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Phenolic Compounds Detected in Rice Blast Lesions

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Abstract. Salicylic, *p*-coumaric, and ferulic acids were detected in the acetone-insoluble cell fraction, presumably protein, extracted from the brown discoloured tissues of rice (*Oryza sativa* L.) leaves infected with *Pyricularia oryzae* CAV. but not from healthy leaves. It is proposed that these phenolic acids are oxidized *in vivo* in blast diseased rice leaves, and forms a protein-quinone complex.

Phenolic compounds and their related oxidases have been associated with the defense mechanisms of plants because of their general accumulation near the wounded and infected tissues and that phenols and their oxidation products are highly toxic to pathogens. A marked post-infectional change in the phenolic compounds and its oxidases have been observed in many host-parasite relationships (URITANI 1961, FARKAS and KIRÁLY 1962, CRUICKSHANK and PERRIN 1964, RUBIN and ARTSIKHOVSKAYA 1964). The lesions caused by *Pyricularia oryzae* CAV. on rice leaves are brown in colour and are presumably containing melanoid pigments (OKU 1964, SUZUKI 1965). Evidently during infection, phenolic compounds are oxidized by peroxidase of rice plants since, rice plants do not contain any polyphenol oxidase (SRIDHAR 1972). Formation of melanoid pigments due to polymerization of quinones has long been known (THOMSON 1964).

The brown pigments of aged burley tobacco was found to contain a protein-phenol complex consisting of caffeic and quinic acids, both constituents of chlorogenic acid (WRIGHT *et al.* 1960, 1964). The oxidation products of phenolic compounds easily polymerize or react on amino group-containing compounds in the infected cells such as proteins and amino acids to produce a melanin-like coagulated substance (URITANI 1961, KOSUGE 1969). Caffeic acid, a hydrolysis product of chlorogenic acid, was shown to be associated with acetone insoluble cell fraction, presumably protein, in the necrotic tissues of tobacco leaves infected with tobacco streak virus but not from

healthy leaves (HAMPTON 1970). In light of the above evidences the brown pigment formed around the blast lesions in rice leaves is investigated.

Materials and Methods

Prechilled leaf tissues from both healthy and diseased (lesions and surrounding tissues) rice cultivar Peta were homogenized in acetone at $-15\,^{\circ}$ C, filtered, and the residue was washed with additional cold acetone. The residue was transferred to a cellulose extraction thimble and extracted for 8 h with acetone in a soxhlet extractor, followed by three successive washes with anyhydrous diethyl ether. The resultant acetone powders were white when healthy tissues were used and a bit brown when diseased tissues were used. The acetone powders were hydrolyzed by refluxing in 2 *N* HCl for a period of 24 h. The hydrolysates were then extracted with equal volumes of anyhydrous diethyl ether for three times, the combined ether fractions were evaporated to dryness under reduced pressure at $45\,^{\circ}$ C and the residue was dissolved in small quantity of 95 per cent ethyl alcohol (HAMPTON 1970).

The phenolic compounds were separated ascendingly by thin layer chromatography. Glass plates of 20 × 20 cm coated with 0.25 mm thickness of silica gel $-$G, were spotted with 50 µl of these extracts and authentic samples of phenolic compounds and developed in various solvents (BLOCK *et al.* 1958, HARBORNE 1961, RANDERATH 1966). The phenolic compounds were located by observing the fluorescence under short wave ultraviolet lamp and by spraying either with one per cent ferric chloride solution, a solution of 0.5 per cent diazotized sulfanilic acid in 10 per cent sodium carbonate solution or with 0.1 *N* potassium permanganate in sodium carbonate solution (RANDERATH 1966).

Results and Discussion

Three spots giving positive reactions to the reagents tested were detected in the chromatograms spotted with the hydrolysate from the diseased leaf

TABLE 1

RF VALUES OF PHENOLIC COMPOUNDS present in the hydrolysates from diseased leaf tissue as compared to that of known compounds.

Solvents	Rf values					
	Authentic samples			Spot No.		
	p-coumaric acid	Ferulic acid	Salicylic acid	1	2	3
Benzene-dioxane-acetic acid (90 : 25 : 4)	0.490	0.521	0.648	0.483	0.513	0·650
Benzene-methanol-acetic acid (45 : 8 : 4)	0.507	0.589	0.679	0.513	0.594	0·688
Butanol-acetic acid-water (4 : 1 : 5, upper layer)	0.746	0.781	0.783	0.767	0.798	0.785
Isopropanol-ammonia-water (8 : 1 : 1)	0.531	0.500	0.565	0.531	0.500	0.563
Tolune-acetic acid-water (4 : 1 : 5, upper layer)	0.000	0.074	0.135	0.000	0.074	0.136
Butanol-pyridine-satd. aq. NaCl (1 : 1 : 2)	0.821	0.792	0.846	0.821	0.793	0.848

tissues but not from the healthy leaf tissues. The flourescence under ultraviolet light of spot no. 1, 2 and 3 appeared as faint violet, green and violet in acid conditions, while, in alkaline conditions they appeared as violet, bluish green and bright blue respectively. The flourescence under ulatraviolet light and Rf values (Table 1) of spot no. 1 was identical to authentic sample of *p*-coumaric acid, spot no.2 to ferulic acid and spot no. 3 to salicylic acid.

The browning of plant tissues in response to infection and injury has long been known to be the result of the oxidation of phenolic compounds by its oxidases, the system of which is accelerated under pathological stress conditions. Phenols may form reversible complexes with protein by hydrogen bonding, whereas quinones form stable covalent linkages (LOOMIS 1969). The *in vitro* formation of a complex between the quinone of chlorogenic acid and protein (bovine albumin fraction V), which yielded caffeic acid upon hydrolysis has been shown (HAMPTON 1970). Detection of three phenolic compounds in the acid hydrolysates of acetone insoluble cell component from the diseased tissue and their absence in the similar fraction from the healthy tissues indicate the *in vivo* oxidation of salicylic, *p*-coumaric, and ferulic acids in rice plants infected with the blast disease and the formation of a complex between the quinone and the acetone insoluble cell fraction. Since this complex is degraded by acid hydrolysis, it is probably a protein-quinone complex. This gives evidence to show that the phenolic oxidation is enhanced in rice leaves infected with the blast disease resulting in the brown pigment around the lesions. In rice cultivars, resistant to blast disease the tissue browning is more intensified and takes much more faster in the infected tissue than in the susceptible varieties. Hence, the time and rate at which the oxidation of phenolics is triggered in the host-parasite relationship would determine the resistance or susceptibility of the rice cultivars to the blast disease.

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R. SRIDHAR, S. H. OU, Oddělení rostlinné pathologie, Ústřední ústav výzkumu rýže, Cuttack, Indie: **Fenolické látky detekované v listech rýže napadené houbou.** — Biol. Plant. **16** : 67—70, 1974.

Kyselina salicilová, kyselina *p*-kumarová a kyselina ferulodá byly detegovány v buněčné frakci nerozpustné v acetonu, převážně bílkovinné, extrahované z hnědě zbarvené tkáně listů rýže (*Oryza sativa* L.) a to pouze z listů infikovaných *Pyricularia oryzae* CAV. a nikoli ze zdravých listů. Dá se předpokládat, že tyto fekolové kyseliny jsou *in vivo* oxidovány v napadených listech a tvoří komplex chinonu s bílkovinou.