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Changes in Poly Phenoloxidase and Peroxidase due to Bacterial Blight Disease in Clusterbean

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ABSTRACT: The activity of polyphenol oxidase and peroxidase enzyme was also disturbed in infected leaves of clusterbean. It was observed that amount of polyphenol oxidase and peroxidase activity was less in extracts prepared from healthy leaf tissues. The activity in infected leaves was more than healthy leaves. Therefore, the activity of polyphenol oxidase and peroxidase increased due to bacterial blight disease in clusterbean. Polyphenol oxidase per cent change in optical density/min/ml maximum was recorded in cultivar RGC-1055 (89.47 & 88.00) followed by RGC-1017 (85.00 & 80.76), RGC-986 (80.95 & 73.91), RGC-936 (45.45 & 71.87) and RGC-1031 (30.00 & 71.42) at 30 and 45 days old plants respectively. Peroxidase per cent change in optical density/min/ml maximum was recorded in cultivar RGC-1055 (52.63 & 51.56) followed by RGC-1017 (44.23 & 38.70), RGC-986 (34.88 & 30.18), RGC-936 (23.95 & 30.00) and minimum in RGC-1031 (9.34 & 17.43) 30 and 45 days old plants respectively.

Keywords: Clusterbean, Bacterial blight, *Xanthomonas*, Cultivar, Enzyme and Polyphenol oxidase.

INTRODUCTION

Clusterbean [*Cyamopsis tetragonaloba* (L.) Taub.] is an important annual legume crop of *kharif* season in arid and semi-arid regions of the Indian subcontinent. It is a self-pollinated, short duration legume crop generally cultivated under resource constrained conditions on marginal and sub marginal lands (Kumar, 2005). Isoenzymes assay such as polyphenol oxidase (PPO) and peroxidase (PO) of clusterbean has special significance in reference to blight resistance. Role of PPO and PO has been implicated in the ultimate enzymatic step of lignin synthesis induced by biotic and abiotic stresses. Bacterial polysaccharides have attracted the attention of phytopathologists because their production in culture is often positively correlated with virulence (Leigh and Coplin, 1992). Therefore, it would be interesting to investigate whether the trend of changes in the activity of enzymes over period of time could be correlated to resistance and susceptibility to bacterial blight. To better understand the mechanisms underlying disease development, the biochemical characteristics of the causal bacterium were studied for providing new information on disease development.

MATERIAL AND METHODS

A. Defense-related anti-oxidative enzymes

Estimation of Polyphenol oxidase (PPO) activity. The pattern of enzymatic activity of polyphenol oxidase (PPO) in five varieties was determined with the method described by Shannon *et al.*, (1966).

Reagents:

1. Sodium phosphate buffer
2. 0.01 M Catechol ($C_6 H_6 O_2$)

Procedure. Two-gram fresh leaf sample was homogenized in four ml of 0.1 M sodium phosphate buffer at pH 6.5 and centrifuged at 6,000 rpm for 15 minutes at 4ºC. The supernatant was used as enzyme source. The reaction mixture consisted of one ml enzyme extract (supernatant) and three ml of 0.1 M sodium phosphate buffer. To start the reaction, 0.5 ml of 0.01 M catechol was added and the change in absorbance was recorded at 30 second intervals up to three minutes at 495 nm.

Estimation of Peroxidase activity. Peroxidase activity was determined by using the method as described by Shannon *et al*., (1966) with minor modifications.

Reagents

1. 0.05 M. pyrogallol ($C_6 H_6 O_3$)
2. One per cent hydrogen peroxide (H_2O_2)

Procedure

Two gram fresh leaf sample was ground in a previously chilled mortar in 10 ml ice-cold 0.1 phosphate buffer at pH 6.0. The homogenate was strained through a two-fold muslin cloth and centrifuged at 16,000 rpm for 20 minutes. The supernatant was used as an enzyme source. The reaction mixture contained 0.05 M sodium phosphate buffer (pH 5.5), two per cent H2O2, 0.05 M guaiacol and 0.1 ml enzyme extract in a final volume of five ml. The reaction was started by the addition of enzyme extract. The formation of tetra-guaiacol was measured at 470 nm wavelength.

RESULT AND DISCUSSION:

*A. **Change in polyphenol oxidase (PPO) activity***

Polyphenol oxidase activity in healthy and infected leaves was estimated at 30 and 45 days old plants by measuring oxidation of catechol in spectrophotometer. Change of absorbance was measured OD/min/ml of enzyme extracts. It was observed that amount of polyphenol oxidase activity was less in extracts prepared from healthy leaf tissues. The activity in infected leaves was more than healthy leaves (Table 1 & Fig. 1). The per cent change in optical density/min/ml was maximum in cultivar RGC-1055 (89.47 &88.00) followed by RGC-1017 (85.00 & 80.76), RGC-986 (80.95 & 73.91), RGC-936 (45.45 & 71.87) and minimum in RGC-1031(30.00 & 71.42) at 30 and 45 days old plants respectively. Increase in this enzymes activities due to bacterial infection was also reported by Farkas and Luvrekovich (1965); Luvrekovich *et al.* (1968); Sridhar and Mahadevan (1972); Obukowizc and Kennedy (1981); Nema (1991); Sharma *et al.* (2012); Sain and Gour (2013); Kalaskar *et al.* (2014); Shobha *et al.* (2014); Prakasha and Umesha (2016).

Table 1: Change in polyphenol oxidase (PPO) in a healthy and diseased leaf of different cultivars of clusterbean at different days.

Sr. No.	Cultivar	Polyphenol oxidase (optical density/min/mg protein)					
		30 days/H*	30 days/D**	% Increase over healthy	45 days/H*	45 days/D**	% Increase over healthy
1.	RGC-1055	0.19	0.36	(+) 89.47	0.25	0.47	(+) 88.00
2.	RGC-1031	0.20	0.26	(+) 30.00	0.21	0.36	(+) 71.42
3.	RGC-986	0.21	0.38	(+) 80.95	0.23	0.42	(+) 73.91
4.	RGC-936	0.22	0.32	(+) 45.45	0.32	0.55	(+) 71.87
5.	RGC-1017	0.20	0.37	(+) 85.00	0.26	0.47	(+) 80.76
SEm±		0.03	0.05	-	0.06	0.04	-
CD at 5%		0.09	0.16	-	0.19	0.13	-
CV%		2.76	3.52	-	4.55	2.50	-

*H- Healthy; **D- Diseased leaf showing ≤50 Per cent disease index; All data are means of four replications

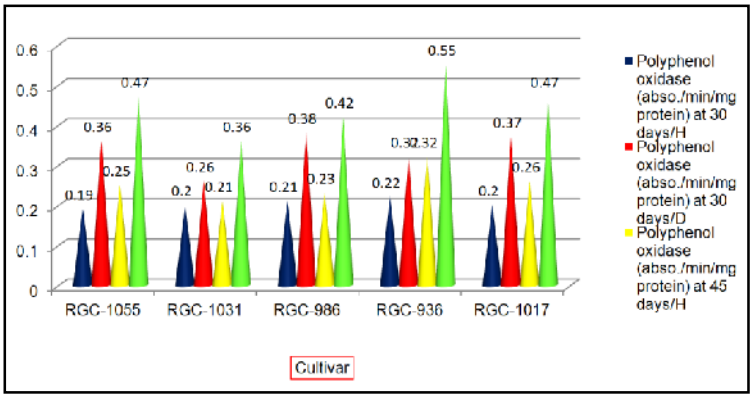

Fig. 1. Change in polyphenol oxidase (PPO) in a healthy and diseased leaf of different cultivars of clusterbean at different days.

B. Change in peroxidase (PO) activity

Peroxidase activity in healthy and infected leaves was also estimated at 30 and 45 days old plants. It was observed that amount of peroxidase activity was less in extracts prepared from healthy leaf tissues. The activity in infected leaves was more than healthy leaves (Table 2 & Fig. 2).

The per cent change in optical density/min/ml was maximum in cultivar RGC-1055 (52.63 & 51.56) followed by RGC-1017 (44.23 & 38.70), RGC-986 (34.88 & 30.18), RGC-936 (23.95 & 30.00) and minimum in RGC-1031 (9.34 & 17.43) at 30 and 45 days old plants respectively.

Increase in this enzymes activities due to bacterial infection was also reported by Farkas and Luvrekovich (1965); Luvrekovich *et al.* (1968); Sridhar and Mahadevan (1972); Obukowizc and Kennedy (1981); Nema (1991); Sharma *et al.* (2012; Sain and Gour (2013); Kalaskar *et al.* (2014); Shobha *et al.* (2014); Prakasha and Umesha (2016).

Table 2: Change in peroxidase (PO) in a healthy and diseased leaf of different cultivars of clusterbean at different days.

Sr.No.	Cultivar	Peroxidase (optical density/min/mg protein)					
		30 days/H*	30 days/D**	% Increase over healthy	45 days/H*	45 days/D**	% Increase over healthy
1.	RGC-1055	0.57	0.87	(+) 52.63	0.64	0.97	(+) 51.56
2.	RGC-1031	1.07	1.17	(+) 9.34	1.09	1.28	(+)17.43
3.	RGC-986	0.43	0.58	(+) 34.88	0.53	0.69	(+) 30.18
4.	RGC-936	0.96	1.19	(+) 23.95	1.00	1.30	(+) 30.00
5.	RGC-1017	0.52	0.75	(+) 44.23	0.62	0.86	(+) 38.70
SEm±		0.06	0.04	-	0.05	0.04	-
CD at 5%		0.20	0.15	-	0.19	0.14	-
CV%		2.42	1.68	-	2.04	1.59	-

*H- Healthy; **D- Diseased leaf showing ≤50 Per cent disease index; All data are means of four replications

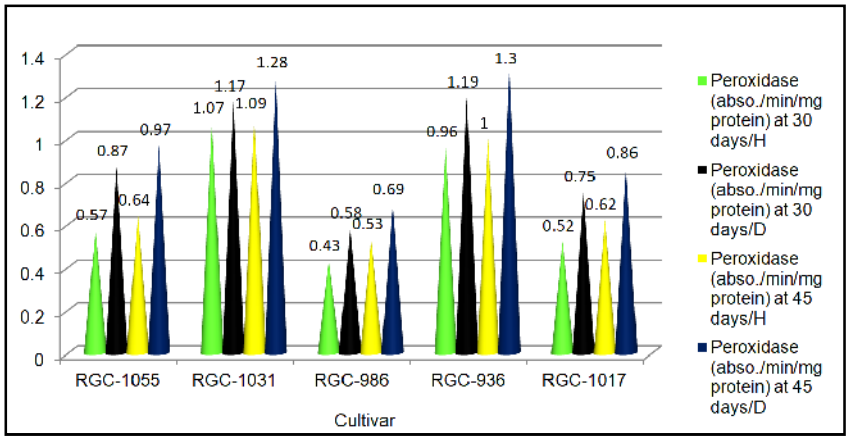

Fig. 2. Change in peroxidase (PO) in a healthy and diseased leaf of different cultivars of clusterbean at different days.

CONCLUSION

As the disease progressed, the polyphenol oxidase activity increased in all the five cultivars. The activity in infected leaves was more than healthy leaves. The per cent change in activity was maximum in infected cultivar RGC-1055 (89.47 & 88.00) and minimum in RGC-1031 (30.0 & 71.42) at 30 and 45 days old plants respectively.

The activity of peroxidase greatly increased in bacterial blight disease infected leaves of cultivar RGC-1055 (52.63% & 51.56%) and minimum in RGC-1031 (9.34% & 17.43%) at 30 and 45 days old plants respectively.

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Conflict of Interest. None.

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