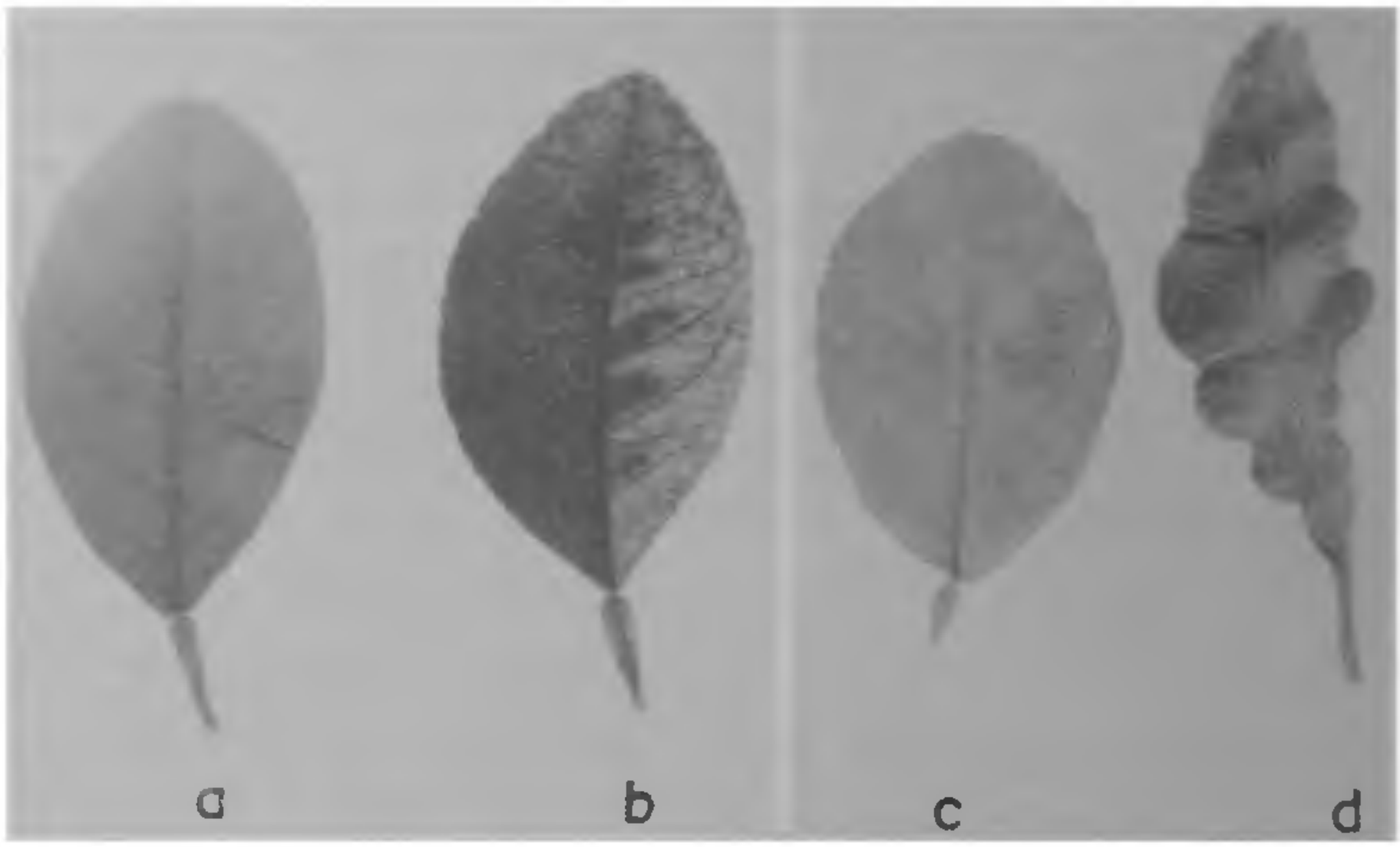

Figure 1a–d. a. Healthy mosambi leaf, **b.** Mature leaf symptoms of mosaic on mosambi, **c.** Young leaf of mosambi showing mosaic symptoms, **d.** Sikkim orange leaf showing wavy margin due to mosaic infection.

Vector transmission is also of help in differentiating these disorders.

The mosaic infection can be assured by close observation of the field trees and can be clearly detected by inoculating on differential host(s) or vector transmission. The transmission of the mosaic disease by aphids, *i.e. M. persicae* and *A. craccivora* has been established for the first time. Kegzi lime and other citrus species were found susceptible to the mosaic.

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1. Murthi, V. D. and Reddy, G. S., *Indian Phytopathol.*, 1975, **28**, 398.
2. Dakshinamurti, V. and Reddy, G. S., *Curr. Sci.*, 1976, **45**, 352.

SIGNIFICANCE OF CELL WALL PHENOLS IN THE RESISTANCE OF RICE AGAINST BLAST

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THE presence of phenolic acids in cell walls has been already reported[1–4]. The cell wall-bound phenols may participate in the resistance of plants against pathogens[5,6]. Correlations between resistance of rice to the blast fungus, *Pyricularia oryzae* and the concentration of ethanol extractable phenols have been shown[7–10]. In this paper, we report the presence of two cinnamic acid derivatives in the leaf blade cell walls of blast resistant and susceptible rice cultivars.

Rice (*Oryza sativa* L) cultivars, Carreon, Tetep and IR-8 resistant and Karuna, CO-13 and Benibhog susceptible to blast were grown from seeds in shallow earthen pots (30 cm in diameter) containing 5 kg of alluvial soil under natural photoperiodic conditions. The disease reaction of plants was confirmed with 21-day-old seedlings artificially inoculated with the fungus. The pots were watered daily. The fully matured third and fourth leaf blades from the 25-day-old plants were harvested. Leaf blade samples (200 g) were

dipped in chloroform (2 × 300 ml; 2 × 20 sec) and cell walls were prepared according to Hartley[2]. The de-waxed leaf blades were homogenized in a blendor with acetone (400 ml), filtered and washed thrice with acetone. The acetone insoluble material consisting of cell walls was washed repeatedly with excess of hot distilled water, acetone and ether, and air-dried.

Cell walls (1 g) were hydrolyzed with N NaOH (50 ml) for 24 hr at 37°C, and filtered through Whatman No. 41 filter paper. The clear filtrate was acidified with 6 N HCl to pH 2.5, and extracted thrice wth 10 volumes of ethyl acetate. The total ethyl acetate extract was evaporated to dryness at 30°C, and the residue dissolved in 5 ml of N NaOH. This is considered as the cell wall extract.

To identify phenols present in the cell wall extract, the extracts from two rice cultivars (Carreon and IR-8) were scanned between 260 and 400 nm in a spectrophotometer (Beckman, U.S.A.). The extract (25 μl) was chromatographically separated using Whatman No. 1 paper and benzene-dioxane-acetic acid (95:25:4, v/v) as solvent system. Chromatograms were examined under UV light and sprayed with diazotized sulphanilic acid reagent to detect phenolic acids[11]. Reference *p*-coumaric and ferulic acids were employed, and identification of the unknowns was confirmed by co-chromatography. Authentic *p*-coumaric and ferulic acids dissolved in N NaOH showed characteristic absorption maxima at 295 and 318 nm, respectively when scanned between 260 and 400 nm in a spectrophotometer. The concentration of *p*-coumaric acid and ferulic acid present in the cell wall extracts (10 ml diluted to 2 ml with N NaOH) was spectrophotometrically determined by measuring the A_{295} and A_{318}, respectively.

Resistance of rice plants to blast is characterised by small lesions and intense tissue browning, which eventually limit the growth and sporulation of the pathogen. Even on susceptible varieties, spread of the individual lesions ceases with browning of the margin. Indeed, the presence of chlorogenic acid in the brown discoloured cell walls of lesions has been shown[12].

The cell wall extracts from two rice cultivars (Carreon and IR-8) yielded two distinct spots (R_f 0.90 and 0.75) when separated on paper. These fluoresced under UV light and developed violet and brick red colour, respectively, with diazotized sulphanilic acid reagent. They were identified as *p*-coumaric acid (R_f 0.75) and ferulic acid (R_f 0.90) by co-chromatography. In addition, UV spectra of the N NaOH extract exhibited two distinct absorption peaks; one at 295 nm and the other at 318 nm. Authentic compounds dissolved in N NaOH also exhibited similar UV spectra. The concentration of *p*-coumaric and ferulic acids as determined by UV absorption present in the cell wall extracts of leaf blades of different cultivars resistant and susceptible to blast is shown in table 1. The ferulic acid concentration of cell wall extracts was higher than that of *p*-coumaric acid irrespective of the cultivars tested. Clearly, the cell wall extracts of leaf blades from three blast resistant cultivars characteristically possessed greater amounts of both *p*-coumaric and ferulic acids than those from the blast susceptible cultivars.

Esters of phenolic acids present in the cell walls form the linkage units between the lignin core and structural carbohydrates[2]. Lignification of cell walls may act as a physical barrier by limiting the diffusion of fungal enzymes and toxins from the infection site to the healthy tissue[13]. In fact, nitrogen fertilization, known to augment the susceptibility of rice plants to blast, markedly decreases the lignin content[14]. However, there is no evidence for increased lignification in the leaves of a susceptible cultivar infected by *P. oryzae*[15]. The phenolic esters are not restricted to lignified cells but also occur in the cell walls of growing tissue[4]. It is possible that esterification of cell wall polysaccharides with phenolic acids may render the former unsuitable as substrates for fungal enzymes[16]. In this respect, the cell wall phenols may be effective as prohibitins[17]. The role of prohibitins in disease resistance has been well documented[18]. In the absence of convincing explanation from the point of phytoalexin theory, the presence of cinnamic acid derivatives in the cell walls may be important in resistance of rice against *P. oryzae*.

Table 1 *Relative amounts of p-coumarate and ferulate present in the cell walls of blast resistant and susceptible rice leaf blades*

Cultivar	Disease reaction	Relative amount *p*-Coumarate (A_{295})	Ferulate (A_{318})
Carreon	R	0.506	0.805
Tetep	R	0.324	0.535
IR-8	R	0 246	0.382
Karuna	S	0.121	0.271
CO-13	S	0 068	0 215
Benibhog	S	0 040	0.157

(Ten μl of the cell wall extract equivalent to 2 mg of cell walls was diluted to 2 ml with N NaOH and *p*-coumarate and ferulate concentrations were determined by measuring the absorbance at 295 and 318 nm, respectively. R, resistant, S, susceptible).

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1. Brown, S. A., *Annu. Rev. Plant Physiol.*, 1966, **17**, 223.
2. Hartley, R. D., *Phytochemistry*, 1973, **12**, 661.
3. Whitmore, F. W., *Plant Physiol.*, 1974, **53**, 728.
4. Harris, P. J. and Hartley, R. D., *Nature (London)*, 1976, **259**, 508.
5. Swain, T., *Annu. Rev. Plant Physiol.*, 1977, **28**, 479.
6. Friend, J., In: *Biochemistry of plant phenolics.* Recent Advances in Phytochemistry Vol. 12 (eds.) T. Swain, J. B. Harborne and C. F. V. Sumere, Plenum Press, New York, 1979, p. 557.
7. Wakimoto, S. and Yoshii, H., *Ann. Phytopathol. Soc. Japan*, 1958, **23**, 79.
8. Ramakrishnan, L., *Phytopathol. Z.*, 1966, **56**, 279.
9. Sridhar, R., *Acta Phytopathol. Hung.*, 1972, **7**, 57.
10. Sridhar, R., *Riso*, 1972, **21**, 25.
11. Mahadevan, A. and Sridhar, R., *Methods in physiological plant pathology*, 2nd edn. Sivakami Publications, Madras, 1982.
12. Suzuki, N., Doi, Y. and Toyoda, S., *Ann. Phytopathol. Soc. Japan*, 1953, **17**, 97.
13. Vance, C. P., Krik, T. K. and Sherwood, R. T., *Annu. Rev. Phytopathol.*, 1980, **18**, 259.
14. Matsuyama, N. and Dimond, A. E., *Phytopathology*, 1973, **63**, 1202.
15. Langcake, P. and Wickins, S. G. A., *Physiol. Plant Pathol.*, 1975, **7**, 113.
16. Friend, J., In: *Biochemical aspects of plant parasite relationships* (eds) J. Friend and D. R. Threfall, Academic Press, New York, 1976, p. 291.
17. Mahadevan, A., *Phytopathol. Z.*, 1970, **68**, 73.
18. Mahadevan, A., *Biochemical aspects of disease resistance. Part I. Preformed inhibitory substances "prohibitins"*. Today & Tomorrow's Printers & Publishers, New Delhi, 1982.

TRAPS TO MONITOR GALL MIDGE POPULATION IN RICE

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THE rice gall midge, *Orseolia oryzae* (Wood-Mason) is one of the important insect pests of rice. Although virgin females of gall midge are being used in monitoring the population of this pest in China[1], no such attempt has been made in India. Therefore, the feasibility of using virgin females to attract males with various types of traps was studied.

Delta sticky trap with virgin gall midge females:

Of the three traps tested, only the delta trap was found suitable. The delta traps supplied by M/s. Pest Control (India) Pvt. Ltd., Hyderabad are made of a thick card board sheet, smeared with sticky adhesive on inner surface (figure 1). Virgin gall midge females were caged in 5 × 4 cm glass tube which was fixed inside the trap. The trap was then suspended from a wooden stake at crop canopy level (60 cm). When the delta trap containing 3 virgin females was placed in the middle of rice fields (November 1981), on an average, 24 males were caught per night, while as many as 125 males were caught with 6 females. No males were trapped in the absence of female gall midges. The results indicate the presence of strong female pheromone.

Gall midge population was monitored with delta trap during 1982 at weekly intervals. The average catches varied from 2.3 males/trap/night during December to 120 males in October, while they were nil in February, May to July. The advantages of this trap over the light trap presently under use for monitoring the pest population are ease of handling and convenience of counting since only gall midges are trapped.

Inverted funnel trap to catch live gall midge males:

This trap consisted of a plastic pot with 36 circular

Figure 1. Gall midge virgin female trap (large number of males are sticking on the inner surface of trap.).