BIOLOGIA PLANTARUM (PRAHA)

33 (3): 211–215, 1991

Changes in Phenol and Peroxidase in the Leaves of *Java citronella* Infected with *Curvularia andropogonis*

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Abstract The contet of phenols, o–dihydroxyphenols and peroxidase activity in healthy and *Curvularia andropogonis* (Zimm.) Boedijn infected leaves of *Java citronella* were determined. As a result of infection, the content of phenols and peroxidase increased two– and four–fold, respectively in necrotic lesions compared to healthy leaves. In surrounding tissue of lesions, their increase was one–and half fold only. The peroxidase activity decreased with the maturity of the necrotic lesions. Necrotic lesions produced in response to infection appear to be the consequence of higher accumulation of phenols and their oxidation by peroxidase.

Leaf blight of *Java citronella* (*Cymbopogon winterianus* Jowitt) has been reported to be caused by *Curvularia andropogonis* (Zimm.) Boedijn (Alam and Husain 1983). The symptoms of the disease are characterized by long elongated reddish brown necrotic lesions along the veins spreading towards the margins and tips of the leaves.

The infection caused by plant pathogens are known to induce changes in phenolic contents and in the activity of oxidative enzymes such as peroxidase, catalase and polyphenol oxidase (Farkas and Kiraly 1962, Kosuge 1969, Fric 1976, Friend 1981, Bashan 1986). Increase in phenolic contents and peroxidase activity as a result of infection is often correlated with resistance (Kuc 1966, Bazzalo *et al.* 1985, Kumar and Sridhar 1985, Werder and Kern 1985 and Srivastava 1987). No information is available on the induced changes in phenol metabolism and peroxidase in *Java citronella* as a result the changes in phenolic contents as well as peroxidase activity at the sit of infection by *C. andropogonis* and their role in the development of necrotic lesions.

Received June 26, 1989; accepted December 12, 1989
CIMAP Publication No. 859

MATERIALS AND METHODS

Healthy plants of *Java citronella* were grown in earthen pots (25 cm) in a glasshouse maintained at a temperature of 25 ± 2°C and relative humidity 70–80 °C. The plants at 6 leaves stage were sprayed with an aqueous spore suspension at the concentration of 1×10^7 spores per ml from ten day old culture of *Curvularia andropogonis* (strain CLB) on PDA. Control plants were sprayed with distilled water. All the plants were covered with polythene bags to maintain high humidity for 48 h and maintained in the glasshouse for development of symptoms of the disease.

Determinations of phenolic contents and peroxidase were carried out using 0.5 g fresh tissues from the site of intection consisting of necrotic lesion, tissues around lesion (0–5 mm) and non–lesion area (1–2 cm apart from lesions) at different times after inoculation. Healthy tissues from control plants were sampled simultaneously.

Total phenols and *o*–dihydroxyphenols were estimated from the methanolic extract following the method of Swain and Hills (1959) and Johnson and Schall (1952), respectively. Determinations were carried out in triplicate.

The enzyme extracts were prepared by grinding 0.5 g fresh tissues in cold 0.01 M sodium phosphate buffer at pH 6.6 for 2 min and homogenates were strained out through four layers of cheese cloth and filtrates were centrifuged at 15 000 *g* for 20 min at 4 °C. The clear supernatant was used for estimation of the enzymes. The activity of peroxidase was determined by the procedure described by Mahadevan (1975). Determinations were carried out in triplicate.

TABLE 1

Changes in total phenolic contents in the leaves of *Java citronella* after infection with *C. andropogonis*

ncubation period [d]	Concentration [mg/g fresh mass ± S.D.]			
	Healthy	Infected		
		Non-lesion area	Around lesion	Lesion
0	8.90 ± 1.20	–	–	–
5	9.50 ± 0.80	13.50 ± 0.30	14.25 ± 0.50	16.50 ± 1.15
7	10.75 ± 0.50	14.75 ± 1.26	16.00 ± 1.40	18.00 ± 1.30
10	10.83 ± 0.37	14.75 ± 1.42	16.75 ± 0.65	20.00 ± 1.45
15	11.00 ± 0.63	15.25 ± 1.10	18.00 ± 1.21	23.50 ± 1.26

Total phenol was calculated as chlorogenic acid equivalent. Results are the averages of three determinations.

RESULTS

Changes in Phenolic Contents

Tables 1 & 2 show the amount of total phenols and dihydroxyphenols accumulated in healthy leaves and in necrotic lesion, around lesion and non–lesion area of infected leaves at various intervals of time. Their accumulation in healthy and non–lesion area of infected leaves was considerably low. The concentration increased towards necrotic lesion and its bordering area. Fifteen days after inoculation,

TABLE 2

Changes in O-dihydroxyphenols in the leaves of *Java citronella* after infection with *C. andropogonis*

Incubation period (d)	Concentration [mg/g^{-1} fresh mass ± S.D.]			
	Healthy	Infected		
		Non-lesion area	Around lesion	Lesion
0	0.75 ± 0.10	–	–	–
5	0.77 ± 0.08	0.90 ± 0.10	0.95 ± 0.06	1.47 ± 0.13
7	0.79 ± 0.06	0.92 ± 0.09	1.05 ± 0.13	1.57 ± 0.12
10	1.07 ± 0.13	1.42 ± 0.15	1.57 ± 0.16	1.84 ± 0.16
15	1.23 ± 0.12	1.57 ± 0.21	1.62 ± 0.12	1.96 ± 0.18

O-dihydroxyphenol was calculated as catechol equivalent. Results are the averages of three determinations.

TABLE 3

Changes in peroxidase activity of different stages of lesion development

Lesion stage	Peroxidase activity* [ΔA/min^{-1} g^{-1} ± S.D.]			
	Healthy	Infected		
		Non-lesion area	Around lesion	Lesion
Young	32.00 ± 1.85	44.40 ± 3.10	65.75 ± 4.28	127 ± 6.45
Intermediate	30.00 ± 2.50	42.00 ± 4.50	68.00 ± 3.40	146 ± 5.80
Mature	28.50 ± 1.60	48.00 ± 2.80	61.00 ± 4.70	110 ± 6.30

*Expressed as absorbance change (A) at 420 nm per minute per gram of fresh tissues. Results are the average of three determinations.

total phenols and dihydroxyphenols in infected leaves were found to be 23.50 mg and 1.96 mg equivalents of chlorogenic acid and catechol, respectively per gram fresh matter. The inoculated leaves accumulated phenolic compounds at a faster rate compared to uninfected controls.

Change in Peroxidase Activity

Table 3 shows the change in peroxidase activity in necrotic lesion, around lesion and non–lesion area of infected leaves. An enhanced peroxidase activity was observed as a result of infection. The increase was greater in necrotic lesions and its surrounding tissues.

DISCUSSION

Total phenol and dihydroxyphenols in infected leaves were found to be higher than in healthy leaves. In necrotic lesions of infected leaves, the peroxidase activity was increased fourfold as compared to healthy leaves. It appears that changes in phenolic contents are accompanied by the alteration in the metabolic activity of the cells under pathological stress.

The browning of the necrotic lesions, produced in response to infection caused by *C. andropogonis*, seems to be due to higher accumulation of phenolic compounds and its oxidation products such as dimers and polymers. Peroxidase plays a significant role in their oxidation, since host tissue lacks polyphenoloxidase. Sridhar and Ou (1974) reported that the phenolic oxidation is enhanced in rice leaves infected with *Pyricularia oryzae* Cag. resulting in brown pigment (melanin type) around the lesion. The oxidation products of phenolic compounds readily polymerize or react with an amino group containing compounds in the infected cells to produce melanin like substances (Uritani 1961).

Acknowledgments

The authors are grateful to Director, Central Institute of Medicinal and Aromantic Plants, Lucknow for facilities and ancouragement.

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