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ACKNOWLEDGEMENT. This article is dedicated to Prof. M. Lakshmanan on his 50th birthday. We thank CSIR, New Delhi for financial assistance. We are grateful to the referee for constructive criticism.

Received 28 December 1998; revised accepted 10 March 1999

Multicomponent coordinated defence response of rice to *Rhizoctonia solani* causing sheath blight

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An excellent multicomponent coordinated defence response of rice plants (cv. IET-2233) to fungal attack has been demonstrated and a plausible relationship among them has been proposed. Some selected defence components such as momilactone 'A' (a rice phytoalexin), β-1,3-glucanases and exo chitinases (both pathogenesis related (PR)-proteins) and an enzyme phenylalanine ammonia lyase (PAL) were employed as biochemical parameters for evaluating the degree of response of rice plants to *Rhizoctonia solani* Kühn, a fungus causing sheath blight disease. A systemic fungicide kitazin which reduced disease significantly also concomitantly activated biosynthesis of momilactone A, induced PR-proteins and increased PAL activity in rice. Treatment of rice leaf sheaths with a PR-protein inhibitor (kinetin + NAA) increased disease markedly but inhibited β-1,3-glucanases and exo-chitinase activities in treated plants. Similarly, amino oxyacetic acid (AOA), a PAL inhibitor also enhanced disease intensity and inhibited PAL activity in treated, inoculated plants. Results confirm the coordinated function of various defence components in rice following infection by *Rhizoctonia* and also after abiotic induction of resistance.

USUALLY a plant responds to a pathogen by mobilizing a complex network of active defence mechanisms. The success of the plant in warding-off the pathogenic attack depends upon the coordination among the different defence strategies and the rapidity of the response. It is generally believed that plants defend themselves against pathogenic fungi by producing fungitoxic substances such as phytoalexins, pathogenesis related (PR)-

proteins, oxidized phenols and several other components. But in most cases the role of a single defence component has been reported at a time while working on disease resistance of a host–pathogen system. Hence, an attempt has been made to determine whether a multicomponent coordinated mechanism is operative in disease resistance of rice with special reference to sheath blight. Initially, the amount of production/accumulation of rice phytoalexin (momilactone 'A'), PR-proteins and phenylalanine ammonia lyase (PAL) activity in non-infected and *Rhizoctonia solani*-infected rice plants were measured under similar conditions. PAL is important since it is associated with the production of fungitoxic phenolics and some phytoalexins (PA). The activities of defence components (PA, PR-proteins and PAL) were also reassessed after chemical induction and suppression of constitutive resistance in order to demonstrate their coordinated functions. In this communication we have shown the probable relationship among PA, PR-proteins, PAL and disease resistance of rice on the basis of our experimental results.

Rice grains (*Oryza sativa* L. cv. IET-2233) were disinfected, and germinated on moist filter paper in sterilized Petri dishes. Ten days after germination, the seedlings were transplanted to pots (25 cm diam.) containing non-infested soil. Two-month-old plants were inoculated by inserting sclerotia (10-day-old) of *Rhizoctonia solani* in the 2nd leaf sheaths (from the top) of the plants (one/leaf sheath) and covered with moist polythene bags for 48 h to provide adequate humidity at the initial stage of infection. Disease intensity was assessed following the method of Bera and Purkayastha[1].

Rice phytoalexin momilactone 'A' was extracted and quantified following the method described by Li *et al.*[2]. The infected portions of rice leaf sheaths were boiled in 70% methanol for 3 min. The methanolic extract was evaporated to aqueous phase in a vacuum evaporator at 40°C. The aqueous fraction was extracted with diethyl ether. The ether fraction was evaporated to dryness in a vacuum evaporator and the residue was dissolved in methanol. The methanolic fraction was added to distilled water and applied on a Bond Elut C-18 column and eluted with 80% methanol. The eluate (80% methanolic fraction) was collected, evaporated to dryness at 60°C and dissolved in 0.1 ml methanol (HPLC grade).

Momilactone 'A' content was estimated by a Shimadzu QP_{1000} GC-MS-SIM (fitted with a column J & W Scientific DB-5, inner diameter of 0.25 mm with linear He (helium) flow at the rate of 20 ml/min; the column temperature was raised from 250°C to 300°C at the rate of 10°C/min and kept at 300°C for 5 min). A standard solution of momilactone 'A' (2 µl of 100 µg ml^{-1} solution) was used as reference.

The leaf sheath tissue (1 g fresh wt) of rice was homogenized with 1 ml of 0.1 M sodium citrate buffer (pH 5.0). The buffer extracts were centrifuged at 9600 *g* for 20 min at 4°C and the supernatant used for analysis by polyacrylamide gel electrophoresis[3]. Protein extracts were concentrated by using lyophilizer and adjusted to 3 mg ml^{-1} following Lowry *et al.*[4] and bovine serum albumin (BSA) as a standard.

SDS-PAGE was carried out according to Laemmli[5] using a 12.5% acrylamide separating gel. The gels were stained overnight with 0.03% coomassie brilliant blue and destained in a methanol:acetic acid:water (40:20:50 v/v) mixture.

The leaf sheath tissue (1 g fresh wt) was homogenized in a prechilled mortar pestle with an appropriate homogenizing buffer (1 ml g^{-1}). The homogenate was clarified by centrifugation at 9600 *g* for 20 min at 4°C. The resultant extracts were used for enzyme assays. Protein contents were measured following the method of Lowry *et al.*[4].

β-1,3-glucanase was extracted with sodium citrate buffer (0.1 M, pH 5.0) and assayed colorimetrically by measuring the rate of reducing sugar production from laminarin[6]. The assay mixture (12.5 g laminarin in 2.5 ml of 50 mM sodium citrate buffer, pH 5.0) was preincubated for 10 min at 37°C. Subsequently, 20 µl enzyme was added to the assay mixture and incubated for 0, 5, 10 and 15 min. Finally, 0.5 ml aliquot in each case was taken from the assay mixture and mixed with 0.5 ml of Somogyi[7] reagent and the mixture was heated at 100°C for 15 min under water-saturated air. After cooling, 0.5 ml of Nelson's chromogenic reagent[8] was added to 3.5 ml water and absorbance measured at 660 nm. Glucose standards and appropriate controls were included. The glucanase activity nanokatal (nkat) was defined as the enzyme catalysing the formation of 1 nmol glucose equivalent sec^{-1}.

Exo-chitinase activity was assayed colorimetrically following the method of Boller *et al.*[9]. The reaction mixture (0.5 ml enzyme, 1 mg colloidal chitin, 0.3 µ mol sodium azide and 14 µ mol sodium citrate buffer (pH 4.5)) was incubated at 37°C for 4 h. After incubation, 0.1 ml sodium borate buffer (0.8 M, pH 9.1) was added and the mixture was centrifuged at 1000 *g* for 15 min. *N*-acetyl glucosamine (Glc NAc) was estimated colorimetrically using 0.5 ml supernatant[10]. Standards of

Table 1. Effect of *Rhizoctonia* infection on momilactone 'A' production

Incubation period (h)	DI/plant	Momilactone 'A' (µg g^{-1} fresh wt)
24	0.15	5.83
48	0.35	10.09
72	0.45	8.06
96	0.78	3.74

Age of the plant 60 days at the time of inoculation.
No phytoalexin was detected in healthy non-infected leaf sheaths.
DI, Disease index.

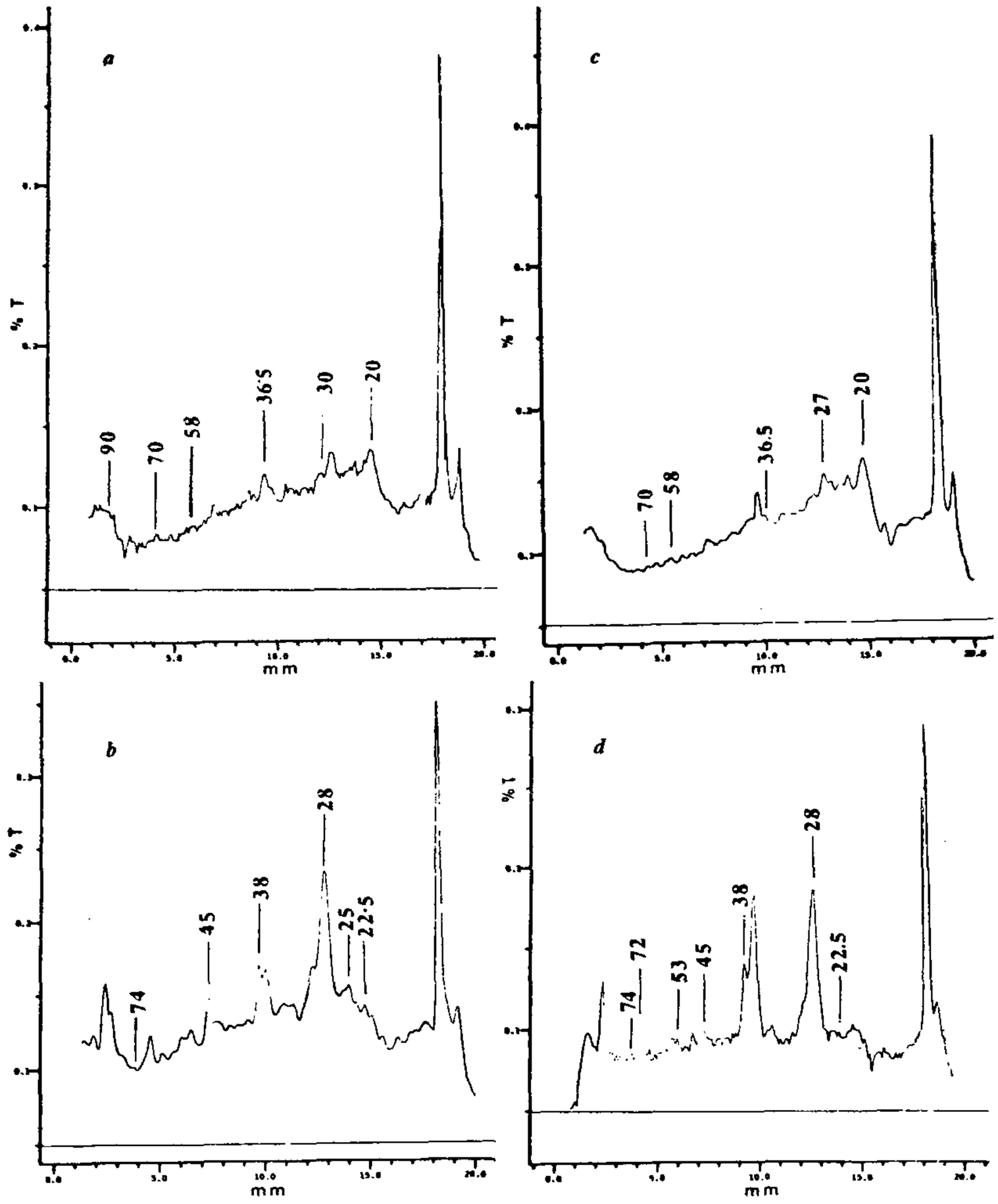

Figure 1. Densitometric scanning of SDS-PAGE showing profiles of defence-related proteins in rice leaf sheaths. *a*, Untreated non-inoculated; *b*, Untreated inoculated; *c*, Kitazin-treated non-inoculated; *d*, Kitazin-treated inoculated.

Glc NAc and appropriate blanks (enzyme, substrate) were also maintained. The enzyme activity nkat was defined as the amount of enzyme producing 1 nmol sec^{-1} Glc NAc equivalent.

In the case of PAL, the extract was prepared by grinding rice leaf sheath in 50 mM Tris-HCl buffer (pH 8.6) containing 10 mM ascorbic acid (1 ml g^{-1} fresh wt). The homogenate was filtered through a cloth, centrifuged at 20,000 *g* for 20 min at 4°C and the supernatant dialysed against 50 mM Tris-HCl buffer (pH 8.6) for overnight at 4°C.

The PAL activity in 1 ml dialysate (containing 3 mg ml^{-1} protein) was assayed spectrophotometrically after adding 1 ml of 30 mM L-phenylalanine dissolved in 50 mM Tris-HCl (pH 8.6). Absorbance at 290 nm was recorded after 2 h of incubation at 30°C against a control of 10 mM D-phenylalanine[11]. Enzyme activity was expressed as µM cinnamic acid mg^{-1} protein min^{-1}.

Leaf sheaths of 60-day-old plants were inoculated, incubated and momilactone 'A' was extracted. Results (Table 1) revealed that *Rhizoctonia*-infected leaf sheaths produced 10.09 µg momilactone 'A' g^{-1} (fresh wt) leaf sheaths while no momilactone 'A' was detected in non-inoculated (control) leaf sheaths.

PR-proteins were also extracted from *Rhizoctonia*-infected rice leaf sheaths following the standard method. The details of the extraction, isolation, identification and characterization of the PR-proteins have been

Table 2. Effect of *Rhizoctonia* infection and kitazin treatment on sheath blight disease development, glucanase and exo-chitinase activities

Treatment	Incubation period (h)	DI/plant[a]	β-1,3-glucanase activity[b]	Correlation coefficient (r)	Exo-chitinase activity[b]	Correlation coefficient (r)
Untreated						
Non-inoculated	0	0	0.539		0.091	
	24	0	0.235		0.112	
	48	0	0.683		0.115	
	72	0	0.475	$r = 0.014^{NC}$	0.115	$r = 0.75^{NS}$
	96	0	0.477		0.112	
	120	0	0.443		0.119	
Inoculated	24	0.23	3.387		0.246	
	48	0.43	4.320	$r = 0.997$	0.336	$r = 0.988$
	72	0.60	5.836	$P < 0.001$	0.543	$P < 0.01$
	96	1.08	7.349		0.627	
	120	1.73	8.580		0.729	
Kitazin-treated						
Non-inoculated	24	0	4.526		0.279	
	48	0	5.664	$r = 0.983$	0.367	$r = 0.965$
	72	0	7.345	$P < 0.01$	0.474	$P < 0.01$
	96	0	7.575		0.462	
	120	0	9.391		0.582	
Inoculated	24	0.08	10.573		0.504	
	48	0.15	12.259	$r = 0.988$	0.706	$r = 0.986$
	72	0.20	14.303	$P < 0.01$	0.909	$P < 0.01$
	96	0.35	15.494		0.960	
	120	0.55	16.336		1.153	

[a]Average of 10 plants/treatment.
Age of the plant 60 days at the time of inoculation.
[b]nkat mg^{-1} protein sec^{-1}; 3 replicates/treatment.
NS, not significant ($P > 0.05$); NC, not correlated.

published earlier[12]. Comparison of the protein profiles of healthy and infected leaf sheaths (Figure 1 *a*, *b*) revealed that inoculated leaf sheaths produced more protein bands (16) than their corresponding non-inoculated leaf sheaths (11 bands). β-1,3-glucanases (known PR-protein, involved in defence reactions of plants) were extracted from the inoculated and non-inoculated rice leaf sheaths. The activity of β-1,3-glucanase was lower (Table 2) in untreated, non-inoculated leaf sheaths than in *Rhizoctonia*-infected leaf sheaths. Approximately 19-fold increase in glucanase activity was observed over the untreated non-inoculated control after 120 h of inoculation. Chitinase (exo-form) activity was relatively low in the untreated, non-inoculated control leaf sheaths, which however, increased 2-fold within 24 h after inoculation and the same activity increased with time up to 120 h (Table 2).

PAL activity of untreated and non-inoculated leaf sheaths was measured after 12, 24, 48, 72 and 96 h of inoculation. A 5-fold increase in PAL activity was observed in inoculated leaf sheaths after 24 h of inoculation (Table 3), which decreased with increase in incubation time.

Fiftysix-day-old plants were sprayed twice at an interval of 2 days separately with different concentrations of kitazin (25 and 480 µg ml^{-1}). Kitazin (480 µg ml^{-1}) was effective (72, 69, 65 and 46% reduction in disease index (DI)/plant after 7, 12, 21 and 28 days, respectively) in controlling sheath blight disease significantly (Figure 2 *f*). It also induced maximum (14.58 µg g^{-1}) and minimum (5.85 µg g^{-1}) amount of momilactone 'A' in inoculated and non-inoculated leaf sheaths respectively (Table 4).

Kitazin-treated, inoculated leaf sheaths showed greater number of PR-proteins compared to non-inoculated kitazin treated leaf sheaths (Figure 1 *c*, *d*). Time course study also confirmed that β-1,3-glucanase activity was higher in kitazin-treated inoculated leaf sheaths than in kitazin-treated non-inoculated ones (Table 2). Exo-chitinase activity was also relatively lower in kitazin-treated non-inoculated leaf sheaths than in kitazin-treated inoculated leaf sheaths (Table 2). Kitazin enhanced PAL activity more in treated inoculated plants than in untreated, non-inoculated control (Table 3).

Table 3. Effect of *Rhizoctonia* infection and kitazin treatment on disease development and PAL activity

Treatment	Incubation period (h)	DI/plant[a]	Specificity activity[b] (μmol cin mg^{-1} protein min^{-1})
Untreated			
Non-inoculated	0	0	0.38
	12	0	0.40
	24	0	0.38
	48	0	0.36
	72	0	0.39
	96	0	0.46
Inoculated	12	0	1.49
	24	0.25	2.03
	48	0.81	1.25
	72	0.92	0.99
	96	1.38	0.77
Kitazin-treated			
Non-inoculated	12	0	1.49
	24	0	1.71
	48	0	1.23
	72	0	1.06
	96	0	0.77
Inoculated	12	0	2.46
	24	0.10	2.70
	48	0.17	2.32
	72	0.25	1.98
	96	0.48	1.52

[a]Average of 12 plants/treatment.
Age of plants 60 days at the time of inoculation.
[b]3 replicates/treatment.

Leaves of 56-day-old rice plants were sprayed twice at an interval of 2 days with a mixture of Kinetin + NAA (1:7.6), a PR-protein inhibitor and inoculated as stated earlier. Healthy, treated and inoculated leaf sheaths were collected after 24, 48, 72 and 96 h, DI/plant was calculated, β-1,3-glucanase and exo-chitinase activities were measured. Although NAA and NAA + kinetin treatment increased sheath blight disease (Figure 2 *c*), there was no marked change in DI for kinetin-treated plants (Table 5). β-1,3-glucanase activity also increased significantly after inoculation in all cases, except NAA + kinetin-treated leaf sheaths where no significant difference in glucanase activity was observed before and after inoculation. The activity slowly decreased in NAA + kinetin-treated, non-inoculated plants with time up to 96 h of incubation.

The results showed that PAL activity increased after inoculation as well as kitazin treatment and that disease intensity decreased in kitazin-treated plants. This suggests that PAL may play a role in the reduction of disease. Hence, an attempt was made to ascertain whether suppression of PAL could alter the disease reaction in rice leaf sheaths. The plants (56-day-old) were sprayed with AOA (amino oxyacetic acid, 500 μg ml^{-1}) twice at an interval of 48 h and inoculated after another 48 h.

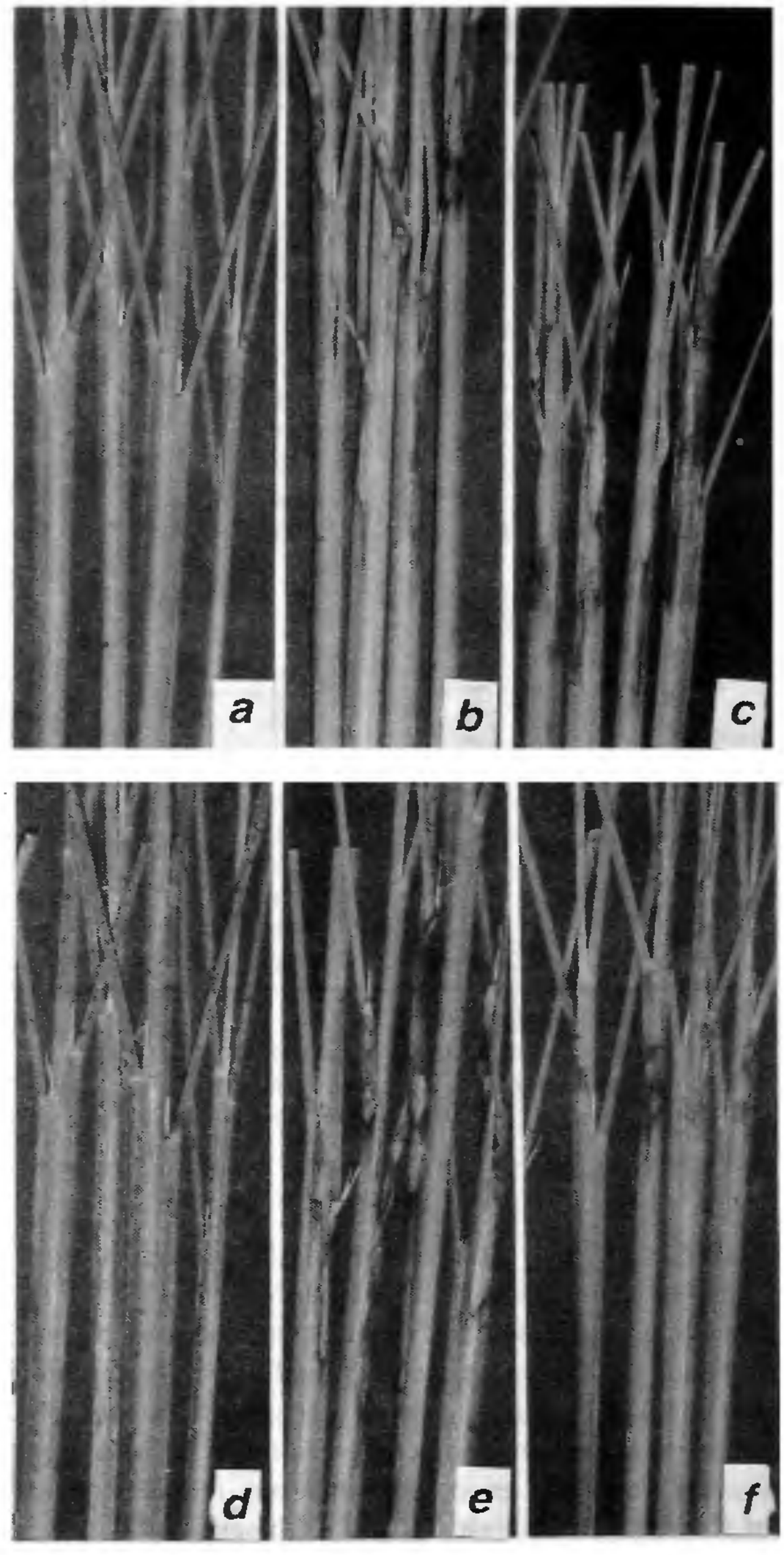

Figure 2. Inducer and inhibitor of PR-proteins affecting development of sheath blight of rice. *a*, *d*, Untreated non-inoculated leaf sheaths; *b*, *e*, Untreated inoculated leaf sheaths; *c*, Inhibitor (kinetin + NAA)-treated inoculated leaf sheaths showing increase in disease intensity; *f*, Inducer (kitazin)–treated inoculated leaf sheaths showing reduction in disease intensity (restricted lesions only).

Healthy untreated, healthy treated, untreated inoculated and treated inoculated leaf sheaths were collected and DI/plant and PAL activity were measured (Table 6). AOA treatment enhanced disease and also reduced PAL expression. However, the enzyme activity was relatively higher in inoculated than in non-inoculated leaf sheaths.

Table 4. Effect of kitazin on the production of momilactone 'A'

Treatment	Momilactone A* (μg g^{-1} fresh wt)
Untreated	
Non-inoculated	0
Inoculated	10.7
Kitazin-treated	
Non-inoculated	5.85
Inoculated	14.58

*48 h after inoculation.

PAL activity and incubation time showed negative correlation for all combinations excepting for untreated non-inoculated leaf sheaths. Results strongly support the involvement of PAL in disease reaction in rice.

Any drastic change in the basic physiology of a plant could influence the development of a disease. In the present communication multicomponent coordinated defence response of rice plants to *Rhizoctonia* infection has been reported for the first time. At the outset, pathogenicity of *R. solani* was tested on 6 rice cultivars of which IET-2233 was the most susceptible and hence an

Table 5. Effect of PR-protein inhibitor (NAA + kinetin) on β-1,3-glucanase and exo-chitinase activities

Treatment	Incubation period (h)	DI/plant[a]	β-1,3-glucanase activity[b]	Correlation coefficient (r)	Exo-chitinase activity[b]	Correlation coefficient (r)
Untreated						
Non-inoculated	0	0	0.605		0.081	
	24	0	0.669		0.112	
	48	0	0.624	$r = 0.503^{NS}$	0.111	$r = 0.17^{NC}$
	72	0	0.774		0.081	
	96	0	0.657		0.088	
Inoculated	24	0.15	2.901		0.445	
	48	0.40	3.973	$r = 0.981$	0.529	$r = 0.865^{NS}$
	72	0.58	4.868	$P < 0.05$	0.552	
	96	1.17	6.532		0.550	
Kinetin-treated						
Non-inoculated	24	0	0.661		0.089	
	48	0	0.785	$r = 0.157^{NC}$	0.098	$r = 0.809^{NS}$
	72	0	0.716		0.093	
	96	0	0.660		0.139	
Inoculated	24	0.21	2.624		0.304	
	48	0.42	3.877	$r = 0.886^{NS}$	0.293	$r = 0.830^{NS}$
	72	0.69	4.264		0.320	
	96	1.33	4.281		0.425	
NAA-treated						
Non-inoculated	24	0	0.677		0.082	
	48	0	0.677	$r = -0.948^{NS}$	0.082	$r = 0.124^{NC}$
	72	0	0.595		0.069	
	96	0	0.545		0.089	
Inoculated	24	0.21	2.490		0.237	
	48	0.50	2.943	$r = 0.986$	0.372	$r = 0.720^{NS}$
	72	0.79	3.986	$P < 0.05$	0.359	
	96	1.25	4.445		0.359	
NAA + kinetin-treated						
Non-inoculated	24	0	0.535		0.037	
	48	0	0.413	$r = -0.973$	0.088	$r = -0.246^{NC}$
	72	0	0.376	$P < 0.05$	0.057	
	96	0	0.207		0.031	
Inoculated	24	0.35	0.629		0.075	
	48	0.69	0.541	$r = -0.982$	0.044	$r = 0.507^{NS}$
	72	1.20	0.488	$P < 0.05$	0.101	
	96	2.21	0.450		0.088	

[a]Average of 12 plants/treatment.
Age of the plant 60 days at the time of inoculation.
[b]nkat mg^{-1} protein sec^{-1}; 3 replicates/treatment.
NS, not significant ($P > 0.05$); NC, not correlated.

Table 6. Effect of amino oxyacetic acid on disease development and PAL activity

Treatment	Inocubation period (h)	DI/ plant[a]	Specific activity[b]	Correlation coefficient (r)
Untreated				
Non-inoculated	0	0	0.26	
	12	0	0.22	
	24	0	0.30	$r = 0.65^{NS}$
	48	0	0.27	
	72	0	0.26	
	96	0	0.35	
Inoculated	12	0	1.48	
	24	0.21	1.61	$r = -0.913$
	48	0.58	1.13	($P < 0.05$)
	72	0.69	1.09	
	96	0.90	0.58	
Amino oxyacetic acid-treated*				
Non-inoculated	12	0	0.27	
	24	0	0.26	$r = -0.0345^{NC}$
	48	0	0.26	
	72	0	0.23	
	96	0	0.28	
Inoculated	12	0	0.72	
	24	0.25	0.86	$r = -0.843^{NS}$
	48	0.69	0.73	
	72	0.88	0.53	
	96	1.04	0.40	

[a]Average of 12 plants/treatment.
[b]µmol cin mg^{-1} protein min^{-1}; 3 replicates/treatment.
*500 µg ml^{-1}.
NS, not significant ($P > 0.05$); NC, not correlated.

attempt was made to control sheath blight disease by a systemic fungicide kitazin. Although kitazin reduced the disease, it also induced phytoalexin momilactone 'A' in rice leaf sheaths. However, the accumulation of phytoalexin was always more in treated inoculated leaf sheaths than in non-inoculated ones. Induction of phytoalexin by a fungicide is not unusual since Reilly and Klarman[13] tested 27 fungicides of which 15 induced detectable quantities of phytoalexin (hydroxyphaseollin) in soybean plants. These results suggest that reduction of disease by some fungicides may be associated with induction of phytoalexin, a known defence component. Apart from phytoalexins, role of PR-proteins in plant defence has also been suggested[14–16]. Therefore, it is not unreasonable to speculate that a relationship might exist between the production of phytoalexins and PR-proteins since both are defence components and are regulated by host genes. To trace the relationship between phytoalexins and PR-proteins, PAL may be considered as an important factor. PAL plays an active role in the biosynthesis of cinnamic acid from phenylalanine; cinnamic acid is closely associated with biosynthetic pathways of some isoflavonoid phytoalexins[17]. Salicyclic acid, an inducer of PR-proteins, is also synthesized from cinnamic acid via benzoic acid[18] and the accumulation of salicylic acid may induce production of PR-proteins. The results of the present study showed that kitazin not only reduced sheath blight but also induced both PR-proteins and rice phytoalexin (RPA). Precursors of RPA can be derived

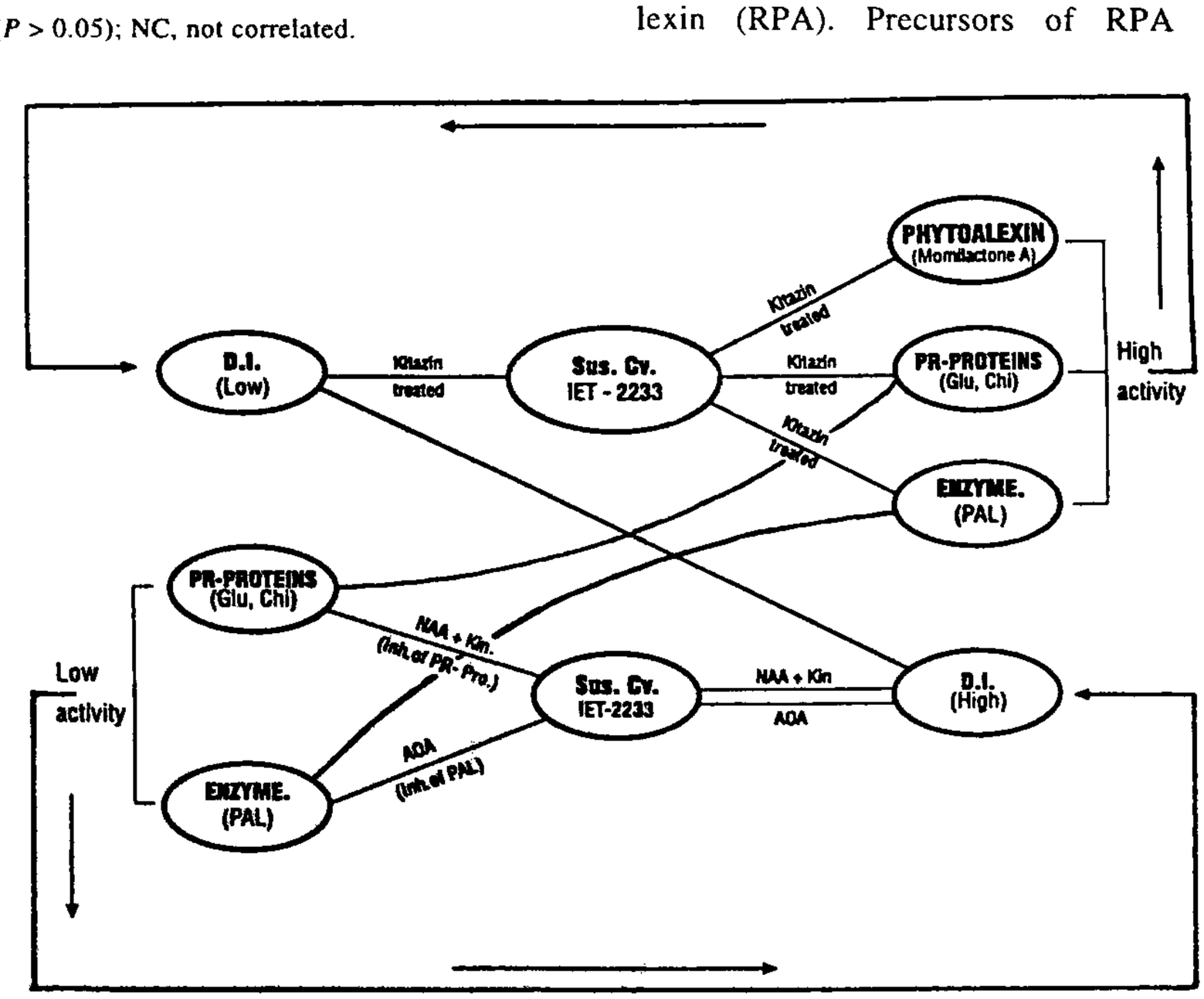

Figure 3. Schematic diagram showing involvement of different defence components in rice plants during development of sheath blight disease. Sus. cv., Susceptible cultivar; Glu-β, 1,3-glucanase; Chi, chitinase; PAL, phenylalanine ammonia lyase; NAA, napthaleneacetic acid; AOA, amino oxyacetic acid; DI, disease index; kin, kinetin.

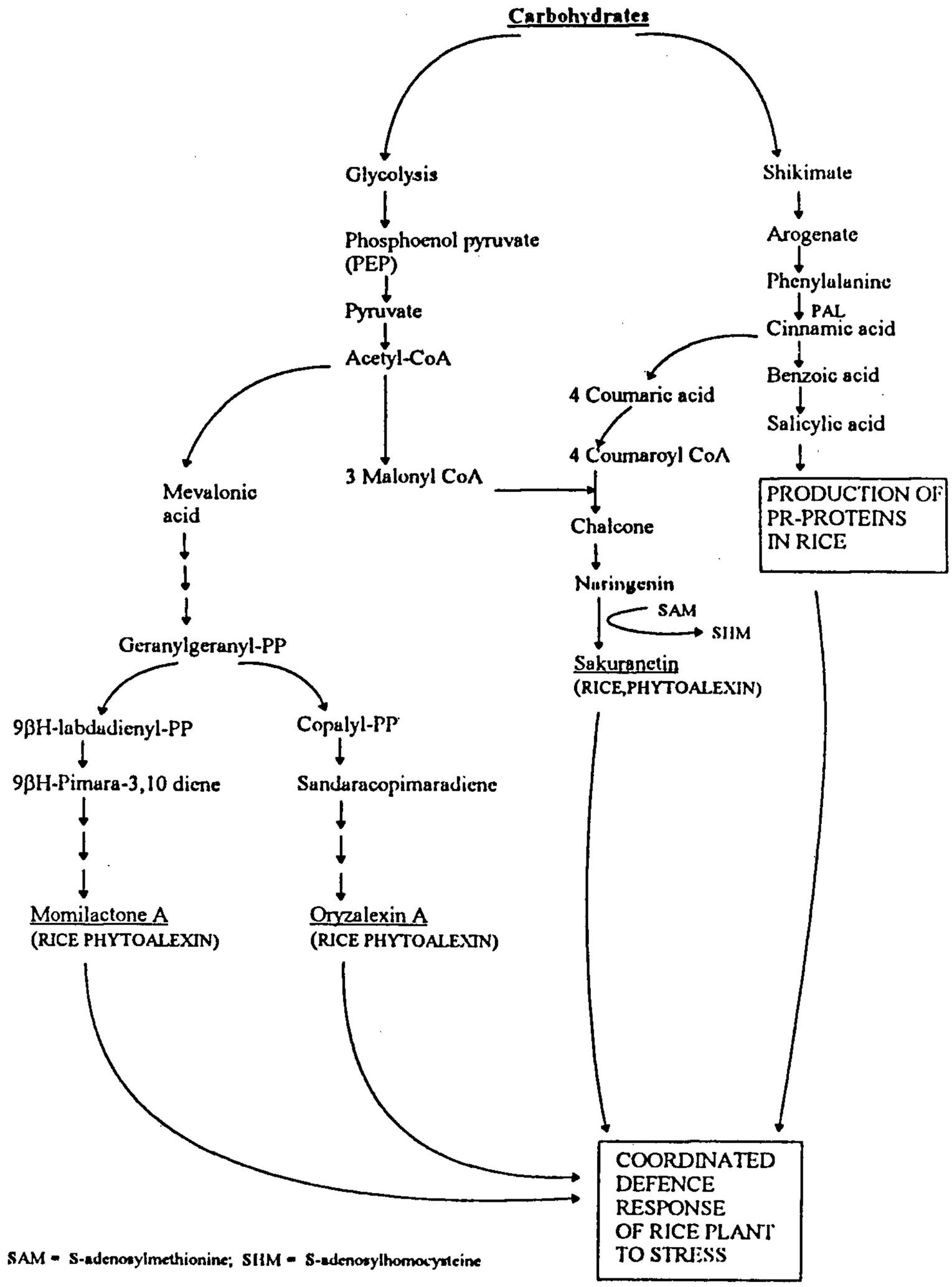

Figure 4. Probable relationship among phytoalexins, PR-proteins, PAL activity and defence response of rice against stress (biotic or abiotic).

from one of the three biosynthetic pathways (i.e. shikimate, acetate-malonate and acetate-mevalonate) or a combination of 2 or 3 pathways. Several key enzymes such as PAL, cinnamate-4-hydroxylase, 4-coumarate coenzyme A, chalcone synthase and chalcone isomerase of phytoalexin biosynthesis may be coordinately regulated. Usually phytoalexin accumulation after infection or treatment with inducers is consistent with mRNA production and enzyme activity[19]. Thus, it is likely that kitazin influences the biosynthetic pathways of RPA and PR-proteins (Figures 3, 4). A relationship between the two has been proposed. To confirm this, rice leaf sheaths were treated with a mixture of kinetin + NAA (1:7.6), which inhibits both glucanase and chitinase. The results showed a significant increase in DI and concomitant decrease in glucanase and chitinase activities. These findings are in sharp contrast with that of inducer (kitazin)-treated rice plants where reduction in disease intensity and increase in glucanase and chitinase activities were noted.

Any host cv. resistant or susceptible has its natural defence to pathogens and as such occurrence of multicomponent defence response of a susceptible rice cv. is not unnatural. But why is this rice cv. IET-2233 susceptible

to the disease in spite of the coordinated defence response? It is likely that the speed of defence response of a susceptible cv. is usually slow or weak and the production of defence components is not sufficient for the total inhibition of growth of the pathogen or the syntheses of certain critical components of defence are not activated by the infection process. The delay in recognition of the pathogen and induction of the defence response, in this case, are also not unlikely. It has been possible to enhance the speed of response to some extent by kitazin which is evident from the higher production of phytoalexin, PR-proteins and higher activity of PAL in treated than in untreated plants.

Multicomponent, coordinated defence response of tobacco was reported earlier by Oelofse and Dubery[20]. The tobacco cells in cultures were treated with heat released soluble cell wall elicitors from mycelial walls of the pathogen (*Phytophthora nicotianae*). The experiments reported here suggest that a multicomponent, coordinated defence mechanism is also operative in rice plants after *Rhizoctonia solani* infection. Since there are practically no known sources of resistance against *R. solani* in the rice germplasm it would be worthwhile to identify potent inducers of multicomponent defence which could be exploited in the control of sheath blight.

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ACKNOWLEDGEMENTS. We thank to Prof. Osamu Kodama, Laboratory of Bioorganic and Pesticide Chemistry, Ibaraki University, Japan for helping us to quantify momilactone 'A'. The financial assistance received from UGC is gratefully acknowledged.

Received 15 October 1998; revised accepted 18 February 1999

Characterization of tobacco mosaic virus isolated from tomato in India

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Tomato mosaic tobamovirus (ToMV) differs from the type strain of tobacco mosaic virus (TMV) in producing local lesions instead of systemic infection on *Nicotiana sylvestris*. An isolate collected from Kolar district of Karnataka which produced this differential host reaction was propagated in the greenhouse on *N. tabacum* cv. Samsun and purified. The virus is a rigid rod shaped particle with a coat protein of molecular weight 18 kDa and genomic RNA of size 6.3 kb. A cDNA library was constructed using a specific primer designed based on the conserved nucleotide sequence at the 3′ non coding region of tobamoviruses. The cDNA library was screened for recombinant clones and the recombinant clone 82 with an insert of size 1.04 kb was sequenced in both directions. This sequence was compared with the genomic sequence of TMV and ToMV which showed 93.1 and 73.7 per cent identity, respectively. The sequence encompassed the 3′ non coding region, the complete coat protein ORF and 467 nucleotides of the movement protein. The deduced amino acid sequence of the coat protein was compared with that of TMV and ToMV. This sequence was nearly identical to TMV with nine amino acid changes whereas thirty-two changes were observed with ToMV. This suggests that the virus under study is a strain of TMV and therefore we have named it as tobacco mosaic virus tomato strain from Karnataka, India (TMV(Tom-K)).

KARNATAKA is a major producer of tomatoes in India. Several tomato hybrids are grown all round the year.

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