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Enzymatic Responses to *SriLankan cassava mosaic virus* infection in cassava plants after grafting

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Abstract

Enzyme response in cassava plants grafted with Srilankan cassava mosaic virusinfected scionhave been investigated at different intervals of time. It was observed that the enzyme activity of defense related enzymes was higher in virus inoculated plants compared to the healthy plants.The activity was found to increase at all stages in case of both healthy and infected plants.Peroxidase (PO), Polyphenol oxidase (PPO) and Phenyl alanine ammonia lyase (PAL) activities increased and reached the peak value on 60thday after inoculation.Grafted plants exhibited maximum activity for all the enzymes.

Keywords: Srilankan cassava mosaic virus, Peroxidase, Polyphenol oxidase, Phenyl alanine ammonia lyase, Grafting

I. Introduction

Cassava, an important tropical tuber crop is affected by a number of diseases, of which Cassava mosaic disease is the most important. This disease is caused by *Cassava mosaic virus* belonging to the family *Geminiviridae*. A short duration cassava variety, VellayaniHraswa released from College of Agriculture, Vellayani is found highly susceptible to the disease, caused by *Srilankan cassava mosaic virus*. The present study involves the response of defense related enzymes like peroxidase, polyphenol oxidase and phenyl alanine ammonia lyase in the healthy and graft inoculated cassava plants of Hraswa variety.

When plants are attacked by pathogens, they defend themselves against the invasion involving active and passive mechanisms.Enzymes involed in phenol metabolism were considered as an important biochemical parameters for disease resistance. Defense-related enzymes namely, peroxidase, polyphenol oxidase are associated with the biosynthesis of lignin, phenolic compounds and phytoalexins, which are important plant defence components. Peroxidase (PO) oxidizes phenolics to quinones and generates H2O2. This is not only antimicrobial in itself, but it also releases highly reactive free radicals which further increases the rate of polymerization of phenolic compounds into lignin like compounds. These substances are deposited in cell walls and inhibited further growth and development of pathogen.Polyphenol oxidase (PPO) catalyses the oxidation of monophenol and orthodihydroxy phenol (Maheshwari*et al.,* 2006). Increased activity of polyphenol oxidase (PPO) results in accumulation of higher concentrations of toxic products of oxidation that participates in the defence reaction of host. Phenylalanine ammonia lyase (PAL),one of the key enzymes in the phenyl propanoid pathway, has a rolein phytoalexin, phenolic compound and salicylic acid synthesis.

II. Materials and Methods

Cassava shoots showing typical systemic symptoms of chlorotic mosaic on leaves were used as the scion. The base of the scion was trimmed to a wedge shape and inserted into a cleft made on the stem of forty day old healthy plants which was used as root stocks. The base of the scion was inserted into the cleft of the stock. The graft was then tied firmly using a polythene strip and kept inside an insect proof cage. Samples were collected at intervals of one, ten, twenty, thirty and sixty days after graft inoculation to estimate the activity of defense related enzymes such as peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase.

A.Extraction of enzymes

Leaf sample of 1 g was homogenized in 5 ml of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The homogenization was done at 40C using a pre-chilled mortar and pestle. The homogenate was filtered through a muslin cloth and centrifuged at 5000 rpm for 15 minutes at 40C. The supernatant was used as the enzyme extract for the assay of POand PPO activity.

B. Assay of Peroxidase(PO)

Peroxidase activity was assayed by a spectrophotometric method as described by Srivastava (1987). The reaction mixture consisting of 1 ml of 0.05 Mpyrogallol and 50 µl of enzyme extract was taken in both reference and sample cuvettes, mixed and kept in a spectrophotometer (Systronics UV-VIS spectrophotometer 118) and the reading was adjusted to zero at 420 nm. To initiate the reaction, one ml of one percent hydrogen peroxide (H2O2) was added to the sample cuvettes and the changes in absorbance were recorded at 30 seconds interval upto 180 seconds. The PO activity was expressed as changes in absorbance min-1 g-1 fresh weight of tissue.

C. Assay of Polyphenol oxidase (PPO)

Polyphenol oxidase activity was determined as per the procedure given by Mayer et al. (1965). The reaction mixture contained one ml of 0.1 M sodium phosphate buffer (pH 6.5) and 50 µl of enzyme extract. The reaction was initiated after adding one ml of 0.01 M catechol. The observations were recorded in a spectrophotometer (Systronics UV-VIS spectrophotometer 118). The change in absorbance was recorded at 495nm at 30 seconds interval upto 180 seconds. PPO activity was expressed as change in the absorbance of the reaction mixture per minute per gram on fresh weight basis.

D. Enzyme extraction and assay of Phenylalanine ammonia-lyase (PAL)

The enzyme extract was prepared by homogenizing one gram leaf sample in 5 ml of 0.1 M sodium borate buffer (pH 8.8) containing a pinch of PVP using chilled mortar and pestle. The homogenate was centrifuged at 10000 rpm for 10 minutes at 40C. The supernatant was used for the assay of PAL activity. PAL activity was assayed spectrophotometrically by assaying the rate of conversion of L- phenyl alanine to trans- cinnamic acid at 290 nm as described by Dickerson et al. (1984). The reaction mixture contained 3 ml of 0.1 M sodium borate buffer (pH 8.8), 0.2 ml enzyme extract and 0.1 ml of 12 mM L-phenyl alanine prepared in the same buffer. The blank contained 3 ml of 0.1 M sodium borate buffer (pH 8.8) and 0.2 ml enzyme extract. The reaction mixture and blank were incubated at 400C for 30 minutes and reaction was stopped by adding 0.2 ml of 3 N hydrochloric acid. The absorbance was read at 290 nm in a spectrophotometer (Systronics UV-VIS spectrophotometer 118). PAL activity was expressed as micrograms of cinnamic acid produced per minute per gram on fresh weight basis.

III. Results and Discussion

Extraction and assay of enzymes

Peroxidase, Polyphenol oxidase and Phenyl alanine ammonialyase activity in healthy and graft inoculated cassava plants at different DAT was estimated (Table 1). A significant increase in PO activity of inoculated samples was noted at all stages of infection compared to healthy uninoculated plants except for 1 DAT. The activity of the enzyme in inoculated plants (3.02 min-1 g-1) was found to be on par with that of uninoculated plants (2.57 min-1 g-1) at 1 DAT. The activity of the enzyme was found to be the highest at 60 DAI in case of inoculated plants (22.25 min-1 g-1) whereas a lower value of 10.92 g-1min-1was recorded in control plants. A significant increase in PO activity was noted at 10 DAT (10.55 g-1min-1) in inoculated plants which was found to be on par with that at 20 DAT (12.48 g-1min-1). Thereafter a significant increase in PO activity was noted.Vasanthi and Shanmugam (2003) observed an increase in the activities of peroxidase, phenyl alanine ammonia- lyase (PAL) in meristem derived cassava regenerants compared with enzyme activities in healthy and infected sett propagated cassava. The increase in peroxidase activity may be due to the susceptible nature of the variety. Ramaiahet al. (1973) reported that the catalase and peroxidase (PO) activity increased inthe leaves of BYVMV infected plants. Meenaet al. (2008) observed higher PO activity in chilli leaf infected with geminivirus as compared to healthy leaf. Increased peroxidase activity was associated with resistance reaction which could be due to increased phenol concentration, where phenols were cofactor of peroxidase and hence influenced resistance in the host. Dienget al. (2011) showed increased PO activity in *Solanum lycopersicum* in the presence of TYLCV.

The activity of polyphenol oxidase in inoculated plants (0.51 min-1g-1) was found to be on par with that of healthy control (0.6 min-1g-1) at 1 DAT. But a significant difference in the activity of the enzyme was noted in the inoculated (4.97 min-1g-1) and uninoculated (1.1 min-1g-1) plants at 60 DAT. The maximum level of activity of the enzyme was found in the inoculated plants at 60 DAT. Gomathiet al. (1993) reported the increased activity of PO, PPO and PAL enzyme activity in banana infected with banana streak virus or banana common mosaic virus.

Results of PAL activity in the virus infected cassava leaves in comparison with healthy leaves indicated higher activity in virus inoculated plants compared to the healthy plants. The activity was found to increase at all stages of treatment in case of both uninoculated and inoculated plants. In healthy plants, PAL activity was 12.04 µg g-1 min-1 at first day after treatment which increased to 18.41 µg g-1 min-1 at 60 DAT. In the case of inoculated plants, PAL activity reached a maximum of 23.61 µg g-1 min-1 at 60 DAI which was found to be on par with the PAL activity at 30 DAI (22.22 µg g-1 min-1).Sindhu (2001) reported an enhanced PAL activity in BlCMV inoculated cowpea plants compared to healthy. Ahmed et al. (1992) suggested that higher amount of phenols and their oxidation products like quinones formed by increased PO and PPO might be responsible for reduced virus multiplication and finally could lead to resistant reaction in yellow vein mosaic virus infected okra.

Table 1. Changes in PO, PPO and PAL activity of cassava leaves in response to virus inoculation

Days after treatment	*PO activity(changes in absorbance min-1g-1 fresh weight)		Per cent increase or decrease over healthy	*PPO activity (changes in absorbance min-1g-1 fresh weight)		Per cent increase or decrease over healthy	*PAL activity (changes in absorbance µg g-1min-1fresh weight)		Per cent increase or decrease over healthy
	Healthy	Inoculated		Healthy	Inoculated		Healthy	Inoculated	

1	2.57	3.02	17.5	0.51	0.6	17.65	12.04	10.55	12.37
10	7.92	10.55	33.2	0.71	0.83	16.9	14.50	16.44	13.38
20	8.81	12.48	41.65	0.83	0.97	16.87	14.92	20.39	36.66
30	12.33	20.92	69.66	1.17	1.52	29.91	16.65	22.22	33.45
60	10.92	22.25	103.8	1.1	4.97	351.8	18.41	23.61	28.25
CD at 5%	Healthy vs Inoculated- 1.22 DAT- 2.113			Healthy vs Inoculated- 0.418 DAT-1.723			Healthy vs Inoculated- 0.846 DAT- 1.465		

* Mean of three replications

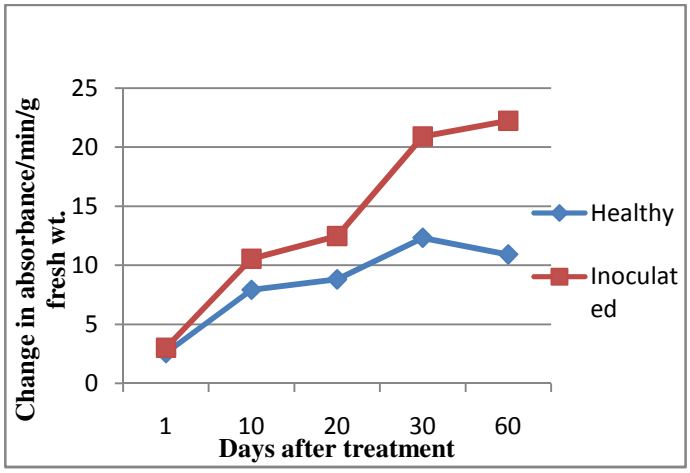

Fig. 1 Changes in peroxidase activity in cassava leaves in response to virus inoculation

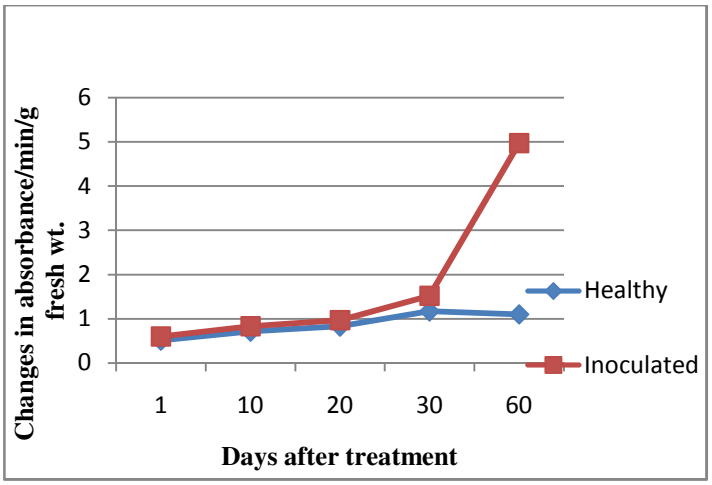

Fig. 2 Changes in polyphenol oxidase activity in cassava leaves in response to virus inoculation

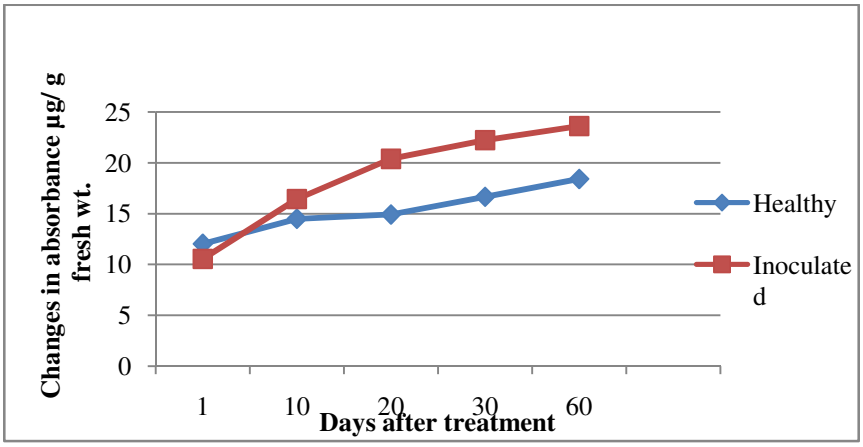

Fig.3 Changes in phenyl alanine ammonia-lyase activity in cassava leaves in response to virus inoculation

IV. Conclusion

Activity of defense related enzymes such as Peroxidase (PO), Poly phenol oxidase (PPO) and Phenylalanine ammonia-lyase (PAL) showed an increasing trend during all stages of graft inoculation (Fig.1 to 3) in the present study.The virus infection on host plants by grafting induces the hosts to produce cell wall bound enzymes like peroxidases and polyphenol oxidases which might have some antimicrobial activity.Defense-related enzymes namely, peroxidase, polyphenol oxidase are associated with the biosynthesis of lignin, phenolic compounds and phytoalexins, which are important plant defense components.Phenylalanine ammonia lyase (PAL) has role in the synthesis of phytoalexin, phenolic compound and salicylic acid.Study of these enzymes involved in phenol metabolism is important in understanding the defense mechanism in plants.

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Bibliography

[1] Ahmed, N., Thakur, M. R., Bajaj, K. L. and Cheema, S. S. 1992. Biochemical basis of resistance to Yellow vein mosaic virus in okra.Pl. Dis. Res. 9: 20-25.
[2] Dickerson, D. P., Pascholati, S. F., Hagerman, A. K., Butler, L. G. and Nicholsn, R. L. 1984. Phenyl alanine ammonia-lyase hydroxyl cinnamalte: CoA Ligase in maize mesocotyls inoculated with Helminthosporiummaydis or Helminthosporiumcarbonum. Physiol. Pl. Path.25: 111-123.
[3] Dieng, H., Satho, T., Hassan, A. A., Aziz, A. T., Morales, R. E., Ab Hamid, S., Miake, F. and Abubakar, S. 2011. Peroxidase activity after viral infection and whitefly infestation in juvenile and mature leaves of Solanumlycopersicum.J. Phytopath. 159: 707- 712.
[4] Gomathi, N., Mariappan, V. and Devanathan, M. 1993. Changes in the oxidative enzymes or PAL in mosaic and streak mosaic virus infected banana plants. S. Indian Hort. 41: 376-378.
[5] Maheshwari, M. U., Suresh, S. and Emmanual, N. 2006. Biochemical basis of resistance in rice hybrids and conventional varities against brown plant hopper.Ann. Pl. Protec. Sci. 14: 69-72.
[6] Meena, R. K., Vidya, P. and Arora, D. K. 2008. Study on phenolics and their oxidative enzymes in Capsicum annum L. infected with geminivirus. Asian J. Exp. Sci.22: 307-310
[7] Ramaih, M., Vidhyasekaran, P. and Kandaswamy, T. K. 1973. Changes in the activities of oxidative enzymes in yellow mosaic disease of Bhendi leaves.Madras Agric.J.60: 609-610.
[8] Sindhu, A. R. 2001. Biochemical basis of resistance against Blackeye cowpea mosaic virus in cowpea (Vignaunguiculata (L.)Walp).MSc (Ag) thesis, Kerala Agricultural University, Thrissur, 65 p.

[9] Srivastava, S. K. 1987. Peroxidase and polyphenol oxidase in Brassica juncea plants infected with Macrophominaphaseolina (Tassi) Goid and their implication in disease resistance. Phytopath.120: 249-254.
[10] Vasanthi, V. J. and Shanmugam, V. 2003. Physiological changes in cassava (Manihotesculenta) infected with Indian mosaic geminivirus. Pl. Dis. Res. 18: 196- 198.