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Association of Polyphenoloxidase with Induced Antiviral Resistance in Two Host-Induced Combinations

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Systemic resistance induction in *Cyamopsis tetragonoloba* (CT) against infection by sunnhemp rosette virus (SRV) was studied subsequent to application of systemic resistance inducers from *Clerodendrum aculeatum* (CA-SRI) *and C. fragrans* (CF-SRI). Resistance developed generally 2 h after SRI application at the site as well as at remote site and attained maximum effect aorund 4-8 h after treatment at both sites. Once induced, the antiviral activity persisted up to 24 h. Reduction in SRV infectivity in both CT-CA and CT-CF was associated with an increase in polyphenoloxidase (PPO) activity. Maximum elevation in PPO activity in all cases occurred 2 h after SRI application, and appears to be commensurate with a high degree of resistance developed at that time. PPO may mediate resistance induction by producing quinones which affect SRV multiplication and/or spread.

Key Words: Induction Phenoloxidase systemic resistance Virus Quinone

Development of systemic resistance against virus infection in susceptible plants by aqueous extracts obtained from higher plant parts is known (Verma & Prasad, 1983). These extracts induce resistance at the sites of application as well as at sites away from it and are termed systemic resistance inducers (SRIs) (Verma & Prasad, 1983). Reduction in virus infectivity is displayed in terms of decreased symptom expression (Verma & Prasad, 1983).

Polyphenoloxidase has been implicated in disease resistance and is increased during systemic resistance induced by virus infection and SRIs (Prasad, 1986; Van Loon, 1983). In this paper systemic resistance induced in *C. tetragonoloba* against SRV infection, subsequent to separate treatments with two different SRIs and the association of such antiviral state with alteration in polyphenoloxidase activity profile is presented.

MATERIALS & METHODS

Seeds of *Cyamopsis tetragonoloba* (L) Taub. were sown in sterilised soil and kept in an insect-free glass house. Plants were used at three leaf stage. Sunnhemp rosette virus (SRV), was cultured on its systemic host *Crotalaria juncea* L.

SRIs were obtained from fresh leaves of *Clerodendrum aculeatum* L. and *C. fragrans* Vent. and applied onto the basal leaves of *C. tetragonoloba*. Antiviral activity was determined by challenge inoculating the treated lower as well as non-treated upper leaves by SRV 30 sec., 2 h, 4 h, 8 h, 16 h and 24 h after SRI application. At the same time interval, leaf discs were isolated and tested for total proteins and polyphenoloxidase activity. Controls were maintained by treating the plants with distilled water. Antiviral activity, enzyme preparation and assay were performed according to Prasad (1986) and total proteins were estimated by the method of Scopes (1982). The experiment was performed in triplicate.

RESULTS CA-SRI induced resistance was not recorded 30 sec after treatment but increased to 71 and 70 per cent at the site and remote site respectively, when challenge inoculated 2 h subsequent to SRI administration. The maximum protection attained between 4 and 16 h was almost maintained up to 24 h at both sites (Table 1). but CF-SRI exhibited antiviral state only 30 sec after its application at site with a 75 per cent decrease in SRV expression. At remote site however, only 17 per cent reduction in virus infectivity was recorded. At both sites in the CT-CF system, antiviral activity reached its maximum at 4 h which was maintained throughout at site but declined slowly at remote site to 75 percent after 24 h of SRI application (Table 1).

Total proteins fluctuated in the treated plants. They increased after 2 h of treatment and reached maximum at 4 h, declined thereafter up to 16 h and increased in 24 h samples. This pattern was consistent at all sites of assay (Table 2).

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Ta l: Systemic Resistance Induction in *C. tetragonoloba* After Application of SRIs from *C. aculeatum* and *C. fragrans*.

Time interval	Percent decrease in lesion number			
	CT - CA		CT - CA	
	At site	At remote site	At site	At remote site
30s	0	0	75	17
2h	71	70	85	60
4h	96	95	98	92
8h	97	97	100	87
16h	99	98	100	85
24h	91	88	100	75

CT: *Cyamopsis tetragonoloba* ; CA: *Clerodendrum aculeatum*; CF: *C. fragrans*

Table 2 Alterations in Total Proteins of *C. tetragonoloba* Treated with SRIs from *C. aculeatum* and *C. fragrans*.

Time interval	Total protein concentration in mg ml^{-1}			
	CT - CA		CT - CA	
	At site	At remote site	At site	At remote site
30s	4.0	3.0	3.7	3.5
2h	4.8	3.9	4.1	4.2
4h	6.1	5.7	6.5	6.1
8h	4.6	3.5	5.2	4.9
16h	4.2	2.8	4.2	4.1
24h	5.9	5.3	6.0	5.7

CT: *Cyamopsis tetragonoloba* ; CA: *Clerodendrum aculeatum*; CF: *C. fragrans*

Table 3 Alteration in Polyphenoloxidase Activity in *C. tetragonoloba* upon Treatment with *C. aculeatum* and *C. fragrans* SRIs.

Time interval	CT - CA				CT - CA			
	At site A		At remote site		At site		At remote site	
	ΔO.D.[a]	Percent increase	ΔO.D[a]	Percent increase	ΔO.D[a]	Percent increase	ΔO.D[a]	Percent increase
Control	.05	—	.01	—	.05	—	.04	—
30s	.05	0	.01	0	.07	40	.03	0
2h	.11	120	.05	400	.13	160	.12	200
4h	.10	100	.02	100	.10	100	.09	125
8h	.06	20	.02	100	.06	20	.06	50
16h	.03	0	.05	400	.06	20	.06	50
24h	.03	0	.01	0	.07	40	.08	100

a: change in absorbance per minute per 500 mg fresh weight;

CT; *Cyamopsis tetragonoloba*; CA: *Clerodendrum aculeatum;*

CF: *Clerodendrum fragrans*.

Polyphenol oxidase displayed no change in profile at both sites 30 sec after administration with CA-SRI. It increased by 120 percent at site 2 h after treatment and declined, comparable to control 16 h onwards (Table 3). The peak in activity at remote site was also at 2 h with a 400 percent increase. Subsequently it declined up to 8 h, thereafter it increased up to 16 h (Table 3).

At site of CF-SRI application, polyphenol oxidase displayed its first peak at 2 h, declined gradually up to 16 h and increased slightly at 24 h. At the remote site, maximum increase in activity occurred 2 h after treatment and the second increase occurred after 24 h (Table 3).

DISCUSSION The SRIs used for resistance induction effectively checked symptom expression by SRV. The pattern of resistance induction was generally similar in the two combinations with the exception of CF-SRI exhibiting a strangely rapid response at site of its application. Progressive development of resistance appears to signify activation or synthesis of a virus interfering agent (VIA), while display of antiviral activity at untreated remote site may denote translocation of this resistance conferring VIA (Verma & Prasad, 1983). Maximum activation/synthesis of VIA takes place within 2 h of SRI administration with a gradual increase between 2 and 4 h. The results are in general agreement with Verma *et al.* (1984).

Increase in total proteins coincided with increase in polyphenol oxidase. Had increase in enzyme activity been exclusively due to activation of existing enzyme, total proteins should have remained at the control level. Therefore it is suggested that SRI molecules contribute towards the derepression of the concerned genetic elements resulting in greater synthesis of the enzyme.

Polyphenol oxidase attained peak activity 2 h subsequent to SRI treatment and paralleled with the development of resistance. After 2 h, the enzyme activity declined but antiviral activity was high. The initial peak in polyphenol oxidase activity may be attributed to SRI administration either directly or by VIA formation. Decline in polyphenol oxidase activity could be a consequence of the depleted substrate pool becoming a limiting factor to put autoregulation processes in motion and once substrates reach an optimum level, the enzyme becomes hyperactive again since the initial stimulus perhaps still remains undegraded within the system. The second peak could also be totally non specific, a consequence not of an undegraded SRI stimulus but of a generalized stress laid upon the system. This may well be true since the second elevation is not always seen, and when present, is to a much smaller extent. An observation which eludes direct explanation is the greater polyphenol oxidase stimulation at untreated remote sites.

In vitro inactivation of plant viruses by *o*- quinones has been reported Mink & Saksena, 1971. An increase in polyphenol oxidase activity subsequent to SRI application signifies greater degradation of phenolic substrates and enhanced production of quinones. Thus upon challenge, SRV is subjected to an environment of elevated quinone concentration. Quinones may not support viral multiplication and/or spread owing to their toxicity to plant cell or by irreversibly degrading virus particles through areas in the protein coat or genomic nucleic acid which are quinone sensitive, since plant viruses are known to possess such sections (Mink, 1965).

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