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Induction of PR-proteins in Co 43 rice plants following antiviral principle application and inoculation with rice tungro virus

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Abstract : The levels of PR-proteins following application of antiviral prinicples (AVPs) and inoculation with rice tungro virus (RTV) at 5, 10, 15, 20 and 25 days after inoculation were assessed by enzyme linked immunosorbent assay (ELISA) technique. Treatment of rice plants with pigeonpea-AVP (PAVP) and mungbean - AVP (MAVP) and inoculation with RTV increased the levels of PR-proteins (PR1a and PR2) to significant levels compared to healthy and RTV-inoculated control plants. The reduction in per cent infection of RTV and restriction of titres of RTD-associated viruses may be as a result of the induction of defense-related compounds induced by the AVPs. *(Key words : PR-proteins, AVP application, RTV).*

Tungro is a composite disease of rice induced by dual infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). The use of antiviral agents as a component of the biological control aimed to reduce the inoculum density or disease-producing activities of the viral pathogens (Baker and Cook, 1974) has attracted the attention of several researchers. Accumulation of PR-proteins which have been described to play a role in induction of resistance to pathogens have been reported by many workers. The AVPs from seed sprouts of pigeonpea (PAVP) and mungbean (MAVP), at 10 per cent concentration were found to be the most effective in reducing the RTV infection. In this paper attempts have been made to study the accumulation of PR-proteins in AVP-treated and RTV-inoculated plants.

Materials and Methods

Using indirect enzyme linked immunosorbent assay (ELISA), PR-proteins *viz.*, PR1a and PR2 in AVP-treated rice plants were detected (Mowat, 1985). The treatments are as follows:

1. Healthy
2. RTV-inoculated
3. AVP-treated
4. AVP-treated and RTV-inoculated

The seed sprout extracts of PAVP and MAVP were sprayed at 10 per cent concentration on 15 days old Co 43 rice plants raised under insect proof conditions. After 24 hr the seedlings were inoculated with viruliferous green leaf hoppers at the rate of two insects per plant.

The plant samples were collected at 5, 10, 15, 20 and 25 days after inoculation and the presence of PR-proteins were detected by using specific antisera to PR1a and PR 2 (Kindly supplied by John Ryals, Ciba-Geigy Corporation). Alkaline phosphatase conjugated goat antirabbit 1gG was used as enzyme conjugate at 1:7000 dilution and p-nitrophenyl phosphate (PNP) was used as substrate. The rabbit IgG was used at 1:100 dilution. Development of bright yellow colour indicated positive reaction. Absorbance at 405 nm was recorded using the ELISA reader (Lab System, Multiscan MS).

Results and Discussion

The levels of PR-proteins following application of AVPs and inoculation with RTV at 5, 10, 15, 20 and 25 days after inoculation were assessed by ELISA technique (Table 1). Inoculation with RTV stimulated the accumulation of PR1a protein to significant levels. This accumulation rapidly increased from 5 days after inoculation and almost doubled at 25 days after inoculation whereas the PR1a protein level was not altered in the healthy plants throughout the period of study. Treatment of rice plants with PAVP and MAVP also rapidly increased the levels of PR1a protein at different period after inoculation. Inoculation of AVP-treated plants further stimulated the accumulation of PR1a protein to significant levels. Such increases in the PR1a protein levels may indicate the possible induction of new messenger RNAs, the production of which is triggered in response to the RTV inoculation.

The change in the levels of PR2 protein, following AVP treatment and inoculation with RTV were assessed at the same intervals as in PR1a protein.

Table 1. Determination by ELISA of levels of PR1a protein induced by antiviral agents in Co 43 rice plants inoculated with RTV.

Sl. No.	Treatments	PR1a protein concentration (OD value at 405 nm)				
		Days after inoculation				
		5	10	15	20	25
1	Healthy	0.207[d]	0.228[d]	0.251[e]	0.260[e]	0.271[e]
2	RTV - inoculated	0.468a (126.09)	0.654[a] (186.84)	0.857[c] (241.43)	0.890[c] (242.31)	0.920[c] (239.48)
3	PAVP - treated	0.457[ab] (120.77)	0.548[c] (140.35)	0.619[d] 146.61)	0.750[d] 188.46)	0.890[dc] 228.41)
4	PAVP - treated + inoculated	0.512[a] (147.34)	0.603[ab] (164.47)	0.970[a] (286.45)	1.074[a] (313.08)	1.240[a] 358.30)
5	MAVP - treated	0.443[c] (114.00)	0.505[c] 121.49)	0.589[d] 134.66	0.720[d] (176.92)	0.910[c] 235.79)
6	MAVP - Treated + inoculated	0.476[a] (129.75)	0.596[ab] 161.40)	0.903[b] 259.76)	0.984[b] (278.46)	1.010[b] (272.69)
7	Blank	0.006[e]	0.004[e]	0.008[f]	0.006[f]	0.004[f]

Mean of three replications

	CD (P=0.05)
Treatments	0.060
Days	0.051
T x D	0.135

In a column, means followed by common letter are not significantly different at 5% level by DMRT. Numbers in the parenthesis are percentage increase over healthy.

Table 2. Determination by ELISA of levels of PR2 protein induced by antiviral agents in Co 43 rice plants inoculated with RTV.

Sl. No.	Treatments	PR2 protein concentration (OD value at 405 nm)				
		Days after inoculation				
		5	10	15	20	25
1	Healthy	0.204d	0.210e	0.235e	0.254d	0.361d
2	RTV - inoculated	0.340e (126.09)	0.554c (159.04)	0.620c (163.83)	0.754b (196.85)	0.825d
3	PAVP - treated	0.402b (97.06)	0.525c 150.00)	0.610c 159.57)	0.740b (191.34)	0.805bc
4	PAVP - treated + inoculated	0.485a (137.75)	0.590a (180.95)	0.725a (208.51)	0.890a	0.975a
5	MAVP - treated	0.410b (100.98)	0.490d (133.33)	0.540d (129.79)	0.665c	0.788c
6	MAVP - Treated + inoculated	0.464a (127.45)	0.580ab (176.19)	0.875a (272.34)	0.920a	0.960a
7	Blank	0.020e	0.060f	0.045f	0.002f	0.004e

Mean of three replications

	CD (P=0.05)
Treatments	0.015
Days	0.013
T x D	0.034

In a column, means followed by common letter are not significantly different at 5% level by DMRT. Numbers in the parenthesis are percentage increase over healthy.

In the healthy plants, PR2 protein contents showed slight variations due to increase in age (Table 2), whereas in the RTV inoculated rice plants there was a dramatic increase in PR2 from 10 days after inoculation. Application of PAVP and MAVP stimulated the accumulation of PR2 proteins still further and the accumulation progressively increased with increase in time after inoculation reaching the maximum at 25 days after inoculation. When the AVP-treated plants were inoculated with RTV remarkable accumulation of PR2 protein was observed at all periods of sampling.

The induction of PR1a and PR2 proteins and their accumulation to very high levels may probably indicate the protective role of these proteins. The reduction in per cent infection of RTV and restriction of titres of RTD associated viruses may be as a result of the induction of defense-related compounds induced by the AVPs.

The PR-proteins are induced in the leaves of tobacco and other plants by a number of stimuli and most notably by pathogen infections that induce the hypersensitive response (VanLoon, 1985). Three of these proteins PR1a, PR1b and PR1c isolated from tobacco cultivars have been characterized chemically and immunologically (Antoniw *et al.* 1985; Matsuoka and Ohashi, 1984) and their accumulation in leaves have been described. Ohashi and Matsuoka (1985) observed the induction of PR-proteins in tobacco leaves in response to infection with TMV. A rabbit antibody to PR1a also reacted with two other PR proteins designated as PR1b and PR1c in double immunodiffusion tests.

Ohashi and Matsuoka (1987) reported that the PR-proteins were induced in tobacco leaves by the treatment with potassium salicylate. Total PR-protein levels increased with time after these treatments and reached maximum at nine days after treatment. Induction of PR-2 proteins with Bet1, 3-glucanase activity was detected in tobacco plants infected with TMV (Kauffmann *et al.* 1987). The function of these novel proteins is the development of resistance to viruses.

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