BIOCHEMICAL CHANGES INDUCED BY ANTHRACNOSE DISEASE IN BETELVINE

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Healthy and infected leaves from ten varieties of Piper betle were taken as the experimental material. The diseased spots, *i.e.* Central black nectronic zone, senescent yellow region and surroundings faded green region and the healthy leaves were analysed biochemically for chlorophyll, carbohydrate, protein, amino acids, phenols and vitamin contents. Comparison was made among contents of infected and healthy leaves in these ten varieties. (Key words: necrotic, senescent, chlorophyll)

Introduction

Betelvine (Piper betle L.) is attacked by an number of destructive diseases of which anthracnose, bacterial leaf spot complex <u>Colletotrichum capsici</u> (Syd.) Butler & Bisby + <u>Xanthomonas compestries</u> **P.V.betlicola** (Patel, Kulkarni; Dhande) Dye has been the most serious menace in betelvine growing tracts of the country.

Host pathogen interaction is a struggle for survival of both, which manifests in the development of visual symptoms. In order to study the chemical changes that occur following infection by anatghracnose complex, the present studies were taken up.

Waterials and methods

Fresh samples from infected leaves with conspicuous spots were taken and fractionize into (1) Nectrotic zone, (2) Yellow zone surrounding the necrosed areas, and (3) Faded greenish area around by Yellow halo. Apparently healthy fresh leaves were taken for analysis and comparison. The samples were biochemically analysed for content of chlorophyll 'a' and chlorophyll 'b' and total chlorophyll (Arnon, 1949), total sugar and starch (Yoshida et al., 1972), reducing sugar (Nelson, 1944), total protein (Jakson, 1958), amino acids (Lee and Takahasi, 1966) and phenols (Bray and Thorpe, 1954), Vitamin 'C' was estimated following the method of Roe and Kuether (Mapson, 1961) and Vitamin 'A' according to Kimble, 1939. The data are presented in Tables – 1 to 4.

Results and discussion:

The biochemical analysis of diseased betel leaves showed the reduction in chlorophyll pigments, which is a characteristic feature of diseased plant tissues and chlorosis is one of the most common symptoms of fungal infection (Mathews, 1981). The present study has shown that the disease development drastically reduced chlorophyll content in black lesions. This effect was also seen in green and yellow regions. Reduction in chlorophyll content was reflected both in chlorophyll 'a' and chlorophyll 'b' fractions. While looking to total chlorophyll content of the healthy tissues, it was minimum with the variety Kanjale and maximum with Maghi.

Host plant is a living culture medium for the pathogen and sugar provides the major source of energy for the parasite. In the present study, disease development reduced the concentration of soluble carbohydrates both reducing and total sugars in nectrotic zone as well as in the yellow region accompanied by a marked accumulation of starch in the healthy tissues and the effect of disease development in green tissues were pronounced only to a less extent. Chaurasia *et al.* (1987) studied the biochemical changes in betelvine leaves affected by bacterial disease and reported the maximum amount of total sugar as well as reducing and non-reducing sugars in the susceptible varieties in comparison to resistant varieties.

TABLE No. 1: Estimation of chlorophyll – 'A' and chlorophyll – 'B' and the total chlorophyll (Mg/Gm. fresh weight) from the leaves of betelvine varieties under disease stress.

	Н	lealthy l	eaf				In	fected l	eaf				
Variety					1*			2**			3***		
	CHL 'A'	CHL 'B'	TOTAL CHL										
Awani	0.421	0.532	0.953	0.081	0.082	0.163	0.136	0.135	0.271	0.341	0.272	0.613	
(Assam)													
Maghi	0.425	0.262	0.687	0.044	0.028	0.072	0.104	0.101	0.205	0.213	0.212	0.425	
Ghanghate (Bangla)	0.258	0.224	0.472	0.049	0.029	0.078	0.038	0.084	0.122	0.125	0.137	0.252	
Mahoba (Bangla)	0.375	0.523	0.898	0.052	0.041	0.093	0.080	0.029	0.109	0.380	0.218	0.598	
Red stem (Bilhari)	0.321	0.201	0.522	0.083	0.082	0.165	0.072	0.021	0.093	0.132	0.231	0.363	
Berhampuri (Sanchi)	0.437	0.682	1.119	0.062	0.088	0.150	0.213	0.128	0.331	0.424	0.392	0.816	
Halisahar (Sanchi)	0.335	0.312	0.647	0.042	0.066	0.108	0.028	0.071	0.099	0.241	0.180	0.421	

Kapuri	0.232	0.147	0.379	0.034	0.045	0.079	0.038	0.084	0.122	0.115	0.135	0.250
Ramtek (Bangla)	0.259	0.271	0.530	0.044	0.045	0.089	0.035	0.045	0.080	0.189	0.188	0.377
Dhob Mahata	0.225	0.262	0.487	0.036	0.034	0.070	0.061	0.025	0.086	0.183	0.168	0.351

1* : Necrotic central black region

2** : Senscent yellow region

3*** : Faded green region.

 $TABLE\ No.\ 2: Estimation\ of\ starch,\ total\ sugar\ and\ reducing\ sugar\ (Gm/\ 100Gm\ fresh\ weight)\ from\ the\ samples\ of\ betelvine\ varieties\ under\ disease\ stress.$

				Tufacted loaf										
	H	lealthy l	leaf	Infected leaf										
Variety					1*			2**		3***				
	Starch	Total Sugar	Redu c sugar	Starch	Total Sugar	Redu c sugar	Starch	Total Sugar	Redu c sugar	Starch	Total Sugar	Redu c sugar		
Awani (Assam)	1.502	1.5	0.9	0.250	0.760	1.200	0.730	0.840	0.885	1.140	1.210	0.890		
Maghi	1.475	2.35	1.225	0.486	1.160	2.008	0.750	1.050	1.130	1.110	2.360	1.190		
Ghanghate (Bangla)	1.684	1.05	0.7	0.680	0.580	1.206	0.817	0.560	0.680	1.180	1.040	0.720		
Mahoba (Bangla)	1.093	1.8	1.125	0.880	0.898	2.228	0.570	0.890	1.010	1.020	1.960	1.090		
Red stem (Bilhari)	2.012	2.4	1.2	1.040	1.020	2.060	1.040	1.210	1.050	2.040	2.220	1.089		
Berhampuri (Sanchi)	1.336	0.65	0.425	0.520	0.310	0.780	0.680	0.330	0.288	1.120	0.510	0.428		
Halisahar (Sanchi)	1.275	0.7	0.46	0.680	0.389	0.986	0.656	0.360	0.388	1.160	0.648	0.350		
Kapuri	1.366	2.6	1.7	0.588	1.236	2.430	0.600	1.250	1.340	1.226	2.300	1.510		
Ramtek (Bangla)	1.548	1.05	0.9	0.798	0.560	1.880	0.750	0.500	0.821	1.250	1.000	0.970		
Dhob Mahata	1.457	0.47	0.535	0.698	0.250	1.010	1.656	0.280	0.407	1.202	0.390	0.490		

1* : Necrotic central black region

2** : Senscent yellow region

3*** : Faded green region.

TABLE No. 3: Estimation of total protein and amino acid percentage in the leaves of betelvine varieties disease stress.

		Prote	eins		Amino-acids					
Variety			Infected				Infected			
	Healthy	1*	2** 3***		Healthy	1*	2**	3***		
Awani (Assam)	0.9125	1.962	0.456	0.856	0.254	0.088	0.125	0.234		
Maghi	0.85	1.769	0.378	0.784	0.288	0.058	0.140	0.185		
Ghanghate (Bangla)	0.8	1.642	0.392	0.766	0.344	0.086	0.168	0.263		
Mahoba (Bangla)	0.975	1.824	0.5	0.864	0.248	0.042	1.121	0.207		
Red stem (Bilhari)	1.15	2.322	0.622	0.988	0.300	0.072	0.153	0.283		
Berhampuri (Sanchi)	1.025	2.186	0.584	0.978	0.272	0.965	0.140	0.320		
Halisahar (Sanchi)	0.9	1.902	0.428	0.852	0.337	0.062	0.159	0.258		
Kapuri	1.075	2.082	0.592	0.978	0.288	0.052	0.145	0.148		
Ramtek (Bangla)	1.000	1.975	1.568	0.964	0.284	0.054	0.146	0.268		
Dhob Mahata	0.95	1.862	1.48	0.88	0.263	0.046	0.160	0.338		

1* : Necrotic central black region

2** : Senscent yellow region

3*** : Faded green region.

 $\begin{tabular}{ll} TABLE~No.3: Estimation~of~total~phenols, vitamin-A~and~vitamin-C~of~betelvine\\ &varieties~under~disease~stress. \end{tabular}$

Variety	Phe	nol (mg/1 wei		resh	Vita	tamin-A mg/100gm Fresh weight Vitamin-C mg/100 Gm F weight						Fresh
			Infected				Infected			Infected		
	Heal- thy	1*	2**	3***	Heal- thy	1*	2**	3***	Heal- thy	1*	2**	3***
Awani (Assam)	0.37	0.271	0.392	0.410	0.255	0.109	0.154	0.262	96	32.20	88.2	95.8
Maghi	0.48	0.285	0.500	0.460	0.338	0.183	0.162	0.345	112	36.75	95.7	111.2
Ghanghate (Bangla)	0.51	0.282	0.623	0.500	0.265	0.129	0.141	0.273	194.0	79.9	167.0	178.6
Mahoba (Bangla)	0.7	0.320	0.804	0.691	0.255	0.112	0.132	0.254	197.0	62.03	128.7	188.8
Red stem (Bilhari)	0.55	0.310	0.601	0.542	0.245	0.119	0.124	0.249	195.0	60.13	130.0	155.4
Berhampuri (Sanchi)	0.410	0.289	0.424	0.516	0.332	0.142	0.174	0.360	126.0	46.08	118.0	119.9
Halisahar (Sanchi)	0.37	0.274	0.469	0.414	0.341	0.135	0.164	0.378	216.5	94.02	108.0	212.6
Kapuri	0.660	0.326	0.580	0.640	0.323	0.142	0.123	0.346	218.0	86.72	126.3	212.9
Ramtek												

(Bang	la)	0.660	0.348	0.721	0.654	0.344	0.168	0.158	0.348	197.1	64.25	109.3	165.6
Dhob Mahat	a	0.63	0.394	0.998	0.680	0.235	0.108	0.121	0.244	175.0	58.33	126.3	172.9

1* : Necrotic central black region

2** : Senscent yellow region

3*** : Faded green region.

Any change in the enzymatic level of the host is directly reflected on the protein metabolism. This study revealed higher accumulation of protein in the necrotic lesions. On the other hand, its concentration decreased in yellow region and ten tended to normalize in green tissues. Accumulation or decrease of amino acids depend upon pathogens and it is related to the protein content and proteolytic activity on the tissues. Amino acids generally decreased in the necrotic lesions and yellow region of the infected tissues. The reduction in concentration of amino acids, particularly in the necrotic lesions corresponds with an increase in protein content. It is to note that the disease induced reduction in the amino acids strictly greater in varieties like Kapuri (Maharastra), Karpuri (T.N) Kariele, Ramtek banglad, Kanjale & Bihari. The denotes that these varieties might have suffered due to this disease.

In the present investigation, lowest level of phenols was observed in the diseased tissues but there was enhanced production of phenols in the adjoining yellowish zone and faded greenish zone over the healthy leaves.

Chaurasia reported the higher amount of phenols in resistant varieties (65 mg/g) than susceptible varieties (50 to 54 mg./g) on dry weight basis in diseased betelvine leaves. Maximum phenol accumulation was observed in Kariele, Mahoba Bangla and Bilhari with Karpuri (T.N) contributing the maximum resistance to the disease probably due to more of ascorbic oxidase as suggested by Mahadevan and Sridhar (1982) working on rice resistancxe to blast.

Betelvine varieties showed a good quantity of Vitamin 'A' (0.212 to 0.312 g./100g.) and Vitamin 'C' (152.3 to 214.0 mg./100g.) Vitamin 'A' increased with the colour of the pigments present in the plant and reached highest with yellow colour pigment. It waas seen that the percentage of Vitamin 'A' gradually decreased from the faded green region to the black lesions. This happened due to maximum accumulation of the pathogens in the black lesions. The yellowish zone showed lower content of Vitamin 'A', which might be due to toxic activity of pathogens.

Vitamin 'C' played an important role in metabolism of plants (Mapson, 1958) Varieties showed greater variation in ascorbic acid concentrations. There was a greater reduction in the ascorbic acid content in necrotic lesions compared to yellow regions. It progressed to normalize in the green tissues.

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