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Production of phenolics by *Rhizoctonia bataticola* (taub.) Butler during pathogenesis

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Abstract

***Rhizoctonia bataticola* is a facultative parasite, which causes damping of seedlings and root rot in vegetables, cereals, fruits, oilseed crops and ornamental plants. The present paper deals with the *in vitro* studies of the production of phenolics by this parasitic fungus.**

Keywords: *Facultative parasite, Fungi, Host-parasite relationship, Plant pathology*

Introduction

Phenolic compounds are characterized with an aromatic ring bearing one or more hydroxyl groups in their chemical constitution. Aromatic compounds are widespread in microorganisms as well as in plants whereas lignins, flavonoids and phenolic glycosides are generally restricted to specific families and species. The initial steps of biogenesis of aromatic compounds are same in fungi as in higher plants. In course of reaction sequences, aromatic amino acids are produced from carbohydrates. These amino acids in turn serve as precursors for the synthesis of phenols.

According to Farkas and Kiraly (1962), the accumulation of aromatic compounds in diseased plants is an extremely widespread phenomenon. The compounds, which accumulate in infected plants include mono and dihydric phenols, phenolic glycosides, flavonoids, anthocyanins, aromatic amino acids and coumarin derivatives. On comparing the resistant and susceptible combinations, it is found that a more rapid accumulation of phenolics takes place in the incompatible host-pathogen complex than compatible ones. However, a comparison of susceptible and resistant infected varieties has not always revealed a positive correlation between phenol content and resistance. Reason for these variations has been partly attributed to variations in models of this enquiry. In many infections, there is increase in phenol oxidizing enzymes accompanying enhanced phenol biosynthesis in diseased tissue (Fuchs and Kotte, 1954). Farkes and Ledingham (1959) and Oku (1960) have reported synthesis of polyphenoloxidase and peroxidase in infected tissue by *Cochliobolus miyabeanus* and *Puccinia gramins tritici.*

Certain plant pathogens are also known to produce phenolic compounds in culture (Reddy and Rao, 1975; Suresh, 1982) and in such cases tissue substances may be produced in host tissue and directly responsible for development of necrotic lesions (Cruickshank and Perrin, 1964). After infection, various types of phenols are observed to accumulate around the site of infection *viz.* simple phenol, hydroxyl aromatic compounds of mono- and polyphenolic types and their derivatives. Higher accumulation of phenols and its altered metabolism after infection in underground and subaerial parts of resistant combination of cotton wilt was recorded by Rubin and Ivanova (1960) and Babajan *et al.* (1955). Phenolic compounds get readily oxidized and may act as donor or acceptor in metabolism of diseased tissue (Manaskaya, 1948). Thus each host pathogen combination is unique in relationship between phenolic levels and disease development. Hence in

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Sapru and Mahajan

order to work out the role of phenolic compounds in root rot and leaf blight of mung bean, attempts were made to detect phenolics in culture and mung bean cultivar K 851.

Materials and Method

Phenolics produced by *R.bataticola root* (R1) and leaf (L1) isolates in *vitro* were analysed both qualitatively as well as quantitatively. These isolates of mung bean cultivar K 851 were employed throughout the study. Culture media for phenolic production was Czapeck solution (having 3% sucrose) for 15 and 25 days. Phenolics were extracted from the culture filtrate following the procedures described by Das and Rao (1964) and Reddy and Rao (1975) and extraction of phenolics from culture filtrate and mycelium was done with the help of Whattman No.1 filter paper. For quantitative analysis of phenolics, the method described by Harborne (1973) was followed for the separation and identification of phenolic compounds. Both paper chromatographic and Thin Layer Chromatographic methods were used to separate simple phenols, phenyl propanoids (hydroxyl coumarins, uranocoumarins, and phenyl propenes) and flavonoids. In quantitative analysis of phenolics, the phenolic extracts of culture filtrate and mycelium were analyzed for total phenols (Bray and Thorpe, 1954), Ortho-dihydric phenols (Arnow,1937) and flavonols (Swain and Hillis, 1959).

Results and Discussion

The results of this study are given in Table 1-9. From Tables 1 to 9, it is indicated that six simple phenol compounds were detected in *R. bataticola* R1 and L1 isolates *in vitro.* Out of which 4 were common in culture filtrate of both the isolates. Of the remaining two, one compound was found in the filtrate of R1 and the other in L1 isolate. Healthy tissue extracts of 1 and 3 day old K851 seedling differentiated into 5 and 6 compounds respectively. Tissue infected with R1 isolate accumulated 3 compounds in young seedlings. The number rose to 6 in 3 days old seedlings. The infected tissue contained 2 compounds (R_f 0.19 and 0.39) which were of fungal origin. Similarly in lesions produced by L1 isolate, one compound of pathogen origin was recorded. In these pathogen-suscept combinations, no phenol compound accumulated which could be due to pathogen-suscept interaction. In K 851 mung bean number of accumulated compounds increased both in healthy as well as in old seedlings although these were not associated with increased resistance. Reddy and Rao (1979) observed the presence of many phenolics including chlorogenic,

Table 1: Simple phenols in 25 day old culture of *R.bataticola* isolates

S.No.	R_f*	Color in			Root isolate (R1)		Leaf isolate (L1)	
		Follin ciocaltue	Follin $+NH_3$	Vanilline + HCl	Filtrate	Mycelium	Filtrate	Mycelium
1	0.05	Blue			+	+	-	+
2	0.19	Blue			+	-	+	-
3	0.25			Brick red	-	+	+	+
4	0.39	Blue			+	+	+	-
5	0.78			Dark pink	+	-	+	-
6	0.79		Blue		+	+	+	-

*** Acetic acid: chloroform (1:9) and ethylacetate: benzene (9:11)**

protocatechuic acid, caffic acid, ferrulic acid and 16 undifferentiated compounds in infected groundnut hypocotyls. Some of these compounds were recorded only from the healthy tissue. This observation favored the inference of the present study that less number of phenols (qualitatively) was present in healthy tissue. Accumulation of phenolics, both quantitatively and qualitatively have been reported in tomato wilt (Pierson *et al.*, 1955), in bean seedling infected by *Colletotrichum lindi muthianum* (Romanowski *et al.,* 1962), *Rhizoctonia* disease of bean (Pierre and Bateman,

1967) and potato infected by *R. solani* (Mall and Suresh,1989). A few records however, showed that total phenols decreased in lesion tissue (Arora and Bajaj, 1978).

Table 2: Phenylpropanoids in 25 day old culture of *R.bataticola* isolates

S. No.	R_f	Hydroxycinnamic* Colour in		Hydroxycoumarin* Colour in		Furanocoumarin>		R1		L1	
		UV	UV+NH_3	UV	UV+NH_3	UV	UV+10% In Methanol	Filtrate	Mycelium	Filtrate	Mycelium
1	0.15				Dark Blue			+	-	+	-
2	0.16					Blue	Intensified	+	+	+	-
3	0.24					Blue	Intensified	-	-	-	+
4	0.28					Blue	Intensified	+	-	+	+
5	0.32	Pink						-	-	+	-
6	0.53			Blue				+	-	+	-
7	0.88		Yellow					+	-	+	+

*** n-butanol: acetic acid :water, 4:1:5 (Top Layer) > Chloroform**

Table 3: Phenolics* in 15 and 25 day old cultures of *R.bataticola* isolates

Isolate No.	Source	15 days			25 days		
		Total Phenol	Ortho-Dihydric phenol	Flavanol	Total Phenol	Ortho-Dihydric phenol	Flavanol
R1	Filtrate	0.08	Nil	Nil	1.32	0.08	0.04
	Mycelium	Nil	Nil	Nil	0.71	0.49	0.04
L1	Filtrate	0.12	0.08	Nil	3.64	1.62	0.09
	Mycelium	Nil	Nil	Nil	1.19	1.29	0.05

***mg/ml of culture filtrate. g/g of mycelial mat.**

Table 4. R_f and colour of simple phenols in healthy seedling tissues of mung bean cultivar K 851 and tissue infected with *R.bataticola* isolates.

S. No.	R_f*	Colour in			Healthy 1 day	Lesion		Healthy 3 day	Lesion	
		Folin Cio-caltue	Folin+ NH_3	Vanilline + HCl		R1	L1		R1	L1
1	0.19	Blue			-	+	-	-	+	-
2	0.39	Blue			-	-	-	-	+	-
3	0.51	Blue			+	-	+	+	-	+
4	0.64	Blue			+	+	-	+	+	-
5	0.67		Blue		+	-	+	+	-	+
6	0.79		Blue		-	-	-	+	+	+
7	0.82	Blue			+	-	-	+	-	-
8	0.83			Pink	-	-	+	+	-	+
9	0.86		Blue		-	+	+	+	+	+
10	0.93		Blue		+	-	-	+	+	+

***Acetic acid : Chloroform (1:9) and ethylacetate benzene (9:11)**

Table 5: R_f and colour of hydroxycinnamic acids in healthy seedling tissues of mung bean cultivars K 851 and tissues infected with *R.bataticola* isolates.

S. No.	R_f*	Colour in UV / UV+NH_3	K 851					
			Healthy		Lesion			
			1 day	3 day	1 day		3 day	
					R1	L1	R1	L1
1	0.28	Dark pink	+	+	-	-	-	-
2	0.44	Mauve	-	-	-	-	-	-
3	0.44	Dark absorbance	-	-	-	-	-	-
4	0.53	Blue	-	-	-	-	-	-
5	0.61	Blue	-	-	-	-	-	-
6	0.88	Mauve	+	+	-	-	-	-
7	0.88	Yellow	+	+	-	+	-	+
8	0.95	Blue	-	-	+	-	+	-
9	0.97	Mauve	-	-	-	-	-	-

*** n-butanol acetic acid water, 4:1:5 (Top Layer)**

Table 6 : R_f and colour of hydroxycoumarins in healthy seedling tissues of mung bean cultivars K851 and tissue infected with *R.bataticola* isolates

S. No.	R_f*	Colour in UV / UV+5%NaOH	K 851					
			Healthy		Lesion			
			1 day	3 day	1 day		3 day	
					R1	L1	R1	L1
1	0.26	Dark pink	+	+	-	+	-	+
2	0.57	Yellow	-	-	-	-	-	+
3	0.61	Blue	+	+	+	-	+	-
4	0.64	Blue	-	-	-	-	-	-
5	0.94	Yellow	+	+	+	-	+	-
6	0.94	Mauve Mauve	-	-	-	-	-	-

*** n-butanol acetic acid water, 4:1:5 (Top layer)**

Table 7: R_f and colour of furocoumarins in healthy seedling tissues of mung bean cultivars K851 and tissues infected with *R.bataticola* isolates

S. No.	R_f*	Colour in		K 581					
		UV	UV+10%KOH in methanol	Healthy Lesion	Lesion			Healthy	
				1 day	R1	L1	3 Day	R1	L1
1	0.11	Blue	Intensified	-	-	-	-	-	-
2	0.21	Blue	Intensified	-	-	-	-	-	-
3	0.51	Blue	Intensified	-	-	-	-	-	-

*** Chloroform**

Table 8: R_f and colour of flavonoid in healthy seedling tissues of mung bean cultivar K 851 and tissue infected with *R.bataticola* isolates

S. No	R_f		Colour in		K 851			
					Healthy		Lesion	
	BAW*	5% Acetic acid	UV	UV+NH_3	1 day	3 day	R1	L1
1	0.32	0.89	Mauve		-	-	+	-
2	0.38	0.80	Mauve		-	-	-	-
3	0.44	0.52	Blue		-	-	-	-
4	0.90	0.43	Light blue	P.yellow	-	-	+	-
5	0.91	0.56	Mauve		-	-	-	-
6	0.92	0.57	Blue		-	-	+	-
7	0.92	0.58	Mauve	Pink	-	-	-	-
8	0.94	0.85	Light blue	Yellow	-	-	+	-
9	0.95	0.52	Light blue	P.yellow	+	+	-	-
10	0.96	0.36	Blue	Mauve	+	+	-	-
11	0.98	0.52	Blue	Dull yellow	+	+	+	+
12	0.99	0.62	Mauve		+	+	-	+

***n butanol acetic acid water, 4:1:5 5% Acetic acid**

Table 9: Estimation of phenolics* in healthy seedling tissues of mung bean K 851 and tissues infected with *R. bataticola* isolates

Isolate No.	Age of seedling	Healthy tissue			Lesion tissue			% change		
		Total Phenol	Ortho - Dihydric phenol	Flavanol	Total Phenol	Ortho - Dihydric phenol	Flavanol	Total Phenol	Ortho - Dihydric phenol	Flavanol
R1	1 day	1.8	1.9	0.02	3.2	2.4	0.02	+77.77	+26.31	00
	3 day	1.8	2.0	0.01	3.2	2.6	0.02	+77.77	+30.00	+100
L1	1 day	1.8	1.9	0.02	2.6	1.7	0.02	+ 44.44	-10.50	00
	3 day	1.8	2.0	0.01	2.5	2.2	0.02	+38.88	+10.00	+100

*** mg/g of fresh weight**

More number of flavanols has also been reported in infected mung bean, compared to healthy hypocotyle (Arora and Bajaj, 1978). Concentration was more in susceptible gram against *R.bataticola* (Singh *et al.,* 1982) and in bacterial leaf spot (Jalali *et al.,* 1976). The above result states that pathogenic interaction between mung bean cultivar and *R. bataticola* R1 and L1 caused enhanced biosynthesis of Total phenols, Ortho di - hydric phenols and Flavanols in infected tissue. This hints the additional aromatization of host plant (Kiraly and Farkas, 1962; Cruickshank and Perrin, 1964; Kuc, 1966). Thus all phenols play an important role during infection and disease development. It was observed that phenolics were accumulated during infection in younger seedlings of the K cultivar K851. However, this accumulation was not sufficient so as to resist the infection.

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