteristics of the fungus, the cultures were incubated at temperatures varying from 20 to 30°C with 80% RH and a 16-h photoperiod (30 $\mu E\ m^{-2}\ s^{-1}$). Growth of the fungus was recorded in terms of the colony diameter (cm), spore concentration and spore dimensions. Each treatment consisted of three petriplates (9.0 cm) and the experiments were repeated twice.

Screening of *Helianthus* species and hybrids against *A. helianthi*

Laboratory screening studies. Plant material comprised of twenty two *Helianthus* species and eight interspecific hybrids that are being maintained in the *Helianthus* species garden at the research farm of the Directorate of Oilseeds Research, Hyderabad, India. Leaves with petiole were detached from plants at similar physiological maturity and immersed in a spore suspension (1 x 10^6 spores/ml) prepared from two-week-old cultures and transferred to petriplates (15 cm) containing two layers of moist filter paper with one set of respective controls. The leaf samples were maintained under similar conditions followed for the culture of the pathogen. Disease severity was recorded by visually examining each leaf after three and seven days of inoculation as % incidence following the pictorial key of Allen *et al.* (1983). Based on their reaction to *Alternaria helianthi*, the wild sunflowers were classified into three groups, resistant (0-10%), moderately resistant (11-30%) and susceptible (31-100%). Each treatment comprised of ten leaves in three replicates and the experiment was repeated thrice. Data were analyzed by the analysis of variance (ANOVA 1) and the Student-Neuman-Keul's multiple range test ($p < 0.001$) was used to compare treatment means.

Field screening. A few branches of healthy plants were sprayed with the spore suspension of *A. helianthi*. Subsequently, the treated parts were covered with polythene bags and the field was irrigated to maintain high humidity until the observations, seven days later.

RESULTS

Cultural characteristics of *A. helianthi* as influenced by the temperature and carbohydrates

The maximum colony diameter (8.7 cm) was recorded on the control (SLEM) medium (Table 1; Figures 1a-d). However, media supplemented with carbohydrates were found better than the control for the growth of the fungus in terms of sporulation and spore size. Among the three carbon sources tested, sucrose promoted a faster growth with maximum sporulation (1.53 x 10^6) and conidiophores of the largest size (168.3-170.0 µm). Medium enriched with glucose and maltose also facilitated production of conidiophores of sizes ranging from 122.2 to 136.4 µm which were significantly different from those on the control medium (67.7 µm) but the colony diameter was significantly smaller than that on the latter. Differences in colony diameter, spore size and spore concentration due to the presence of various carbohydrates in the medium were highly significant.

Temperature had significant influence on colony diameter while it showed no positive effect on spore size and spore concentration (Table 1). The radial growth of the fungus was maximum at 30°C on the control medium, and at 25 to 27.5°C on carbohydrate supplemented media. Temperature and carbohydrate interaction

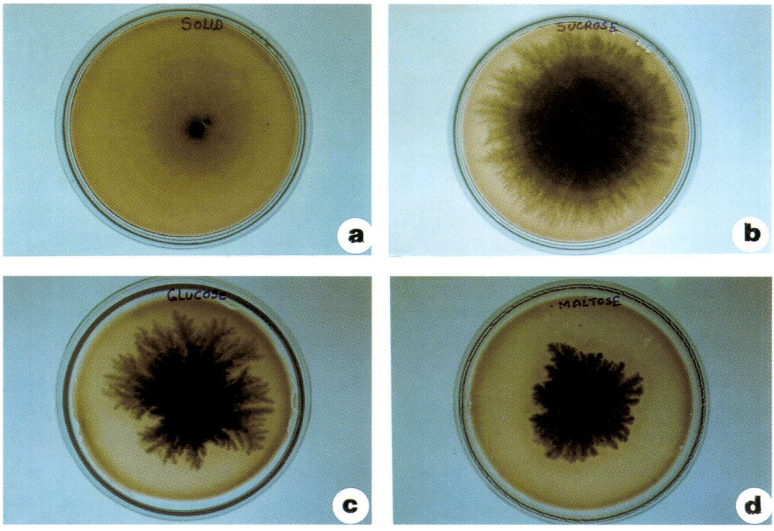

Figure 1. Cultural characteristics of Alternaria helianthi as influenced by various carbohydrates. a) Sunflower leaf extract medium (SLEM-control); b) SLEM + sucrose; c) SLEM + glucose; d) SLEM + maltose

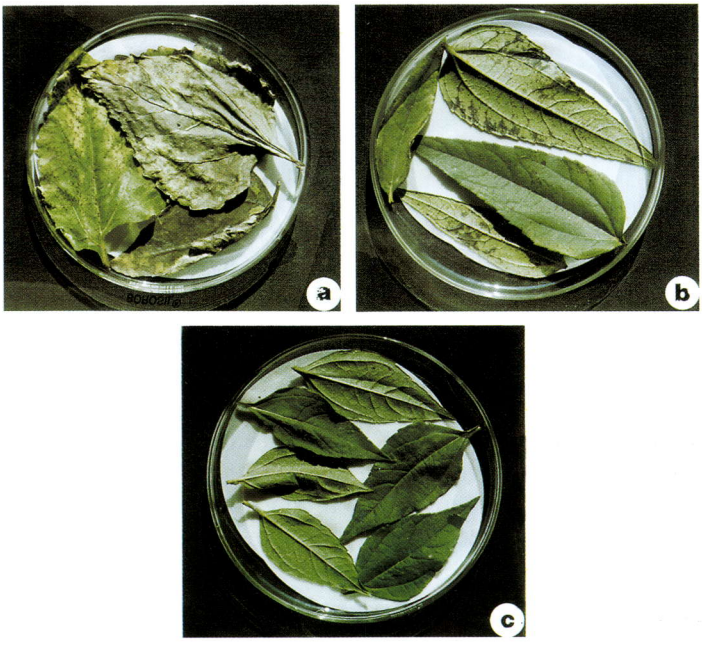

Figure 2. Reaction of wild sunflowers to Alternaria helianthi using the detached leaf technique. a) Susceptible (H. annuus wild); b) Moderately resistant (H. hirsutus); c) Resistant (H. tuberosus)

Table 1: Effect of carbohydrates and temperature on the cultural characteristics of *A. helianthi*

Temperature (°C)	Carbohydrate	Colony diameter (cm)	Spore length (µm)	Spore conc. (x 10^6)
20.0	-	6.7 c	71.7 c	0.47 f
	Sucrose	5.2 fg	149.3 ab	1.12 bcde
	Glucose	4.4 hi	132.3 b	0.99 bcde
	Maltose	4.0 i	120.0 b	1.00 bcde
22.5	-	7.5 b	65.0 c	0.42 f
	Sucrose	5.6 ef	141.7 b	1.19 bcd
	Glucose	5.2 fg	136.7 b	1.06 bcde
	Maltose	4.2 i	123.3 b	1.05 bcde
25.0	-	8.0 b	70.0 c	0.51 f
	Sucrose	7.3 b	170.0 a	1.25 bc
	Glucose	6.5 cd	135.7 b	1.08 bcde
	Maltose	4.7 ghi	122.7 b	1.15 bcd
27.5	-	8.1 b	66.7 c	0.44 f
	Sucrose	7.7 b	168.3 a	1.31 b
	Glucose	6.2 cde	137.7 b	1.28 b
	Maltose	5.1 fgh	123.3 b	0.89 de
30.0	-	8.7 a	65.0 c	0.54 f
	Sucrose	6.8 c	145.0 b	1.53 a
	Glucose	5.9 de	139.7 b	0.82 e
	Maltose	4.6 ghi	121.7 b	0.93 cde
CV (%)		5.2	8.8	10.3
F-test		Level of significance		
Temperature (a)		***	ns	ns
Carbohydrate (b)		***	***	***
a x b interaction		***	ns	***

Experimental design: Two factor ANOVA with 40 DF;
Means in a column followed by same letters are not significantly different according to Student-Nueman-Keul's multiple range test at $p < 0.05$.
*** $p < 0.001$, ns = nonsignificant

effects were highly significant for colony diameter and spore concentration while it was non-significant for spore size.

Screening *Helianthus* species against *Alternaria helianthi*

Under artificial assay conditions the susceptible leaves showed slight yellowing with black spots all over the lamina that enlarged greatly and thereby coalesced resulting in complete blackening and wilting of the entire leaf (Figure 2a). Moderately resistant types showed localized chlorosis with brown to black spots (Figure 2b) while the leaves of resistant types showed no symptoms or developed a few black specks that were sparsely distributed (Figure 2c).

Differences in the percentage of leaf infection caused by *Alternaria helianthi* on wild sunflowers were highly significant (Table 2). All annual species were highly susceptible and died within 48 to 72 h after inoculation. The resistant category included diploids - *H. mollis*, *H. maximiliani*, *H. divaricatus* and *H. simulans*; tetraploids - *H. decapatalus* and *H. pauciflorus* and hexaploids - *H. tuberosus* and *H. resinosus*, which are all perennial.

Table 2: Reaction of *Helianthus* species and certain hybrids to *Alternaria helianthi*

Species	Category	Disease index
H. mollis	Resistant	1.4 f
H. maximiliani		4.1 f
H. divaricatus		2.7 f
H. simulans		4.8 f
H. decapetalus		1.3 f
H. pauciflorus		5.4 f
H. resinosus		5.1 f
H. tuberosus		2.7 f
H. grossesseratus	Moderately resistant	14.2 ef
H. debilis		15.4 ef
H. strumosus		25.3 def
H. hirsutus		12.9 ef
H. praecox ssp	Susceptible	66.8 bcd
H. nuttalli		70.3 bc
H. annuus wild		92.9 abc
H. annuus cultivated		95.0 abc
H. niveus		95.2 abc
H. neglectus		95.6 abc
H. praecox ssp		96.0 abc
H. argophyllus		97.4 ab
H. deserticola		100.0 a
H. petiolaris		100.0 a
Interspecific hybrids		
H. praecox x H. annuus (wild)		58.7 cde
H. praecox x H. simulans		75.1 abc
H. argophyllus x H. petiolaris		88.3 abc
H. praecox x H. petiolaris		92.0 abc
H. petiolaris x H. annuus		100.0 a
H. argophyllus x H. annuus		100.0 a
H. argophyllus x H. annuus (wild)		100.0 a
H. annuus x H. debilis		100.0 a

Combined average disease index of 30 leaves;
Percentage values were arcsin angular transformed prior to analysis;
Means in a column followed by same letters are not significantly different according to Student-Neuman-Keul's multiple range test at $\alpha = 0.001$;
Data scored after one week of inoculation.

Field screening. There were no symptoms of leaf spot on the *Helianthus* species under natural conditions. However, all the interspecific hybrids involving cultivated sunflower as the parent showed moderate levels of infection. Under artificial epiphytotic conditions, the reaction of the wild sunflowers to *Alternaria* had a close agreement with that obtained with laboratory screening. Interestingly, the leaf spot symptoms were seen only on plant parts sprayed with the inoculum and covered with polythene bags to create a favorable environment leaving the unsprayed parts healthy. In fact, the field reaction of the *Helianthus* species except for *H. argophyllus* was not as severe as that observed under laboratory conditions.

DISCUSSION

Yield losses due to *Alternaria* leaf spot are a major constraint in sunflower production in India (Reddy and Gupta, 1977; Balasubrahmanyam and Kolte, 1980). Attempts to develop genotypes resistant to this highly devastating disease met with little success owing to the nonavailability of resistant sources. This investigation attempts at identification of durable sources of resistance from *Helianthus* species through development of a simple assay system. Before attempting at large scale screening, it was felt necessary to optimize the culture conditions for *Alternaria helianthi* since the nutrition of the pathogen assumes paramount importance in enabling successful colonization.

For isolation of *A. helianthi*, potato-dextrose agar (PDA) has been used by several workers (Narain and Saksena, 1973; Anil Kumar *et al.*, 1974; Mukewar *et al.*, 1974). Mukewar *et al.*, (1974) reported a slow growth of the fungus with abundant sporulation on PDA. Tosi and Zazzerini (1991) reported no growth of the pathogen on PDA and malt-agar. Kolte (1985) has reported better mycelial growth on sunflower leaf extract medium (SLEM) than on PDA. In the present study also, maximum radial growth was obtained on the SLEM medium. However, sporulation was not very abundant, the spores were small and the growth was slow. Nevertheless, incorporation of carbohydrates, sucrose, glucose and maltose, in the SLEM medium favored faster growth of the pathogen with abundant sporulation. Of the three carbon sources tested sucrose proved to be superior. While the role of carbon sources in nutrition of plant pathogen is well documented, its influence on the metabolic pathway of the fungus is yet to be understood. Incubation temperature of 30°C facilitated maximum radial growth on the control medium and at 25-27°C on carbohydrate supplemented medium. The results are consistent with the earlier reports where the favourable temperature for *Alternaria helianthi* was reported to be 25-28°C (Islam and Marić, 1978; Li and Chi, 1996)

In vivo and *in vitro* techniques to screen sunflower genotypes for *A. helianthi* tolerance are laborious and time consuming (Morris *et al.*, 1983; Theertha Prasad *et al.*, 1996). An assay system using detached leaves has been developed to assist in field based selections for *Alternaria* tolerance. The technique is simple and the *in*

vitro reaction has a 1:1 correlation with that of the field reaction under artificial epiphytotic conditions. Further, a large number of genotypes can be screened in a single season with limited space. It also avoids accumulation of pathogen load in the environment as occurs in the case of field screening techniques. Since most of the resistant species are perennial in habit, leaf material will not be limiting and screening can be undertaken at the same stage of physiological maturity in different seasons.

Screening under laboratory and field conditions revealed certain reliable sources of resistance in the *Helianthus* species. The resistant reaction of many of the species was in agreement with that reported by Morris *et al.*, (1983) using field screening technique. However, certain diploid species rated as resistant in the present investigation were categorized as susceptibles or highly susceptibles by Morris *et al.*, (1983) in spite of using a 10 to 100 fold lesser spore concentration than that used (1 x 10^6 spores/ml) in the present study. This variation in reaction could be due to either the differences in the accessions or the pathotype used in the two studies. Further, Morris *et al.*, (1983) inoculated plants at the four to ten leaf stage unlike in our study where leaves from field established plants were utilized for screening.

The resistant sources included species predominantly from the section Divaricati comprising of all the three ploidy levels. Studies of Lipps and Herr (1986) also confirmed *H. tuberosus* as a good source of resistance to *A. helianthi*. Interspecific hybridization studies indicated cross compatibility of cultivated sunflower with *H. tuberosus, H. mollis* and *H. decapetalus* (Bohorova and Atanassov, 1990). Hence, our future attempts are directed towards utilization of these sources of *Alternaria* resistance in the improvement of cultivated sunflower.

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REACCIÓN DE ESPECIES DE *Helianthus* SILVESTRE Y HÍBRIDOS INTERSPECÍFICOS PARA RESISTENCIA PROVOCADA POR *Alternaria helianthi*

RESUMEN

Se evaluaron veintidos especies de *Helianthus* silvestre y ocho híbridos interespecíficos para comprobar su resistencia a la mancha foliar provocada por *Alternaria helianthi*. Asímismo, se estudiaron las características del patógeno fúngico a temperaturas que oscillaban entre 20-30°C y sobre un medio con distintos hidratos de carbóno. El desarrollo de colonias de *Alternaria helianthi* fue máximo en un medio de extracto de hoja de girasol (MEHG) carente de hidratos de carbóno mientras que se obtuvo la máxima concentración de esporas con una máxima longitud del conidioforo (154,9 µm) en MEHG con 2% de sucrosa. La temperatura ejerció un efecto significativo en el desarrollo de las colonias pero no influyó en la concentración de esporas ni en el tamaño de las esporas. Se ha desarrollado una técnica de laboratorio sencilla, rápida y fiable utilizando las hojas separadas como método para detectar el germoplasma frente a la mancha foliar con el fin de favorecer la selección en el campo de tipos resistentes. Se detectaron diferencias significativas entre varias especies en cuanto a su reacción a Alternaria según medidas obtenidas del porcentaje de la superficie de la hoja infectada. Se comprobó que todas las plantas anuales diploides y sus híbridos fueron sumamente susceptibles. *H. mollis*, *H. maximiliani*, *H. divaricatus*, *H. pauciflorus*, *H. tuberosus*, *H. resinosus* and *H. simulans* proporcionaron la máxima resistencia. La evaluación en el campo en condiciones epifitóticas artificiales mostraron una conformidad casi total en la reacción del girasol silvestre a A. helianthi tanto en condiciones de laboratorio como en las del campo.

RÉACTION DES TOURNESOLS SAUVAGES ET D'HYBRIDES INTERSPÉCIFIQUES À *Alternaria helianthi*

RÉSUMÉ

Vingt deux espèces sauvages d'*Helianthus* et huit hybrides interspécifiques ont été évalués pour leur résistance à la maladie des taches foliaires occasionnée par l'*Alternaria helianthi*. On a également étudié les caractéristiques de culture du champignon pathogène à la température de 20-30°C sur un milieu contenant divers hydrates de carbone. La croissance des colonies d'Alternaria helianthi est maximale sur un milieu composé d'extraits de feuilles (SLEM) dépourvu d'hydrates de carbone, alors que l'on obtient la concentration maximale en spores et la plus grande longueur de conodiophores (154.9 µm) avec 2% de saccharose. La température exerce un effet sur la croissance de la colonie mais n'a pas d'influence sur la concentration ou la dimension des spores. Une technique de laboratoire simple et fiable, utilisant des feuilles excisées pour le criblage du germplasm contre l'alternaria a été développée pour aider la sélection de génotypes résistants au champ. On a mis en évidence des différences significatives entre diverses espèces pour leur réponse à l'alternaria estimée par le pourcentage de la surface foliaire infectée. Tous les diploïdes annuels et leurs hybrides se sont révélés très sensibles. Le maximum de résistance est apporté par *H. mollis*, *H. maximiliani*, *H. divaricatus*, *H. pauciflorus*, *H. tuberosus*, *H. resinosus* et *H. simulans*. Une évaluation au champ en conditions d'infestation artificielle montre une bonne concordance entre la réponse des tournesols sauvages à l'*A. helianthi*, à la fois en conditions de laboratoire et de champ.