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CULTURE CHARACTERISTICS AND HISTOLOGICAL CHANGES IN LEAF TISSUES OF CULTIVATED AND WILD SUNFLOWERS INFECTED WITH *Alternaria helianthi*

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SUMMARY

The cultural characteristics and histochemical variations following infection with *Alternaria helianthi* was studied in six wild *Helianthus* species of three ploidy levels (diploid, tetraploid and hexaploid) possessing different degrees of resistance to the pathogen and in cultivated sunflower (*H. annuus* cv. CO-4, susceptible check). Of these, the species *H. occidentalis* and *H. tuberosus* were found to be highly resistant while *H. hirsutus* was moderately resistant. Media supplemented with leaf extracts of wild species, with the exception of *H. grosseserratus*, supported less growth and sporulation of *A. helianthi* than media supplemented with leaf extract of cultivated sunflower. Reduced infectivity of the pathogen was recorded when grown on leaf extract media of *H. occidentalis, H. hirsutus* and *H. tuberosus*. Furthermore, abnormalities in the shape of conidia were noticed on supplemented media with leaf extracts of *H. occidentalis* and *H. tuberosus*. RAPD analysis of the fungal DNA isolated from the pathogen grown on leaf extract media of cultivated and wild *Helianthus* species revealed no polymorphism. Histochemical studies showed restriction of the pathogen to epidermal cells in resistant wild sunflowers as well as an increased accumulation of phenols.

Key words: ***Alternaria helianthi*, *Helianthus* species, RAPD**

INTRODUCTION

Sunflower (*Helianthus annuus* L.), a source of vegetable oil and proteins, is an important oilseed crop in the world. The major threat for sunflower cultivation worldwide is the susceptibility of the improved cultivars to foliar diseases like *Alternaria* leaf blight, *Sclerotinia* leaf spot, rust and downy mildew. Among these dis-

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eases, leaf spot incited by *Alternaria helianthi* (Hansf.) Tubaki et Nishihara is economically important in India and other tropical countries. The disease is reported to reduce seed and oil yields by 27 to 80% and 17 to 33%, respectively, leading also to germination losses varying from 23 to 32% (Reddy and Gupta, 1977; Balasubrahmanyam and Kolte, 1980). Chemical, biological and phytosanitary methods are being adopted for the management of this disease with varied success, but host plant resistance seems to be economically the most viable option. Wild sunflowers constitute a vast reservoir of desirable characteristics including disease resistance (Seiler, 1992). There is a need to discriminate between host and non-host type of reaction before undertaking introgressive breeding. The present investigation was undertaken to study the variability in reaction of wild sunflowers to *Alternaria helianthi* and the histological variations associated with pathogen infection as these studies provide information as to the localized reaction in the cells.

MATERIAL AND METHODS

Plant material

Six wild *Helianthus* species of three ploidy levels (*H. occidentalis, H. maximiliani* - diploids; *H. grosseserratus, H. hirsutus* - tetraploids; *H. resinosus, H. tuberosus* - hexaploids) and the highly susceptible cultivated sunflower (*H. annuus*, cv. CO-4), were obtained from the *Helianthus* species garden maintained at the Research Farm of Directorate of Oilseeds Research (DOR), Rajendranagar, Hyderabad. The leaves at the third node of newly emerged branches were used in all experiments. For cultivated sunflower, the leaves at the third node of 30-day-old plants were used.

Reaction to *A. helianthi*

The cultivated and wild sunflowers were screened for their reaction to *A. helianthi* by using a detached leaf technique (Sujatha *et al.*, 1997) at different spore concentrations (10^3, 10^4, 10^5 and 10^6 conidia ml^{-1}). Disease intensity was recorded 2, 4 and 6 days after inoculation (d.a.i.) using the pictorial key of Allen *et al.* (1983).

Cultural characteristics

SLEM consisting of 20% leaf extract of cultivated sunflower was found to support better growth of the pathogen (Sujatha *et al.*, 1997). In order to test the effect of leaf extracts of different *Helianthus* species on the pathogen, *A. helianthi* was grown on the leaf extract media obtained from cultivated and wild sunflowers. Observations of colony diameter, color and sporulation were recorded at three-day intervals up to 21 days. Conidial morphology was studied for isolates grown in different leaf extract media using a research microscope (Leitz Wetzlar, Diaplan, Germany).

Virulence studies

Variations were detected in the cultural characteristics of the pathogen when grown on media supplemented with leaf extracts of wild sunflowers. The virulence of the pathogen isolated from leaf extract media of cultivated and wild sunflowers was tested by inoculating susceptible cultivated sunflower with different spore concentrations (10^3, 10^4, 10^5 and 10^6 conidia ml^{-1}) and observations made 2, 4 and 6 d.a.i.

RAPD analysis

Total fungal genomic DNA was extracted according to Dellaporta *et al.* (1983) from mycelium of 15-day-old colonies of *A. helianthi* grown on leaf extract media of cultivated and selected wild sunflowers where low virulence (*H. occidentalis, H. hirsutus* and *H. tuberosus*) was recorded. PCR was carried out in 10 μl of a reaction mixture containing 25 ng of *A. helianthi* genomic DNA, 1 μl 10 x PCR buffer (Genei), 1.2 μl of random primers (OPA and OPB series) (Operon Technologies, USA) and 3U of *Taq* DNA polymerase. PCR amplification was carried out following a hot start at 94°C for 5 min followed by 45 amplification cycles of 94°C for 1 min., 36°C for 1 min., 72°C for 2 min., with a final extension step for 72°C for 10 min. Amplification products were electrophoresed on 1.4% agarose gel at a constant voltage of 60 V for 45 min. in 1% TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) and photographed under UV illumination after staining in ethidium bromide (0.5 mg l^{-1}).

Histochemical studies

The uninoculated (control) and inoculated (2 d.a.i.) leaves of each *Helianthus* species were fixed in formalin : acetic acid : ethyl alcohol (6 : 1 : 14) for 48 h and stored in 70% alcohol. Standard methods for dehydration in TBA series and embedding in paraffin wax were followed (Johansen, 1940). Sections of 10-12 μm thickness were cut on a rotary microtome then stained with 0.1% toluidine blue O.

Statistical analysis

Experiments for evaluating the reaction of wild *Helianthus* species to *A. helianthi* and the cultural characteristics and virulence of the pathogen when grown on media supplemented with leaf extracts of wild sunflowers were done in three replicates. Each replicate consisted of 3 petri plates (9.0 cm) with two leaves for each cultivated sunflower and three leaves for each wild species. A two-way analysis of variance was done to test the significance of the treatment effects.

RESULTS

Reaction to *A. helianthi*

Significant differences were observed with respect to percent disease severity at different spore concentrations and d.a.i. among the *Helianthus* species (Table 1) and the disease severity increased with time at all the tested spore concentrations. At the spore concentration of 10^3 conidia ml^{-1}, symptoms spread was 100% in cv.

Table 1: Reaction of cultivated and wild *Helianthus* species to *A. helianthi*

Species	Percent disease severity*																Mean
	10^3 conidia ml^{-1}				10^4 conidia ml^{-1}				10^5 conidia ml^{-1}				10^6 conidia ml^{-1}				
	2 d.a.i.	4 d.a.i.	6 d.a.i.	Mean	2 d.a.i.	4 d.a.i.	6 d.a.i.	Mean	2 d.a.i.	4 d.a.i.	6 d.a.i.	Mean	2 d.a.i.	4 d.a.i.	6 d.a.i.	Mean	
H. annuus (cv.CO-4)	25.0	100.0	100.0	75.0[a]	37.5	100.0	100.0	79.2[a]	62.5	100.0	100.0	87.5[b]	100.0	100.0	100.0	100.0[a]	85.4[a]
H. occidentalis	0	0.2	0.6	0.3[c]	0.6	0.8	5.0	2.1[c]	1.5	2.0	17.5	7.0[d]	17.5	37.5	87.5	47.5[b]	14.2[c]
H. maximiliani	37.5	100.0	100.0	79.2[a]	62.5	100.0	100.0	87.5[a]	100.0	100.0	100.0	100.0[a]	100.0	100.0	100.0	100.0[a]	91.7[a]
H. grosseserratus	37.5	100.0	100.0	79.2[a]	50.0	100.0	100.0	83.3[a]	62.5	100.0	100.0	87.5[b]	100.0	100. 0	100.0	100.0[a]	87.5[a]
H. hirsutus	1.5	7.5	42.5	17.2[b]	7.5	75.0	100.0	60.8[b]	37.5	100.0	100.0	79.2[bc]	87.5	100.0	100.0	95.8[a]	63.3[b]
H. tuberosus	0	0.1	0.1	0.1[c]	7.5	87.5	100.0	65.0[b]	10.0	100.0	100.0	70.0[c]	100.0	100.00	100.0	100.0[a]	58.8[b]
Mean	16.9	51.3	57.2	41.8	27.6	77.2	34.2	63.0	45.7	83.3	86.3	71.9	84.2	89.6	97.9	90.6	

[a] Means within a column followed by the same letter are not significantly different according to LSD at P=0.05.
[b] Percentage values are arcsine angular transformed prior to analysis.
[c] Values are means of three replications.
[d] d.a.i. - days after inoculation.

CO-4 and wild sunflowers, *H. maximiliani* and *H. grosseserratus* at 4 d.a.i. The mean disease severity was greatest in *H. maximiliani* and *H. grosseserratus*, which was the same as in cv. CO-4 and significantly different from the other wild sunflowers studied. The lowest level of disease symptoms was recorded in *H. tuberosus* and *H. occidentalis*. Using inoculum containing 10^4 conidia ml^{-1} similar trends were observed but disease incidence in *H. tuberosus* was *on par* with that of *H. hirsutus* while *H. occidentalis* recorded significantly lower disease incidence than any other of the tested *Helianthus* species. With inoculum containing 10^5 conidia ml^{-1}, maximum disease severity was observed in *H. maximiliani* and was significant while cv. CO-4, *H. grosseserratus* and *H. hirsutus* were *on par* with each other. Minimum percent leaf infection was observed in *H. occidentalis*. At the highest spore concentration (10^6 conidia ml^{-1}), 100% disease incidence was recorded at 2 d.a.i. in all the genotypes with the exception of *H. hirsutus* and *H. occidentalis*. All *Helianthus* species with the exception of *H. occidentalis* were *on par* with each other in their mean disease severity.

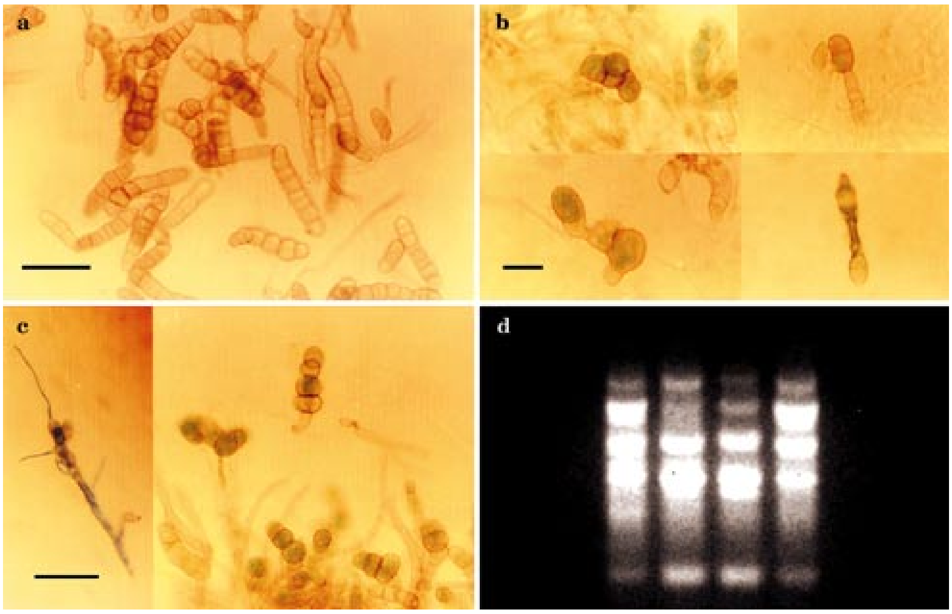

Figure 1: Variations in conidia and DNA polymorphism in A. helianthi grown on media supplemented with leaf extracts of cultivated sunflower (cv. CO-4) and wild Helianthus species.
a. *Normal spores of A. helianthi on medium supplemented with leaf extract of cultivated sunflower cv. CO-4 (bar=50 µm).*
b. *Conidial abnormalities of A. helianthi on media supplemented with leaf extract of H. occidentalis and H. tuberosus (bar=25 µm).*
c. *Precocious germination on the conidiophore and abnormalities of conidia of A. helianthi on medium supplemented with leaf extract of H. occidentalis and H. tuberosus (bar=50 µm).*
d. *RAPD amplification profile with OPA 4 primer of the DNA isolated from A. helianthi grown on media supplemented with leaf extract of cultivated sunflower (Lane 1), H. occidentalis (Lane 2), H. hirsutus (Lane 3) and H. tuberosus (Lane 4). (Lanes left to right)*

Cultural characteristics

Colony color was grayish-white on leaf extract media of all *Helianthus* species. Colony diameter on different leaf extract media ranged between 1.6 and 5.3 cm and was maximum on media with leaf extract of cv. CO-4 followed by *H. grosseserratus* (Table 2). Minimum radial growth was observed in *H. occidentalis* and was *on par* with that of *H. tuberosus* and *H. resinosus*.

Table 2: Cultural characteristics of *A. helianthi* grown on media supplemented with leaf extracts from cultivated sunflower and wild *Helianthus* species

Species	Colony diameter (cm)	Length (μm)	Breadth (μm)	Septa (no.)
H. annuus (cv. CO-4)	5.3[a]	173.0[a]	33.7[a]	9.0[a]
H. occidentalis	1.6[d]	157.5[c]	26.8[c]	8.1[e]
H. maximiliani	4.3[bc]	117.2[f]	21.0[d]	6.8[g]
H. grosseserratus	5.2[ab]	145.2[d]	26.0[c]	8.3[d]
H. hirsutus	3.6[c]	121.8[e]	21.2[d]	7.6[f]
H. resinosus	2.4[d]	147.7[d]	28.3[b]	8.8[b]
H. tuberosus	2.5[d]	163.0[b]	26.3[c]	8.6[c]

[a] Means within a column followed by the same letter are not significantly different according to LSD at P=0.05.
[b] Observations on colony diameter were recorded at 11 d.a.i. and the other parameters at 21 d.a.i. (days after inoculation).
[c] Values of colony diameter are the means of 3 replications.
[d] Values of length, breadth and septation are the means of 100 spores.

The length and breadth of the conidia produced on different leaf extract media varied from 117 to 173 μm and 21 to 33.7 μm, respectively. The mean septation ranged from 6.8 to 9.0. Maximum length, breadth and septation were recorded in leaf extract medium of cv. CO-4 and minimum values in *H. maximiliani*. Abnormalities in the shape and germination of conidia on conidiophore itself were observed in leaf extract media of *H. occidentalis* and *H. tuberosus* (Figures 1a to c).

Virulence studies

Significant differences were observed in the virulence of *A. helianthi* grown on leaf extract media of cultivated and wild sunflowers at different spore concentrations and time intervals (Table 3). At 10^3 conidia ml^{-1} spore concentration, 100% leaf infection was observed when inoculated with the pathogen grown on leaf extract media of CO-4 and *H. maximiliani* at 4 d.a.i. while the same was observed at 6 d.a.i. in *H. grosseserratus*. Minimum percent leaf infection was observed when inoculated with the pathogen grown on *H. resinosus* followed by *H. hirsutus*, *H. tuberosus* and *H. occidentalis*. At spore concentration of 10^4 conidia ml^{-1}, complete disorganization of tissue was observed when inoculated with *A. helianthi* grown on all the *Helianthus* species except *H. resinosus* and *H. hirsutus* at 4 d.a.i. At 10^5 conidia ml^{-1}, minimum percent disease spread was observed when inoculated with the pathogen grown on *H. hirsutus* followed by *H. resinosus* at 2 d.a.i. At

Table 3: Virulence of *A. helianthi* conidia produced on leaf extract media of *Helianthus* species

Species	Percent disease severity																Overall mean
	10^3 conidia ml^{-1}				10^4 conidia ml^{-1}				10^5 conidia ml^{-1}				10^6 conidia ml^{-1}				
	2 d.a.i.	4 d.a.i.	6 d.a.i.	Mean	2 d.a.i.	4 d.a.i.	6 d.a.i.	Mean	2 d.a.i.	4 d.a.i.	6 d.a.i.	Mean	2 d.a.i.	4 d.a.i.	6 d.a.i.	Mean	
H. annuus (cv. CO-4)	25.0	100.0	100.0	75.0[a]	37.5	100.0	100.0	79.2[a]	75.0	100.0	100.0	91.7[a]	100.0	100.00	100.0	100.0[a]	86.5[a]
H. occidentalis	3.0	62.5	87.5	51.0[c]	17.5	100.0	100.0	72.5[ab]	37.5	100.0	100.0	79.2[b]	50.0	100.0	100.0	83.3[c]	71.5[b]
H. maximiliani	10.0	100.0	100.0	70.0[a]	50.0	100.0	100.0	83.3[a]	75.0	100.0	100.0	91.7[a]	87.5	100.0	100.0	95.8[ab]	85.2[a]
H. grosseserratus	1.0	87.50	100.0	62.8[b]	7.5	100.0	100.0	69.2[bc]	37.5	100.0	100.0	79.2	75.0	100.0	100.0	91.7[bc]	75.7[b]
H. hirsutus	0.1	10.0	17.50	9.2[e]	2.6	17.5	50.0	23.4[d]	3.0	100.0	100.0	67.3[c]	5.0	100.0	100.0	68.3[d]	42.1[d]
H. resinosus	0	0.1	0.6	0.2[f]	7.5	87.5	100.0	65.0[c]	10.0	100.0	100.0	70.0[bc]	100.0	100.0	100.0	100.0[a]	58.0[c]
H. tuberosus	3.0	17.5	50.0	23.5[d]	17.5	100.0	100.0	72.5[ab]	75.0	100.0	100.0	91.7[a]	100.0	100.0	100.0	100.0[a]	71.9[b]
Mean	6.0	53.9	65.1	41.7	20.0	81.4	92.9	66.4	44.6	100.0	100.0	81.5	73.9	100.0	100.0	91.3	

[a] Means within a column followed by the same letter are not significantly different according to LSD at P=0.05.
[b] Percentage values are arcsine angular transformed prior to analysis.
[c] Values are means of three replications.
[d] Mean for a given concentration is the average of 2, 4 and 6 days after infection

10^6 conidia ml^{-1} minimum infection was observed in the case of *H. hirsutus* followed by *H. occidentalis* and leaf disorganization was observed in the case of CO-4, *H. resinosus* and *H. tuberosus* at 2 d.a.i. Complete browning of the leaves was observed in all cases at 4 d.a.i. at both 10^5 and 10^6 conidia ml^{-1}. Averaged over conidial concentration and d.a.i., the pathogen isolated from medium supplemented with leaf extract of *H. hirsutus* showed low virulence followed by that isolated from *H. resinosus.*

RAPD analysis

The DNA profiles of *A. helianthi* grown in different leaf extract media with the random primers tested independently showed no distinct variability (Figure 1d) and hence, no further analysis for genetic diversity has been done.

Histochemical studies

Mycelium of the pathogen stained deep red or purple and the host chemical constituents such as polyphenols, lignin and suberin were stained turquoise blue/ blue green with toluidine blue O (TBO). Microscopic examination of the transversally sectioned leaf samples, uninoculated and inoculated (sampled 2 d.a.i.) revealed various degrees of colonization of the pathogen and accumulation of phenols, lignin and suberin. In all the cases, the mycelial growth was both inter- and intra-cellular. Mycelial growth was observed in all the leaf tissues including xylem and phloem vessels of vascular bundles in highly susceptible sunflower cv. CO-4 and also revealed significant cell disorganization characterized by complete collapse of the host tissues (Figures 2a and b). A similar pattern of colonization was observed in *H. grosseserratus* whereas mycelial growth in all the tissues with disorganization of the lower epidermal cells was observed in *H. resinosus.* Intense colonization by the pathogen associated with formation of lysogenic cavities was observed in *H. maximiliani* (Figure 2e) while in *H. occidentalis* (Figure 2c), *H. hirsutus* (Figure 2d) and *H. tuberosus* (Figure 2f), colonization was restricted to the epidermal cell layers. The intense blue-green color reaction observed in *H. occidentalis, H. hirsutus* and *H. tuberosus* indicates a higher polyphenol deposition in the host tissues surrounding the colonization whereas a light blue-green color reaction indicating a low polyphenol deposition was observed in cultivated sunflower.

DISCUSSION

Significant differences were observed among the *Helianthus* species for their reaction to *Alternaria* blight of sunflower. *H. occidentalis* and *H. tuberosus* were found to be highly resistant while *H. hirsutus* was rated as moderately resistant. The results are in agreement with those of Morris *et al.* (1983), Lipps and Herr (1986) and Sujatha *et al.* (1997).

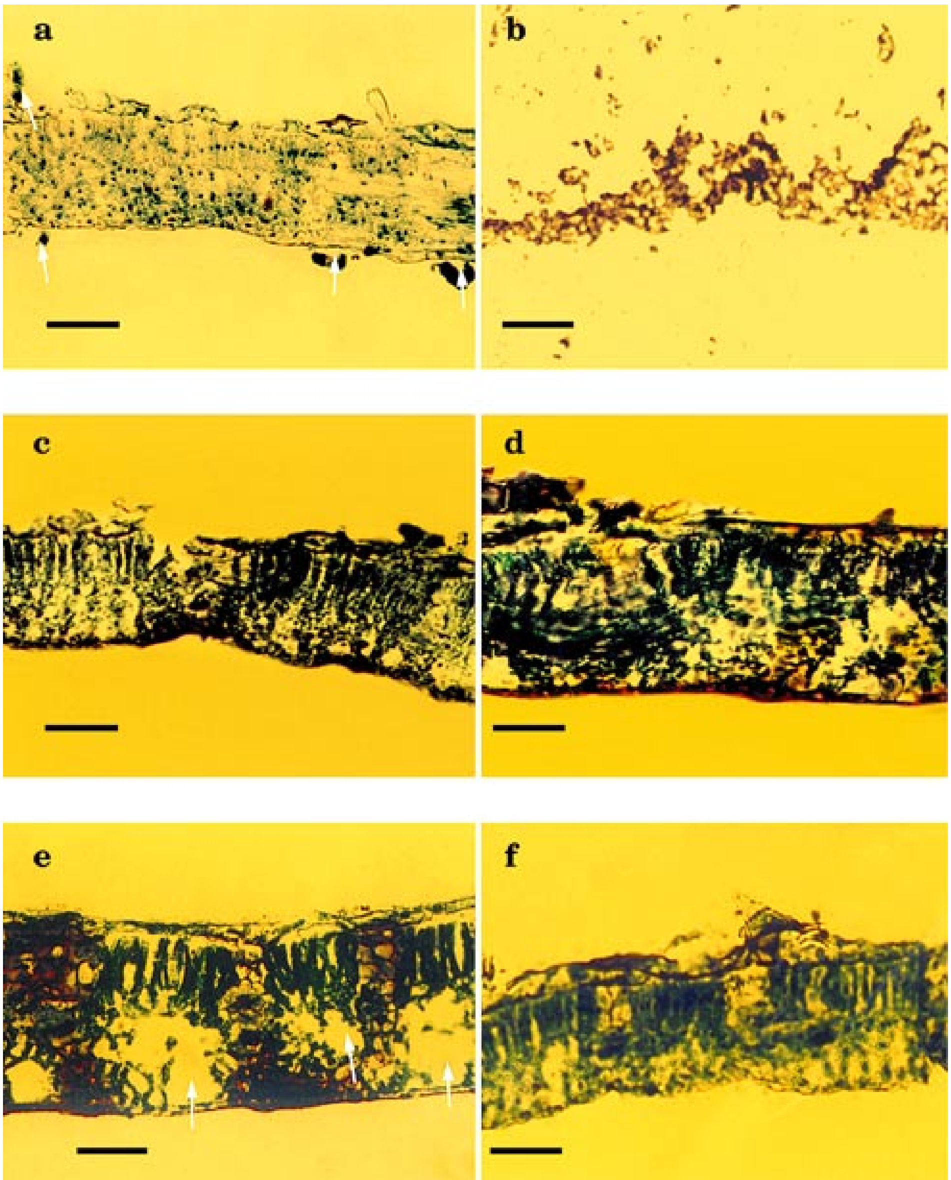

Figure 2: Histochemical variations in transverse sections of leaves of cultivated sunflower (cv. CO-4) and wild Helianthus species stained with toluidine blue at 2 d.a.i. with conidia of A. helianthi.

***a,** H. annuus (cv. CO-4) immediately after infection with A. helianthi showing conidia of the pathogen; **b,** H. annuus (cv. CO-4) showing complete disorganization of the leaf tissue; **c,** H. occidentalis; **d,** H. hirsutus; **e,** H. maximiliani showing formation of lysogenous cavities (pointed by arrow heads); **f,** H. tuberosus. (bar = 400 μm).*

Artificial media supplemented with leaf extracts of different *Helianthus* species caused differences in colony growth and conidial morphology of *A. helianthi.* Leaves of certain plant species contain antimicrobial and biochemical constituents that strongly inhibit the growth of fungal pathogens (Chattopadhyay, 1999). Interestingly, reduced colony growth and spore abnormalities in terms of shape and germination on the conidiophore itself was observed on media supplemented with leaf extracts of *H. occidentalis* and *H. tuberosus*. Such abnormalities were detected in the case of *A. helianthi* grown on media supplemented with fungicides using poison food technique (Srinivas, 1997). These observations reveal that there could be certain antagonistic/antibiotic compounds present in leaf extracts of *H. occidentalis* and *H. tuberosus*, which inhibit normal growth of *A. helianthi*. Characterization of wild *Helianthus* species native to North America revealed antimicrobial and autotoxic properties in addition to the presence of rare diterpenoids in the leaves of *H. occidentalis* which may be the reason for slow growth, spore abnormalities and low virulence (Rogers *et al.*, 1982).

A 1:1 correlation of the toxin level and virulence was reported in several crops with different pathogens (Strange, 2003). The virulence of the pathogen grown on medium supplemented with leaf extracts of *H. occidentalis, H. hirsutus* and *H. tuberosus* was low when tested on highly susceptible sunflower cv. CO-4. Under unfavorable conditions of growth and the influence of antagonistic compounds, toxin production by the pathogen could be drastically affected which in turn lowers the virulence. This might also be due to the differences in the nutritional status or absence of certain nutrients that are required for the virulence of the pathogen. In the present study, RAPD patterns of the DNA isolated from the pathogen grown on media supplemented with different leaf extracts revealed no polymorphism. The differences in virulence of the pathogen as influenced by different leaf extracts could be due to some variation at the transcriptional or translational level, which needs studies on germinability and spore characteristics in subsequent generations.

Histochemical studies revealed anatomical variations in cultivated and wild sunflowers following infection with *A. helianthi*. Complete disorganization of leaf tissues was observed in cv. CO-4 and *H. grosseserratus* while intense colonization and lysogenic cavities were observed in *H. maximiliani* and *H. resinosus.* Colonization of the pathogen was restricted to the epidermal layer in *H. occidentalis*, *H. hirsutus* and *H. tuberosus*, which is indicative of the resistant reaction to *A. helianthi*. Similar structural changes were reported by Shankar *et al.* (1998). Induction of phenols at high levels in the epidermal and mesophyll parenchymatous cells was observed in *H. occidentalis* and *H. hirsutus* following infection with *A. helianthi* and at a lower level in *H. tuberosus*. Accumulation of phenolic compounds including ortho-dihydroxy phenols in host-parasite reactions is a general phenomenon of disease resistance and the rate of accumulation and breakdown of phenolic compounds determine the degree of resistance (Tomiyama, 1963). Several workers have implicated phenols as resistance factor as they are highly reactive upon oxidation and may result in the formation of substances highly toxic to the pathogen or which inactivate enzymes including hydrolytic enzymes produced by plant pathogenic fungi (Patil and Dimond, 1967). There may be a similar stimulation of active defense reactions by the oxidation of phenols in the resistant species, while such mechanisms may be feeble in the susceptible variety.

The present study clearly indicates that wild *Helianthus* species could be exploited in breeding programs aimed at incorporation of resistance to *A. helianthi* in cultivated sunflower. However, detailed biochemical investigations need to be undertaken to distinguish the active and passive mechanisms of resistance against *A. helianthi.*

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CARACTERÍSTICAS CULTIVADORAS Y CAMBIOS HISTOLÓGICOS DE LOS TEJIDOS FOLIARES DE GIRASOL CULTIVADO Y SALVAJE INFECTADO CON EL HONGO *Alternaria helianthi*

RESUMEN

Las características cultivadoras y las variaciones histoquímicas, como consecuencia de la infección con el hongo *Alternaria helianthi*, fueron estudiadas en seis especies salvajes de *Helianthus*, en tres niveles de ploidía (diploide, tetraploide y hexaploide) que poseen diferentes grados de resistencia hacia el patógeno, y el girasol cultivado (*H. annuus* cv. CO-4, control sensible). De las especies investigadas, para *H. occidentalis* y *H. tuberosus* fue determinada alta resistencia, mientras que *H. hirsutus* tenía la resistencia mediana. Los suelos enriquecidos con los extractos foliares de las especies salvajes, con la excepción de *H. grosseserratus*, limitaban el crecimiento y la esporulación *A. helianthi* en mayor medida, en relación con los suelos enriquecidos con los extractos foliares del girasol cultivado. La reducida infectividad del patógeno, fue observada en las condiciones de cultivo en el suelo con los extractos foliares *H. occidentalis*, *H. hirsutus* y *H. tuberosus*. Además, las anormalidades en forma de conidias, fueron observadas en las condiciones de cultivo en los suelos con los extractos foliares de las especies *H. occidentalis* y *H. tuberosus*. El análisis RAPD de la DNA fungosa, aislada del patógeno cultivado en los suelos con los extractos foliares del cultivado y de las especies salvajes de *Helianthus*, no ha mostrado la presencia de polimorfismo. El estudio histoquímico demostró una distribución limitada del patógeno en las células epidérmicas, en las especies salvajes de girasol resistentes, tanto como la acumulación incrementada de fenol.

CARACTÉRISTIQUES DE LA CULTURE ET CHANGEMENTS HISTOLOGIQUES DANS LES FEUILLES DU TOURNESOL DE CULTURE ET DU TOURNESOL SAUVAGE INFECTÉS PAR l'*Alternaria helianthi*

RÉSUMÉ

Les caractéristiques de la culture et les variations chimiques histologiques dues à une infection par l'*Alternaria helianthi* ont été étudiées à trois niveaux (diploïde, tétraploïde et hexaploïde) dans six espèces d'*Helianthus* sauvages possédant différents degrés de résistance au pathogène et dans le tournesol de culture (*H. annuus* cv. CO-, contrôle sensible). Il a été établi que *H. occidentalis* et *H. tuberosus* étaient très résistants tandis que *H. hirsutus* était moyennement résistant. A l'exception de celles qui avaient été enrichies de *H. grosseserratus*, les bases enrichies d'extraits de feuille des espèces sauvages ont limité la croissance et la sporulation d'*A. helianthi* dans une plus grande mesure que les bases enrichies d'extraits de feuille de tournesol de culture. Le pathogène s'est montré moins infectieux quand il était cultivé sur une base d'extraits de feuille d'*H. occidentalis*, *H. hirsutus* et *H. tuberosus*. De plus, des anormalités en forme de conidia ont été observées ans la culture sur base enrichie d'extraits de feuille de *H. occidentalis* et *H. tuberosus*. L'analyse RAPD de l'ADN fongique isolé du pathogène cultivé sur la base d'extraits de feuille de tournesol de culture et de tournesols sauvage des espèces *Helianthus* n'a pas montré de polymorphisme. Les observations chimiques histologiques ont montré une diffusion limitée du pathogène sur les cellules épidermiques des espèces de tournesol résistantes ainsi qu'une plus grande accumulation de phénol.