

ALTERNARIA BLIGHT OF *CUMINUM CYMINUM* L.

PHYSIOLOGY OF PATHOGENESIS

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The susceptibility of the cumin plant to blight (*Alternaria burnsii*) only after flowering stage and not earlier posed an interesting problem regarding its pathogenesis. To understand this, investigations on the sugar and amino acid make-up of plant at various stages of growth and physiology of the pathogen were undertaken which revealed certain interesting facts that possibly this disease is also a "low-sugar disease".

Studies have shown that maltose and sucrose are absent in plants before flowering while they appear at flowering stage and disappear again in mature healthy plant and seed. On the other hand D-mannose and an unknown substance (Benzedene positive) are present in plants before flowering and the same are absent at flowering and diseased stages of the crop. Maltose was found to be the best carbon source followed by sucrose, both for growth and sporulation during physiological studies of the pathogen. Although the fungus could grow on D-mannose, it was found to be poor carbon source for growth and sporulation. The detection of maltose and sucrose after flowering and in the diseased stage and their ability to support good growth of pathogen *in vitro* studies suggest that they might be playing an important role in the plant metabolism to make it susceptible at flowering stage.

Regarding distribution of free amino acids, DL-serine was found present only after flowering stage and is absent before flowering and in mature plant. Phenylalanine, although is present in all stages of crop, its quantity appears to rise after flowering and declines with the progress of disease. DL-serine and phenylalanine have been observed to be good nitrogen sources for growth and sporulation during physiological studies of the pathogen. Hence, there are reasons to believe that possibly DL-serine and phenylalanine are involved in creating favourable conditions for development of disease by assisting in proliferation of the pathogen.

INTRODUCTION

A thorough knowledge of the nutrition of the pathogen has a basic significance in understanding parasitism on the host, which involves a nutritional relationship between the two. The kinds of nutrients, the pathogen utilises *in vitro*, may indicate what it takes from the host plant. Further, the resistance and susceptibility of the host is considerably determined by what the host has to offer by way of nutrients, because for successful pathogenesis the parasite must obtain at site of its localization on the host the kinds and amount of various nutrients for its proliferation and fruiting. In this case the host may be considered as growth medium for the parasite (Garber 1956). This ecological concept to explain biochemical nature of parasitism has been

proposed by various investigators as 'Balance hypothesis of parasitism' (Lewis 1953), 'Nutrition inhibition hypothesis' (Garber 1956, 1960) and 'Nutrition hypothesis' (Dewey *et al.* 1950).

The susceptibility of the cumin plant (*Cuminum cyminum* L.) to *Alternaria* blight (*A. burnsii* Uppal, Patel and Kamat) only after flowering stage and not before, posed an important question regarding the physiology of pathogenesis at flowering stage. To understand this, investigations on the physiology of the pathogen *in vitro* and on the sugar and amino acid make-up of the host plant at various stages of crop growth and in diseased conditions so also the nitrogen, protein and ash content of healthy and diseased plant were undertaken which revealed certain interesting facts and correlations about the physiology of the pathogenesis of the host plant and the same are presented here.

MATERIALS AND METHODS

The response of different sugars and their effect on growth and sporulation was studied in Richards' medium wherein sucrose was replaced by various carbon compounds to supply 11.4 g of carbon/litre. The solutions were distributed in the volumes of 20 ml in 100 ml flasks, autoclaved and single germinating conidium inoculated and incubated at room temperature (26°-28°C) for 12 days. The mycelial mat was harvested, weighed and sporulation studied.

The above method was followed to study the response of nitrogen sources and in this case potassium nitrate was substituted with different amino acids so as to supply 1.4 g of nitrogen/litre. Four replicates were kept for each case and a fifth flask was utilised for recording the final pH of the medium. The total nitrogen, proteins and ash were estimated by standard chemical methods

To estimate amino acids and sugars by paper chromatographic methods, 10 g each of healthy and diseased seeds, healthy and diseased stems with inflorescence were separately macerated in a waring blender with 70 per cent alcohol. The extract so obtained was decanted and filtered through Whatman filter paper No. 1 and collected in small vials. A drop of toluene was added to protect the same from the attack of moulds, etc.

0.1 per cent solution of amino acids and sugars in 70 per cent methyl alcohol and water, respectively was also prepared for comparison of Rf values with the unknown samples of healthy and diseased tissues. Equal amounts of plant extracts were spotted on Whatman filter paper No. 1 (chromatographic) and air-dried. Known amino acids and sugars were also spotted in a similar way. The chromatograms were then run in a mixture of butanol-acetic acid-water (4:1:5). After 16 hr the chromatograms were air-dried for 2 hr. For identification of amino acids, the chromatograms were sprayed with the indicator ninhydrin (0.3 per cent in absolute alcohol), air-dried and kept in incubator at 60°C for 10 min. Different spots of various amino acids were detected and their Rf values calculated.

For sugars, the chromatograms were sprayed with a mixture of benzedene (0.5 g) + glacial acetic acid (10 ml) + absolute alcohol (80 ml); air dried and put in oven at 100°C for 5 min. Various sugars were then detected and their Rf values calculated.

RESULTS

It will be observed from Table I that maximum growth and sporulation was observed when maltose was used as a carbon source. Good growth was also observed when glucose, fructose and sucrose were used as carbon source. Although the fungus could grow on D-mannose and D-galactose, they were found to be poor sources for growth and sporulation. No growth was observed when carbon was absent in the medium. The results suggest that presence of maltose, glucose and sucrose in the host metabolism may induce susceptibility while presence of D-mannose and D-galactose may be able to provide some tolerance to the plant against the disease.

TABLE I
Dry weight of mycelial mat in mg and sporulation of Cumin Alternaria in different carbon sources

Carbon source	Dry wt of mycelial mat	Sporulation	Initial pH	Final pH
D-mannose	40.0	++	7.0	7.9
D-glucose	56.5	+++	7.0	8.7
Sucrose	53.2	+++	7.0	8.1
Maltose	65.5	++++	7.0	8.8
D-galactose	40.0	++	7.0	8.2
Fructose	53.5	+++	7.0	8.5
Richards' medium without carbon (Control)	No growth			

— No sporulation, + Poor sporulation, ++ Fair sporulation

+++ Good sporulation, ++++ Very good sporulation

It will appear from Table II that phenylalanine, aspartic acid and DL-serine are good nitrogen sources both for growth and sporulation. The fungus grows well on potassium nitrate, asparagine and histidine; while β -alanine and cystine are poor sources. Methionine and tryptophane have shown inhibitory effect on sporulation. The results suggest that presence of phenylalanine, DL-serine and aspartic acid in the host metabolism may be able to help in infection process while β -alanine, cystine, methionine and tryptophane may provide some tolerance to the plants by creating unfavourable circumstances for the pathogen to proliferate.

It will appear from Table III that nitrogen and protein content of cumin plant tends to increase with the increase in disease intensity. The ash content on the other hand reduces with the onset of infection but again rises with the increase in disease intensity. Besides this, the oil content of cumin seed is greatly influenced by the disease.

As far as the sugar make-up of the cumin plant at various stages of its growth is concerned, it can be seen from Table IV that maltose is absent in plants before

TABLE II

Dry mycelial weight (in mg), sporulation and final pH of Cumin Alternaria grown on Richards' medium supplemented by amino acids

Source of nitrogen	Initial pH	Final pH	Dry wt of mycelium	Sporulation
KNO ₃	7.3	7.8	313	+++
Asparagine	7.4	7.7	374	++
Phenylalanine	7.4	7.0	455	++++
Methionine	7.4	4.9	219	+
Aspartic acid	6.5	7.8	449	++++
Tryptophane	7.3	4.8	290	—
Histidine	6.3	6.3	365	++
DL-serine	7.3	7.4	421	++++
β-alanine	7.3	5.7	190	+++
L-cystine	7.2	5.5	245	++
Control (No nitrogen)	7.0	7.1	16	—

TABLE III

Effect of Alternaria blight on total nitrogen, proteins and ash content of cumin plant (in percentage) (About 10 days before harvest)

Degree of infection	Protein	Total N	Ash	Oil
Average crop	3.5	0.56	6.22	8.02
Low infection	11.0	1.76	4.01	4.81
Medium infection	11.93	1.91	7.13	5.12
Medium+infection	13.68	2.19	9.78	5.04
Severe infection	14.00	2.24		5.48

flowering stage, while it appears after flowering and continues to be present during diseased conditions and again disappears in healthy seed and healthy plant and attaining maturity. So also, sucrose is present in plants in flowering stage, while the same is absent before flowering stage. On the other hand, D-mannose is present in plants before the flowering stage and in the healthy seed; and is absent at flowering and in diseased stages of crop. Raffinose is present only in plants suffering from powdery mildew. An unknown substance (Benzedene positive of the Rf value of 0.130) has been found to be present in plants only before the flowering stage, and fully matured plants and healthy seed, while the same is absent in stages after flowering and diseased state. The results suggest that the presence of D-mannose and an unknown substance in plants before the flowering stage and in the healthy seed and their absence in plants after the flowering and in diseased state, provide conditions

TABLE IV
Distribution of sugars in healthy and diseased (*Alternaria blight*) plants of *cumin*

Stage of Crop	Glu- cose	Mal- tose	Suc- rose	D- Man- nose	D- Galac- tose	Raffi- nose	Unknown Substance A B	Fruc- tose	Lac- tose	D- Arabi- nose	L- Arabi- nose	D- Xy- lose	L- Rham- nose	D- Ri- bose	L- Sor- bose	D- Melli- birose	Glyco- gen
Just flowering (Healthy)	+	+	+	+	-												Absent
After flowering "	-	+	+	+	+												"
Before flowering "	-	-	-	+	T	+											"
Severe diseased seed	+	+	T														"
Diseased plant blight	-	+	+	-			+										"
Healthy plant	-	-	-	+	+		+										"
Healthy seed	+	-	-	+	+												"
Diseased seed																	"
P.M. + Blight	+	+	+		+												"

A = 0.130 Rf, B = 0.029 Rf, B.A.W. (4:1:5) Benzene positive substance

TABLE V
Distribution of free amino acids in healthy and diseased (*Alternaria blight*) plants of *cumin*

Stage of Crop	Methio- nine	DL- Trypto- phane	DL- Ala- nine	DL- Serine	Phenyl- alanine	L- Gluta- mine	L- Aspara- gine	L- Histi- dine	L- Leu- cine	Hydroxy- proline	Argi- nine	L- Lysine	L- Glycine	Aspartic acid	Unknown substance
Just flowering (Healthy)	+	+	+	+	+		+	T	+	+	+	+	+		
After flowering "	+	+	T	+	+	=		-	+	T	+	+	T		
Before flowering "	+	+	+	-	+	+	+	+	+	+	+	+	+	+	
Severe diseased seed	+	+	+	+	+	T	+	-	+	T		+	+		
Diseased plant blight	+	+	+	+	+	+	+	-	+	+	+	+	+		
Healthy plant	+	+	+	-	+	+	+	-	+	+	+	+	+		
Healthy seed	+	T	+	-	+	+	T	+	+	+	+	+	T		0.378*
Powdery mildew and blight			+	+	+	+	-	-	+	+	+	+	+		

= B.A.W. (4:1:5) Ninhydrin positive

+ Small spot, ++ Medium spot, +++ Minute spot

not conducive for the development of the disease. These substances may play an important role in building up tolerance against the disease in the plant metabolism. On the other hand, the detection of maltose and sucrose after flowering and in diseased states and their absence before flowering and in healthy plant signified that they might be playing an important role in plant metabolism to make it susceptible to the disease at the flowering stage by providing favourable circumstances for the development and proliferation of the pathogen.

Further, regarding the distribution of free amino acids in various stages of crop growth of cumin plant it can be observed from Table V that DL-serine is present only after flowering stage and continues to be present in diseased conditions while the same is absent before flowering and in healthy plants and seed. This suggests that its presence might be playing an important role in creating environments favourable for the disease. Phenylalanine, on the other hand, is present in all stages of the crop including diseased state. But its quantity appears to rise after flowering and again declines with the onset of the disease which suggests that the amino acid is possibly involved for the attack by the pathogen and assists in its proliferation by providing favourable conditions for growth. Methionine is present only in healthy plants in flowering stage and in healthy seed but is absent before flowering and in diseased state. In the same way, hydroxyproline and arginine are absent before flowering while they are present in flowering stages. Tryptophane is present in all the stages of crop including diseased state, but its quantity appears to rise at flowering stages and decline in diseased and maturity states. The absence of hydroxyproline and arginine before flowering and their presence soon afterwards suggests that these may be helping the infection process by way of providing nutrition to the pathogen. Their absence further suggests that these might have been utilised by the pathogen during its metabolism.

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