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Enzyme activities of agarwood (*Aquilaria malaccensis* Lamk.) stem under pathogenesis

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Abstract

Cellulase, pectinase, peroxidase and polyphenol oxidase activities were determined in healthy, naturally infected and inoculated agarwood (*Aquilaria malaccensis*) plant parts at various time intervals to study the changes in activities of these enzymes during pathogenesis. Samples infected naturally with *Chaetomium globosum* and *Fusarium oxysporum* exhibited higher activity of all the enzymes compared to healthy samples.

Keywords: agarwood, *Aquilaria malaccensis*, *Chaetomium globosum*, enzyme activity, *Fusarium oxysporum.*

Aquilaria malaccensis Lamk. (Family: Thymalaeaceae) bears resinous patches of fragrant wood known as 'agar'. Agar is considered to be a pathological product produced by fungal invasion of the host. Many workers have studied agar formation and reported that the agar zones are associated with mould and decay fungi (Bhattacharyya *et al*. 1952; Tamuli *et al*. 2000). Among the different fungal species associated with agar zones, a few could exhibit pathogenesis while others seem to be saprophytic. One of the most common symptoms in the fungal disease is the disorganization of host tissues brought about by enzymes secreted by the pathogens which attack the host walls.

Changes in cellulolytic and pectinolytic enzyme activity during pathogenesis have been reported by various workers (Prasad *et al*. 1988; Reddy & Reddy 1988). Increase in peroxidase and polyphenol oxidase activity in tissues of various plants following infection by various pathogens have been reported (Loebenstein & Linsey 1961; Addy & Goodman 1972). The present investigation was undertaken to study the changes in cellulase, pectinase, peroxidase and polyphenol oxidase activity during pathogenesis in agarwood.

Artificial inoculation of fungal isolates

The most frequently isolated fungi (*Chaetomium globosum* Kunze. and

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Fusarium oxysporum Schlecht.) were inoculated (in combination also) to the healthy plant by artificial boring on to the plants (Tamuli *et al.* 2000). Inoculation was made with these two fungi and their combination.

Enzyme activities were measured in healthy, naturally infected and artificially inoculated plant samples at an interval of 10, 20, 30 and 40 days after inoculation. For estimation of cellulase activity, wood chips were ground in 15 ml of 0.5 M NaCl solution, and activity was determined according to the method described by Verma & Singh (1975) and specific activity was calculated. Pectinase activity was determined according to Dubey & Mathur (1975). The reaction mixture consisted of 1% pectin and 1 ml of enyme extract. One unit of enzyme activity was expressed as 1% loss of viscosity after 30 min. Peroxidase was extracted following the method of Purohit *et al*. (1979) and the activity determined by the method of Chakravarti & Nandi (1978). The activity of polyphenol oxidase was determined by the method of Purohit *et al*. (1979); catechol was used as substrate.

Cellulase and *pectinase* activity

Maximum activity of these enzymes were recorded in plants 20 days after inoculation which, however, reduced at 40 days (Tables 1 & 2). Specific activity of celluase and pectinase was highest in naturally infected wood, and in healthy wood.

Peroxidase activity

Proxidase activity in healthy samples remained more or less unchanged up to 40 days of incubation (Table 3). The highest specific activity (0.077) was recorded in naturally infected wood and the lowest (0.052) in healthy wood.

Polyphenol oxidase activity

In healthy samples, polyphenol oxidase activity did not increase considerably with prolongation of incubation (Table 4). However, considerable increase in polyphenol oxidase activity was observed in infected samples with increase in days of incubation. Specific activity was maximum (0.119) in naturally infected wood and minimum (0.054) in healthy wood.

The investigation indicated that all the treatments showed maximum activities of both the enzymes on the 20th day of incubation, while on the 30th and 40th day of incubation, activities of both the enzymes declined (Tables 1 & 2). Thus, higher cellulase and pectinase activities observed in infected wood might be responsible for disorganization of host tissues leading to the colonization of pathogens. Prasad *et al*. (1988) reported alteration in the enzyme activities of seeds in finger millet due to

Table 1. Effect of fungal inoculation on cellulolytic enzyme activity of *Aquilaria malaccensis* during different periods of incubation

Treatment	10 days	20 days	30 days	40 days
H	2.42 ± 0.53	3.23 ± 0.47	2.74 ± 0.38	2.65 ± 0.31
NI	4.40 ± 0.57	6.97 ± 0.45	6.07 ± 0.51	6.06 ± 0.29
CG	3.08 ± 0.44	4.84 ± 0.49	3.93 ± 0.46	3.61 ± 0.32
FO	3.35 ± 0.72	5.01 ± 0.62	4.24 ± 0.48	4.08 ± 0.24
CG + FO	3.49 ± 0.61	5.45 ± 0.53	4.79 ± 0.54	4.77 ± 0.32
	Time (t)	Treatment (tr)	t x tr	
SEd (Specific activity)	0.118	0.132	0.217	
CD P=0.05	0.239	0.267	0.440	
P=0.01	0.320	0.358	0.588	

Values indicate specific activity
H=Healthy; NI=Naturally infected; CG=*Chaetomimum globosum*; FO=*Fusarium oxysporum*

Table 2. Effect of fungal inoculation on pectinolytic enzyme activity of *Aquilaria malaccensis* during different periods of incubation

Treatment	10 days	20 days	30 days	40 days
H	3.35 ± 0.41	5.18 ± 0.34	4.38 ± 0.27	4.24 ± 0.44
NI	4.66 ± 0.48	7.92 ± 0.48	6.71 ± 0.31	6.55 ± 0.43
CG	3.49 ± 0.34	5.61 ± 0.21	4.95 ± 0.24	4.86 ± 0.36
FO	3.67 ± 0.38	5.40 ± 0.38	4.78 ± 0.52	4.65 ± 0.43
CG + FO	3.71 ± 0.37	6.02 ± 0.43	5.18 ± 0.33	4.96 ± 0.41
	Time (t)	Treatment (tr)	t x tr	
SEd (Specific activity)	0.118	0.132	0.217	
CD P=0.05	0.239	0.267	0.440	
P=0.01	0.320	0.358	0.588	

Values indicate specific activity
H=Healthy; NI=Naturally infected; CG=*Chaetomimum globosum*; FO=*Fusarium oxysporum*

Aspergillus flavus. They found the activities of pectolytic and cellulolytic enzymes were enhanced due to the influence of *A. flavus*. They suggested that higher per cent of pectolytic and cellulolytic enzymes are used as a tool for penetration of fungi as these enzymes are responsible for permeability damage of the cell. Similar results were also obtained by Reddy & Reddy (1988) in fruit rot of coccinia. They suggested that increased activities of these enzymes in infected tissues compared to healthy tissues may be attributed to pathogen activity.

The activities of peroxidase and polyphenol oxidase increased along with the incubation period in all the treatments (Tables 3 & 4). However, maximum activity of these enzymes were recorded in naturally infected samples and minimum in healthy samples. Peroxidase and polyphenol oxidase activities in apple leaves inoculated with *Erwinia amylovora* Burril was studied by Addy & Goodman (1972). They noted an increased activity of both the enzymes in inoculated leaves as compared to control. Loebenstein & Linsey (1960) suggested that the higher peroxidase activities in infected plants might be the consequence of greater breakdown of carbohydrates through monophosphate shunt due to which precursors of phenolic compounds are produced which are oxidised by peroxidase to quinones in the presence of H_2O_2 to overcome the pathogen.

Table 3. Effect of fungal inoculation on peroxidase activity of *Aquilaria malaccensis* during different periods of incubation

Treatment	10 days	20 days	30 days	40 days
H	0.050 ± 0.002	0.051 ± 0.003	0.050 ± 0.002	0.052 ± 0.003
NI	0.064 ± 0.003	0.073 ± 0.002	0.075 ± 0.004	0.077± 0.004
CG	0.052 ± 0.002	0.057 ± 0.002	0.059 ± 0.002	0.062 ± 0.003
FO	0.054 ± 0.004	0.055 ± 0.002	0.058 ± 0.002	0.060 ± 0.003
CG + FO	0.053 ± 0.002	0..057 ± 0.002	0.060 ± 0.003	0.062 ± 0.002
	Time (t)	Treatment (tr)	t x tr	
SEd (Specific activity)	0.002	0.003	0.007	
CD P=0.05	0.004	0.006	0.014	
P=0.01	0.005	0.008	0.019	

Values indicate specific activity
H=Healthy; NI=Naturally infected; CG=*Chaetomimum globosum*; FO=*Fusarium oxysporum*

Table 4. Effect of fungal inoculation on polyphenol oxidase activity of *Aquilaria malaccensis* during different periods of incubation

Treatment	10 days	20 days	30 days	40 days
H	0.052 ± 0.003	0.052 ± 0.003	0.054 ± 0.002	0.054 ± 0.002
NI	0.064 ± 0.003	0.084 ± 0.003	0.095 ± 0.004	0.119± 0.008
CG	0.055 ± 0.002	0.066 ± 0.003	0.074 ± 0.004	0.085 ± 0.003
FO	0.054 ± 0.003	0.068 ± 0.002	0.079 ± 0.002	0.083 ± 0.003
CG + FO	0.055 ± 0.002	0.067 ± 0.002	0.077 ± 0.004	0.090 ± 0.002
	Time (t)	Treatment (tr)	t x tr	
SEd(Specific activity)	0.003	0.003	0.007	
CD P=0.05	0.006	0.006	0.014	
P=0.01	0.008	0.008	0.019	

Values indicate specific activity
H=Healthy; NI=Naturally infected; CG=*Chaetomimum globosum*; FO=*Fusarium oxysporum*

The changes in activity of various enzymes in naturally infected agarwood indicated that these enzymes were involved in the infection process and development of symptoms of the disease in agarwood plants.

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