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Change in activity of few host enzymes under challenge-inoculation of viruses in tomato *(Lycopersicon esculentum* Mill.)

K. PRADHEEP AND D. VEERARAGAVATHATHAM
Horticultural College and Research Institute, TNAU, Coimbatore - 641 003 (T.N)

Abstract: Investigation was carried out at Horticultural College and Research Institute, TNAU, Coimbatore to understand the biochemical defense mechanism underlined in two virus tolerant hybrids namely H 24 x CLN 2123A and H 24 x LCR 1 in tomato *(Lycopersicon esculentum* Mill.). There existed significant differences in the activities of all the three host enzymes studied *viz.* peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activity among different treatments. Both the hybrids recorded significantly higher peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activity than their parents and susceptible check under both challenge-inoculated and control condition. Between the hybrids, rapid increase in phenylalanine ammonia lyase activity under challenge-inoculation of viruses was noticed only in H 24 x LCR 1. The possible reasons involved are discussed.

Key words : Tomato, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, virus tolerance.

Introduction

Host enzymes like peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) play an important role in disease resistance in plants. Bradley, Kjellborn and Lamb (1992) correlated the increased PO action with resistance in many plants owing to its involvement in the polymerization of proteins and lignin or suberin precursor into cell walls or movement through vessels. PAL induces synthesis of salicylic acid which induces systemic resistance in many plants. Two tomato *(Lycopersicon esculentum* Mill.) hybrids namely H 24 x LCR 1 and H 24 x CLN 2123A were selected in the Department of Vegetable Crops, Horticultural College and Research Institute, TNAU, Coimbatore as tolerant to two serious viruses *viz.* Tomato Leaf Curl Virus (TLCV) and a Tospovirus (Tv) infecting tomato in South India. In order to understand the biochemical defense mechanism underlined in these virus tolerant hybrids, this study was taken up.

Materials and Methods

Investigation was carried out at Horticultural College and Research Institute, TNAU, Coimbatore during February-March, 2003. The hybrids *viz.*, H24 x LCR-1 and H24 x CLN 2123, their parents and a check susceptible to both the viral diseases (CO 3) were included in the experiment. Recently mature physiologically active leaf (fifth leaf from the top) of five randomly selected 60 day old plants was used for the assay of enzymes. For studies on PO and PPO enzymes activity, the samples were collected at 0, 24, 48, 72, 96 and 120 hr after graft inoculation of both the viruses simultaneously in different parts of the stem. In case of PAL enzyme activity, samples after

Table 1. PO, PPO and PAL activity in the hybrids, their parents and susceptible check CO 3

Cross/parent	Condition	P0 (changes in OD /min/g fresh weight tissue)							PP0 (changes in OD/min/g fresh weight tissue)						PAL
		0 hr	24 hr	48 hr	72 hr	96 hr	120 hr	Mean	0 hr.	24 hr	48 hr	72 hr	96 hr	120hr	Mean	48hr
H 24 x LCR 1	Ino	5.28	5.85	6.67	7.88	8.72	8.19	7.10	0.610	0.771	0.833	0.880	0.969	0.960	0.837	0.937
	Con	4.60	4.77	4.93	5.08	5.07	5.30	4.96	0.588	0.595	0.596	0.605	0.615	0.619	0.603	0.837
H 24 x CLN 2123A	Ino	6.09	5.69	7.13	7.95	8.84	9.22	7.49	0.711	0.749	0.798	0.829	0.866	0.885	0.806	0.850
	Con	4.54	4.53	4.64	4.89	5.01	5.03	4.77	0.661	0.674	0.673	0.676	0.684	0.685	0.676	0.837
H24	Ino	4.92	5.59	6.27	6.81	7.42	7.22	6.37	0.564	0.611	0.642	0.694	0.735	0.720	0.661	0.740
	Con	4.54	4.53	4.64	4.89	5.01	5.03	4.77	0.546	0.563	0.576	0.586	0.593	0.594	0.576	0.757
CLN 2123A	Ino	4.12	5.77	6.89	7.85	8.69	8.21	6.92	0.666	0.682	0.740	0.799	0.879	0.872	0.773	0.787
	Con	4.26	4.39	4.54	4.66	4.83	4.78	4.58	0.661	0.672	0.675	0.682	0.689	0.695	0.679	0.637
LCR1	Ino	4.88	5.28	6.03	6.75	6.90	6.70	6.09	0.598	0.652	0.683	0.749	0.781	0.781	0.707	0.720
	Con	4.52	4.57	4.64	4.74	4.76	4.66	4.65	0.555	0.563	0.569	0.578	0.587	0.592	0.574	0.543
CO 3	Ino	3.38	3.82	3.98	4.34	4.98	5.02	4.25	0.488	0.579	0.602	0.638	0.680	0.658	0.608	0.510
	Con	3.36	3.46	3.74	3.75	3.77	3.40	3.64	0.456	0.464	0.478	0.486	0.488	0.499	0.478	0.413

*unit: μmol min^{-1} g^{-1} fresh weight.

Treatment	**CD (0.05) for PO**	**CD (0.05) for PPO**	**CD (0.05) for PAL**
Genotype	0.102	0.0048	0.0564
Hour	0.102	0.0048	0.0326
Virus	0.059	0.0028	-
Genotype at hour	0.249	0.0117	-
Hour at virus	0.144	0.0068	-
Genotype at virus	0.144	0.0068	0.0797
Genotype at hour at virus	0.352	0.0168	-

48 hr alone were used. Plants with no inoculation made were kept as control. The study was repeated two times and their mean value was taken into account. Leaf sample (200 mg) obtained at different time interval was homogenized in chilled pestle and mortar with 1 ml of cold 0.1 M phosphate buffer (pH 6.5). The extract was centrifuged at 6000 rpm for 10 minutes at 4°C in a refrigerated centrifuge and the supernatant was used as enzyme extract. PO and PPO activity were assayed following the method described by Srivastava (1987) and were expressed as changes in absorbance per minute per g fresh weight. PAL activity was determined as per the procedure given by Dickerson *et al.* (1984) and was expressed as μmol min^{-1} g^{-1} fresh weight.

Results and Discussion

There existed significant differences in both PO and PPO activity among the genotypes, hours after inoculation and between virus-inoculated and control treatment. Similar is the case with PAL activity. The increase in PO activity was rapid up to 96 hr after inoculation and later on declined. Among the hybrids, H 24 x CLN 2123A recorded maximum mean PO activity followed by H 24 x LCR 1 under inoculated conditions (Table 1). In control, the differences were not so high although they exhibited significant differences in PO activity. The hybrid H 24 x LCR 1 exhibited maximum PPO activity followed by H 24 x CLN 2123A. The susceptible check variety CO 3 recorded the lowest values in either of the case and the parents possessed intermediate values.

According to Kosuge (1969) and Shankarand Jindal (2001) activity of the peroxidase and polyphenol oxidase enzymes is directly related to resistance in the host, which could be due to the conversion of the enzymes into quinones, which were toxic to pathogen. He further stated that these oxidative enzymes might have catalyzed the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure. Hence high induction of these enzymes *viz.,* PO and PPO could have helped these hybrids to trigger the defense system thereby induce resistance/tolerance. Such parallel increase in PO activities with the development of systemic resistance has been observed by Simon and Ross (1970, 1971). Rapid changes in the activities of these enzymes after the stress induction may indicate their possible role in the defense mechanisms.

PAL was reported to be involved in phytoalexin or phenolic compound synthesis. This enzyme has been correlated with defense mechanism against pathogens in several plants (Bashan, Okon and Henis, 1985; Beauodoin-Eagan and Thorpe, 1985). In the present study although both the hybrids (H 24 x LCR 1 and H 24 x CLN 2123A) had higher level of PAL activity before inoculation of viruses, rapid increase in PAL activity was noticed only in H 24 x LCR 1 when both the viruses were simultaneously inoculated. Perhaps this might be by earlier induction of PAL in response to the virus inoculation in that hybrid than the other one. High PAL activity in the above hybrid might have produced precursors of phenolics (cinnamic acid) and lignin synthesis thereby aiding in forming mechanical and chemical barrier against the invading pathogen compared to CO3.

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