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Induction of resistance in rice to rice tungro virus using horsegram (*Vigna unguiculata* Walp. *Sub* sp. *unguiculata*) seed sprout extrac

R. RABINDRAN, P. MUTHULAKSHMI, T. GANAPATHY AND SABITHA DORAISWAMY
Dept. of Pl. Patho., Centre for Pl. Prot. Studies, Tamil Nadu Agrl. Univ., Coimbatore-641 003, Tamil Nadu

Abstract: The antiviral principles (AVPs) from various non-host plant species were tested for their ability to induce resistance in rice against rice tungro virus (RTV). The extract from horsegram seed sprout at 10 per cent concentration reduced the infection to 44.00 per cent followed by 56.00 per cent in 5 per cent extract. The ELISA test also revealed that there was a distinct reduction in the concentration of virus particles (RTBV and RTSV) at both concentrations. The mean titre of RTSV was 0.111 and 0.100 in 10 and 5 per cent concentration respectively of horsegram seed sprout extract treated plants. Thus the horsegram seed sprout extract has interfered with the multiplication of rice tungro virus particles. The AVPs do not appear to have any effect on leaf hopper feeding.

Key words : Rice, RTV, Horsegram, Induction of Resistance.

Introduction

Rice tungro disease (RTD) is one of the important diseases among the 15 virus diseases affecting rice which causes serious losses in India and South East Asian countries. In the recent years, scientists have realized the need to find out effective alternatives to conventionally used chemicals which are known to be responsible for environmental pollution in addition to inducing resistance in pathogens to chemicals. Indian sub continent is a treasure of antiviral principles. The antiviral principles from the seed sprouts of pigeonpea and mungbean have been shown to be effective in reducing RTV infection (Narayanasamy and Viswanathan, 1992; Muthulakshmi and Narayanasamy, 1996). Serological evidences for the effectiveness of horsegram (*Vigna unguiculata* Walp *Sub. sp. unguiculata*) extract against the RTBV and RTSV are provided here under.

Materials and Methods

The efficacy of seed sprout extracts from various plants in reducing RTD infection were tested in pot culture at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore during 1997. The plant species are listed in Table 1. The seeds were soaked in water for 72 hr. and the sprouts were macerated. After maceration, the extracts were diluted with distilled water to get a 10 per cent concentration (wt/vol). Extracts were sprayed at 5 and 10 per cent concentration on twenty-five number of 15 days old TN 1 rice seedlings. Teepol was added to the spray fluid @ 1 ml/litre. Plants sprayed with distilled water and teepol served as control. Rice plants were infested 24 hr after imposing the treatments @ of two viruliferous green leafhoppers (*Nephotettix virescens* Distant) per plant. The experiment was conducted in a completely randomized design. The percentage of infected plants and the per cent reduction in infection over the control was calculated.

Leaf samples were collected 25 days after inoculation and tested by indirect ELISA to determine the presence of rice tungro bacilliform (RTBV) and rice tungro spherical virus (RTSV).

The DAC indirect enzyme-linked immunosorbent assay (DAC-ELISA) was performed to assay the presence of RTBV and RTSV using specific Polyclonal Antibody. RTV antigen was diluted to 1:100 in carbonate bicarbonate buffer (pH 9.6), coated at 100 µl/well of ELISA plate (Corning modified flat bottom polystrene) and incubated over night at 4°C. The plate

ble 1. Effect of non-host seed sprout extracts on RTV infection

No.	Treatments	Concentration	Percent infection	Percent reduction over control
	Horsegram	10%	44.00 (41.54)	54.17
		5%	56.00 (48.69)	41.67
	Sesbania sesban	10%	64.00 (53.30)	33.33
		5%	72.00 (58.37)	25.00
	Sesbania rostrata	10%	72.00 (68.53	25.00
		5%	76.00 (63.10)	20.83
	Soundal	10%	76.00 (63.10)	20.83
		5%	88.00 (72.44)	8.33
	Sunnhemp	10%	96.00 (81.44)	0.00
		5%	96.00 (81.44)	0.00
	Control	-	96.00 (81.44)	-

ble 2. Determination of RTSV and RTBV titre in the rice plants following horsegram application and RTV inoculation through ELISA

No.	Treatments	Virus concentration (Absorbance at 405 nm)			
		RTSV		RTBV	
		Range	Mean	Range	Mean
1.	Horsegram 10% sprayed and inoculated	0.057-0.321	0.111	0.055-0.145	0.103
2.	Horsegram 5% sprayed and inoculated	0.062-0.141	0.100	0.049-0.197	0.105
3.	RTV-inoculated control	0.121-0.801	0.184	0.115-0.741	0.156
4.	Healthy	0.062-0.075	0.067	0.060-0.089	0.072
5.	Blank	0.061-0.073	0.0630	0.060-0.099	0.069

was washed 3 times with Phosphate buffer saline-T (PBS-T). The rabbit IgG antiserum specific to RTBV and RTSV was diluted to 1:200 with PBS-T buffer and incubated for 30 min at 37°C. Then the plates were washed 3 times with PBS-T. The alkaline phosphatase conjugated goat anti-rabbit IgG was diluted to 1:7000 and delivered to the well and incubated for one hour at 37°C. Then the plate was washed 5 times with PBS-T. Freshly prepared substrate solution containing p-nitropheyl phosphate (PNP) (4 mg/ml in 10% diethanolamine buffer) was added and incubated at room temperature for 1 hr. The reaction was stopped with 3 M NaOH. Absorbance at 05 nm was recorded using the ELISA reader (Lab System, Multiscan MS).

Results and Discussion

The reduction of RTV infection by AVPs from plant sources have been reported earlier

by several workers (Srinivasulu and Jeyarajan, 1986; Aiyanathan and Narayanasamy, 1988; Selvaraj, 1990; Narayanasamy and Viswanathan, 1992; Muthulakshmi and Narayanaswamy, 1996). The seed sprouts of pigeonpea mungbean, blackgram, cowpea, lab lab and soybean were quite effective in reducing RTV infection. Muthulakshmi and Narayanasamy (1996) reported that the seed sprout extracts of pigeonpea and mungbean were most effective in reducing the RTV infection.

The concentration of both viruses (RTBV and RTSV) were significantly reduced in extract and mungbean seed sprout extract prior to inoculation (Muthulakshmi and Narayanasamy, 1996).

Reduction in virus infection might be brought out because of different kinds of action of AVPs either directly on the virus or indirectly on the host plants. The AVPs might act as competitive inhibitors of virus infection by occupying the infectible sites which become unavailable for virus particles to initiate the infection as suggested by Regalti and Weintraub (1962). AVPs might block the mRNA activity of viral RNA and inturn affect virus transcription (Owens *et al.* 1973).

The results indicated that extract from horsegram seed sprout at 10 per cent concentration reduced infection to 44.00 per cent followed by 56.00 per cent in 5 extract (Table 1).

The ELISA test results of the horsegram treated leaves revealed that there was a distinct reduction in the concentration of virus particles both RTBV and RTSV at both the concentration *viz.* 5 and 10 per cent. The mean titre of RTSV was 0.111 and 0.100 and RTBV was 0.103 and 0.105 in 10 and 5 per cent concentration of horsegram seed extract treated plant respectively.

The virus concentration was reduced in horsegram treated plants at both concentrations for both viruses (RTBV & RTSV) as compared to RTV inoculated control plants.

Thus application of horsegram seed sprout extract even at 5 per cent concentration protected the plants against both RTSV and RTBV infecti as reflected by the reduction in per cent infecti and the ELISA results, which showed that t concentration of both viruses were reduced AVP-treated plants even if infected by the virus (Table 2). The above finding revealed th the horsegram seed sprout extract has interfer with the multiplication of rice tungro vir particles (Table 2). Studies in the AVP's a molecular biology of resistance induced by t AVP's in rice are in progress.

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