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Biochemical changes in agarwood tree (*Aquilaria malaccensis* Lamk.) during pathogenesis

P Tamuli[1], P Boruah & R Samanta[2]

Division of Plant Sciences and Ecology
Regional Research Laboratory
Jorhat - 785 006, Assam, India

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Abstract

Post-infection changes in sugars, ascorbic acid, phenols and protein of agarwood tree (*Aquilaria malaccensis*) were investigated after inoculation with fungi such as *Chaetomium globosum* and *Fusarium oxysporum*. In healthy trees, the biochemical constituents increased, whereas in infected trees they decreased after inoculation with fungi.

Key words: agarwood tree, *Aquilaria malaccensis*, ascorbic acid, biochemical changes, phenols, protein, sugars.

Introduction

The oil obtained from the agarwood tree, *Aquilaria malaccensis* Lamk. (Family : Thymelaeaceae) is used in cosmetic and pharmaceutical industries. Agar is considered to be a pathological product produced by fungal invasion of the host (Shu-Yuan *et al.* 1992). The agar bearing tree shows various symptoms including a dark tinge on the wood compared to the pale brown buff colour of normal wood of healthy trees. A few workers have studied agar formation and reported that the agar zones are associated with mould and decay fungi (Bose 1938; Bhattacharyya *et al.* 1952; Jalaluddin 1970, 1977; Tamuli *et al.* 2000). Among the various fungal species associated with agar zones, a few could cause pathogenesis while others seem to be of saprophytic nature in different eco-geographical conditions. The ultimate impact of host-pathogen interaction is manifested in alteration of composition of nutritional and structural metabolites in the host. Metabolic changes due to fungal infection have been reported in various plants (Reddy *et al.* 1984; Khatri *et al.* 1985; Prasad *et al.* 1988; Nema 1989). The present investigation was undertaken to study the changes in sugar, ascorbic acid, phenol and protein contents during pathogenesis in *A. malaccensis*.

Materials and methods

Artificial inoculation with fungal isolates

The most frequently isolated fungi (*Chaetomium globosum* and *Fusarium oxysporum*) were cultured on PDA plates. Ten day old cultures of *C. globosum* and *F. oxysporum* were inoculated alone and in combination to healthy plants by artificial boring on to the plants (Tamuli *et al.* 2000). Ob-

[1]Present address: Department of Botany, Darrang College, Tezpur – 784 001, Assam, India.
[2]Department of Life Sciences, Dibrugarh University, Dibrugarh – 786 004, Assam, India.

servations on inoculated plants were made at an interval of 10 days up to 40 days after inoculation.

Estimation of biochemical constituents

Sugars

The soluble sugars were extracted by boiling 5 g of sample with 80% alcohol (Dayal & Joshi 1968). Total and reducing sugars were estimated by Lane and Eynon's method using methylene blue as internal indicator (AOAC 1945). Non-reducing sugars (sucrose) were calculated by subtracting the value of reducing sugar from the total acid hydrolysable sugars and these were multiplied by 0.95 to correct for the water taken up during hydrolysis.

Ascorbic acid

Wood chips (5 g) were extracted with 5% metaphosphoric acid solution and made up to 50 ml. Ascorbic acid content was estimated volumetrically using 2, 6 - dichlorophenol indophenol as indicator (Bessey & King 1933).

Phenols

Phenolic compounds were extracted by boiling 5 g plant material with 80% alcohol for 5 min (Chandramohan *et al.* 1967). Total phenols in the alcohol extract was estimated by employing Folin-Ciocalteau reagent (Bray & Thorpe 1955) and Catechol was used as standard.

Protein

Protein content of plant samples was estimated by the method of Lowry *et al.* (1951) and Wildman & Jogendorf (1952) using bovine albumin as standard.

Results and discussion

Sugar content

The reducing and non-reducing sugar contents increased from 10 days (18.80 and 10.71 mg g^{-1} dry wt., respectively) to 40 days of incubation (20.23 and 11.43 mg g^{-1} dry wt., respectively) in healthy plants (Table 1). However, in naturally infected and inoculated wood, reducing sugar contents decreased with increase in days of incubation. In naturally infected wood, it decreased from 10.92 mg g^{-1} (10 days of incubation) to 9.67 mg g^{-1} (40 days of incubation). In wood samples inoculated with *C. globosum* and *F. oxysporum*, reducing sugar contents decreased from 19.46 to 17.41 mg g^{-1} and 18.74 to 17.63 mg g^{-1}, respectively. In the wood infected with dual culture of *C. globosum* and *F. oxysporum*, it decreased from 18.43 to 17.22 mg g^{-1} during the same period. Similarly, non-reducing sugars in infected wood also decreased with increase in days of incubation. The total sugar content was maximum (31.66 mg g^{-1}) in healthy wood and minimum in naturally infected wood (17.20 mg g^{-1}) at 40 days after inoculation. Artificially inoculated plants contained 26.89, 26.97 and 26.59 mg g^{-1} of total sugars when inoculated with *C. globosum* and *F. oxysporum* alone and as dual cultures, respectively.

A similar trend of decreased sugar levels in diseased plants was observed by Prasad *et al.* (1988) and Nema (1989). The depletion of sugars during host-parasite interaction might be due to increased respiration or utilization of sugars by the fungi which depends on the capability of fungi to secrete carbohydrate degrading enzyme (Prasad *et al.* 1988). Nema (1989) suggested that reduction in sugars during disease development might be due to utilization of sugars probably for energy and synthetic reactions involved in multiplication of the pathogen.

Ascorbic acid content

Ascorbic acid content in healthy wood increased during the test period (10 to 40 days). In healthy wood, ascorbic acid content increased from 9.21 to 10.04 mg g^{-1} during the test period (Table 2). In infected samples ascorbic acid content decreased with inoculation period. The decline in ascorbic acid content was maximum in naturally infected wood which was followed by the wood inoculated with *F. oxysporum*, *C. globosum* and *F. oxysporum* in combination and *C. globosum* alone. The decreased level of ascorbic acid in infected plants might be due to ascorbic acid

Table 1. Effect of fungal inoculation on sugar content of *Aquilaria malaccensis* plants (stem) during different periods of incubation

Sugar	Treatment	Sugar content (mg g^{-1} dry wt.)			
		10 days	20 days	30 days	40 days
Reducing sugar	H	18.80 ± 1.03	19.20 ± 0.97	19.63 ± 0.58	20.23 ± 1.12
	NI	10.92 ± 0.92	10.71 ± 0.72	9.83 ± 0.84	9.67 ± 0.63
	CG	19.46 ± 0.74	18.22 ± 0.57	17.84 ± 1.20	17.41 ± 0.86
	FO	18.74 ± 0.58	18.55 ± 0.73	18.02 ± 0.68	17.63 ± 0.92
	CG + FO	18.43 ± 0.72	17.96 ± 0.66	17.74 ± 0.91	17.22 ± 0.83
		Time (t)	Treatment (tr)	t x tr	
	SEd	0.173	0.192	0.387	
	CD (P=0.05)	0.300	0.389	0.784	
	CD (P=0.01)	0.469	0.521	1.050	
Non-reducing sugar	H	10.71± 0.88	10.85 ± 1.04	11.19 ± 0.67	11.43 ± 0.82
	NI	8.19 ± 1.12	7.72 ± 0.77	7.64 ± 0.81	7.53 ± 0.47
	CG	10.76 ± 0.87	10.51 ± 0.67	10.23 ± 0.62	9.48 ± 0.74
	FO	10.72 ± 0.66	10.54 ± 0.72	9.63 ± 0.67	9.34 ± 0.53
	CG + FO	10.53 ± 0.93	10.38 ± 0.76	9.74 ± 0.82	9.37 ± 0.65
		Time (t)	Treatment (tr)	t x tr	
	SEd	0.164	0.188	0.382	
	CD (P=0.05)	0.332	0.381	0.774	
	CD (P=0.01)	0.445	0.510	1.036	
Total sugar	H	29.53 ± 0.57	30.06 ± 0.93	30.82 ± 1.14	31.66 ± 1.02
	NI	19.11 ± 0.73	18.43 ± 0.67	17.47 ± 1.06	17.20 ± 0.84
	CG	30.22 ± 0.76	28.73 ± 0.89	28.07 ± 0.52	26.89 ± 0.71
	FO	29.46 ± 0.92	29.09 ± 1.12	27.65 ± 0.47	26.97 ± 0.85
	CG + FO	28.96 ± 0.81	28.34 ± 0.73	27.48 ± 0.83	26.59 ± 0.87
		Time (t)	Treatment (tr)	t x tr	
	SEd	0.178	0.197	0.392	
	CD (P=0.05)	0.361	0.399	0.794	
	CD (P=0.01)	0.483	0.534	1.063	

H=Healthy; NI=Naturally infected; CG=*Chaetomium globosum*; FO=*Fusarium oxysporum*

Table 2. Effect of fungal inoculation on ascorbic acid content of *Aquilaria malaccensis* plants (stem) during different periods of incubation

Treatment	Ascorbic acid content (mg g^{-1} dry wt.)			
	10 days	20 days	30 days	40 days
H	9.21 ± 0.30	9.35 ± 0.32	9.67 ± 0.24	9.80 ± 0.22
NI	8.49 ± 0.42	7.45 ± 0.49	7.37 ± 0.83	6.96 ± 0.44
CG	9.19 ± 0.20	9.06 ± 0.29	8.98 ± 0.14	8.70 ± 0.45
FO	9.25 ± 0.37	8.90 ± 0.24	8.80 ± 0.39	8.04 ± 0.54
CG + FO	9.23 ± 0.48	8.78 ± 0.36	8.74 ± 0.42	8.13 ± 0.62
	Time (t)	Treatment (tr)	t x tr	
SEd	0.178	0.199	0.398	
CD (P=0.05)	0.360	0.403	0.806	
CD (P=0.01)	0.482	0.539	1.078	

H=Healthy; NI=Naturally infected; CG=*Chaetomium globosum*; FO=*Fusarium oxysporum*

Table 3. Effect of fungal inoculation on phenol content of *Aquilaria malaccensis* plants (stem) during different periods of incubation

Treatment	Phenol content (μg g^{-1} dry wt.)			
	10 days	20 days	30 days	40 days
H	32.12 ± 1.63	32.45 ± 1.70	35.04 ± 1.70	35.22 ± 2.87
NI	30.14 ± 8.16	29.23 ± 1.25	29.26 ± 1.70	27.31 ± 9.43
CG	31.06 ± 1.41	30.31 ± 9.42	29.33 ± 8.16	29.31 ± 1.25
FO	30.22 ± 4.71	29.41 ± 8.16	28.14 ± 9.43	27.22 ± 4.71
CG + FO	31.04 ± 4.71	29.24 ± 4.71	28.11 ± 1.41	27.34 ± 4.71
	Time (t)	Treatment (tr)	t x tr	
SEd	0.154	0.168	0.312	
CD (P=0.05)	0.312	0.340	0.632	
CD (P=0.01)	0.418	0.456	0.846	

H=Healthy; NI=Naturally infected; CG=*Chaetomium globosum*; FO=*Fusarium oxysporum*

degenerating enzymes either by the fungus alone or by the activity of the host-pathogen complex. Reddy *et al.* (1984) observed a gradual loss in ascorbic acid content in infected fruits of acid lime (*Citrus aurantifolia*).

Phenol content

Phenol content increased from 10 days (32.12 μg g^{-1}) to 40 days (35.22 μg g^{-1}) of incubation in healthy wood. But a reverse trend was noticed in naturally infected and inoculated wood (Table 3). Khatri *et al.* (1985) observed that the amount of total phenols declined in rice leaves due to infection by *Entyloma oryzae*. He suggested that the reduction in concentration of phenolic compounds might be associated with susceptibility of the host to the invading pathogen. Nema (1989) suggested that in betel vine, the leaf spot pathogen is inhibited by the phenolic compounds but when the pathogen is successful in causing the disease, the phenolic compounds in highly susceptible cultivar depleted the most. It is also probable that the infected host is unable to produce phenolic compounds in the presence of the pathogen.

Protein content

In healthy plants, slight increase in protein content (8.23 to 8.40 mg g^{-1}) was observed with increase in days of incubation (Table 4). All infected samples exhibited a decreasing trend in protein content with incubation. Among the infected samples, naturally infected wood contained minimum protein content (6.13 mg g^{-1}). Decrease in protein content might be due to degradation of the host proteins by the proteolytic enzymes secreted

Table 4. Effect of fungal inoculation on protein content of *Aquilaria malaccensis* plants (stem) during different periods of incubation

Treatment	Protein content (mg g^{-1} dry wt.)			
	10 days	20 days	30 days	40 days
H	8.23 ± 0.12	8.27 ± 0.33	8.37 ± 0.21	8.40 ± 0.24
NI	6.83 ± 0.29	6.30 ± 0.08	6.23 ± 0.12	6.13 ± 0.25
CG	8.20 ± 0.29	7.83 ± 0.21	7.67 ± 0.12	7.57 ± 0.17
FO	8.13 ± 0.31	7.93 ± 0.12	7.70 ± 0.29	7.63 ± 0.19
CG + FO	8.07 ± 0.31	7.77 ± 0.33	7.50 ± 0.43	7.37 ± 0.31
	Time (t)	Treatment (tr)	t x tr	
SEd	0.119	0.133	0.267	
CD (P=0.05)	0.242	0.270	0.541	
CD (P=0.01)	0.324	0.362	0.724	

H=Healthy; NI=Naturally infected; CG=*Chaetomium globosum*; FO=*Fusarium oxysporum*

by the pathogens. Similar results were also reported by Prasad *et al.* (1988) in muskmelon fruits infected with fruit-rot fungi.

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