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Resistance in tea plants towards *Glomerella cingulata* with reference to peroxidase profile

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Pathogenicity of *Glomerella cingulata* [(Stoneman) Spauld & Schrenk] causing brown blight, was assessed following detached leaf technique on 10 tea varieties. TV-30 was found to be most resistant, while UP-2 and TV-22 were found to be most susceptible. Peroxidase (POD) activity and isozyme profile were studied in relation to disease development. POD is an important scavenger of active oxygen species (AOS), which, combined with other defence responses, are assumed to lead to incompatibility between host and pathogen. Higher POD activity was found following infection in susceptible varieties. However, analysis of POD isozymes by PAGE using benzidine as a substrate, revealed that there is a definite change in isozyme profile with progress of infection. Two isozymes (R_m=0.06 and R_m=0.66) were found to be specifically associated with resistance. Present study gives some insight into mechanism of action of this important enzyme.

Key words : *Camellia sinensis, Glomerella cingulata,* peroxidase isozymes

INTRODUCTION

Brown blight of tea (*Camellia sinesis* (L.) O. Kuntze) caused by a cosmopolitan genus *Colletotrichum gloeosporioides* Penz. (Sacc.), [telomorph *Glomerella cingulata* (Stoneman) Spauld. & Schrenk] is a common foliar pathogen in all tea-growing areas. Since even slight damages to tea leaves reduces quality and quantity of tea production this foliar pathogen is very important in agriculture. Besides, it has a very wide host range, affecting coffee, avocado, papaya, apple, sorghum, etc. Its isolates are also highly variable.

Peroxidase, a part of PR-9 family, catalyzes the last enzymatic step in the biosynthesis of lignin at the expense of H_2O_2. It is also believed to be responsible for generation of H_2O_2 from O_2 and NADH, thus acting as a regulatory enzyme in lignin biosynthesis. Thus, it serves as a scavenger of active oxygen species (AOS) produced in infection and its activity increases with advance of infection in both compatible and incompatible combinations. The present study has been undertaken as an attempt to elucidate the mechanism of action of peroxidase and its role in resistance mechanism of tea.

MATERIALS AND METHODS

Plant Material

Detached leaves (3th and 4th) of nature (6–7 years old) tea bushes of 10 varieties, collected from three different geographical locations of India, were used, which included five (TV-18, TV-22, TV-26, TV-29 and TV-30) the clonal varieties from Tocklai Experimental Station, Jorhat, Assam, two (CP-1 and S-449) –the seed varieties from Darjeeling Tea Research Station, Kurseong, Darjeeling, while three (UP-3, UP-9, BSS-2) the seed varieties from UPASI Tea Research Station, Valparai, Tamilnadu. Plants were maintained in Phytopathological Experimental Garden of Botany Department. North Bengal University, as suggested by Barbora (1988).

Fungal Pathogen

Glomerella cingulata (Stoneman) Spauld. & Schrenk (GC) isolated from naturally infected tea plants (TV-25) was identified at the International Mycological Institute, UK and was designated as GC-a (IMI mumber 256806). The identity of the organism was confirmed by completing Koch's

postulates. The culture was maintained on Richard's Medium Agar (RMA) slants at 28±2^{0}C.

Inoculation procedure

A detached leaf inoculation technique as described by Dickens and Cook (1989) was followed. Leaves were detached from plants and placed in trays lined with moist blotting paper. Light scratches were made on the adaxial surface of each leaf and inoculated with 20µl droplets of spore suspension (6.4 × 10^6 conidia ml^{-1}) of *G. cingulata* (prepared from 10-day-old culture). Spore suspensions were placed on the adaxial surface of each leaf on the scraches. In control sets, drops of sterile distilled water were placed instead of suspension.

Assessment of disease intensity

Per centage of drops that resulted in lesion production was calculated after 48 h and 72 h of inoculation as described by Chakraborty *et al.*, (2002b). Observations were made based on 50 inoculated leaves for each treatment in average of 3 separate experiments.

Extraction of peroxidase

To extract peroxidase, method as described by Chakraborty *et al.* (2002a) was used with slight modifications. Tea leaf tissue (lg) was crushed in mortar with pestle in 5 ml of 0.1 (M) sodium phosphate buffer (p^H7.0) on ice with addition of a pinch insoluble PVP and sea sand. The homogenate was centrifuged at 15,000 r.p.m. for 20 min. at 4^{0}C. After centrifugation, the supernatant was collected and used immediately for assay and isozyme analysis.

Assay of peroxidase

For determination of peroxidase activity, method of Chakraborty *et al.* (2002a) was followed. Specific activity was assessed spectrophotometrically at A_{460} by monitoring oxidation ofo-dianisidine in presence of H_2O_2 and expressedas increase in absorbance at 460 nm g^{-1} fresh weight tissue min^{-1}.

POD isoxyme analysis by PAGE

Polyacrylamide gel electrophoresis was performed according to the method of Davis (1967), followed by staining of the gel with benzidine. Isozyme analysis was done in 4 selected verieties (TV-30, TV-22, S-449 and UP-3) after 24 h and 48 h of inoculation in both healthy (control) and infected leaves.

RESULTS

Pathogenicity test of G. cingulata on different tea varieties

Among the ten tea varieties tested against *G. cingulata,* TV-30, BSS-2 and TV-18 were found to be resistant, while S-449 and TV-29 were moderately resistant (Table1). The most susceptible varieties were UP-9 and TV-22, followed by TV-26, CP-1 and UP-3, which are grouped as moderately susceptible.

Table 1 : *Pathogenicity test of G.cingulata (isolate GC-a) on detached tea leaves.*

Variety	Lesion Production (%) 48h	72h	Response (72h)
TV-30	0.0	13.5±3.7	R
BSS-2	0.0	18.5±2.0	R
TV-18	5.0±1.9	21.0±2.5	R
S-449	7.2±1.5	31.0±3.1	MR
TV-29	12.9±2.3	35.5±2.1	MR
TV-26	19.0±2.7	39.0±2.6	MS
UP-3	11.3±3.1	50.0±4.1	MS
CP-1	9.7±2.2	53.2±3.2	MS
TV-22	55.0±4.5	66.0±4.8	S
UP-9	24.0±2.2	75.6±3.8	S

Data are the mean of 200 inoculum droplets made on 50 leaves of each variety Means ± SE, n=3
R-Resistant (0–25%) ; MR-Moderately resistant (26%-45%)
S-Susceptible (>65%) ; MS-Moderately susceptible (46%-65%)

Assay of peroxidase

Time-course accumulation of peroxidase in control and infected leaves was determined. Results (Table 2 and Fig. 1) revealed that POD activities were higher in susceptible varieties than in resistant varieties. The highest activity was recorded in infected TV-22 (60.5 g^{-1} tissue min^{-1}). There was a characteristic reduction in POD activities after 24 h of inoculation in resistant and moderately resistant varieties except in case of BSS-2. In the susceptible varieties, the activity increased suddenly after 24 h challenge, but reduced sharply thereafter. Since healthy control leaves were also scratched, these

Table 2. *POD activities in tea varieties at various time intervals after inoculation with G. cingulata (GC-a)*

Variety	0h		24h		48h		72h	
	H	I	H	I	H	I	H	I
TV-30	27.5 ± 0.9	23.0 ± 0.5	25.5 ± 0.2	13.5 ± 0.4	38.2 ± 0.2	49.3 ± 0.1	37.2 ± 0.9	43.0 ± 0.8
BSS-2	42.3 ± 0.2	37.6 ± 0.3	27.7 ± 0.4	31.6 ± 0.3	33.1 ± 0.4	52.5 ± 0.7	34.1 ± 0.3	35.2 ± 0.5
TV-18	35.9 ± 0.7	37.3 ± 0.4	35.1 ± 0.2	34.5 ± 0.9	34.0 ± 0.5	46.6 ± 0.3	17.2 ± 0.1	20.5 ± 0.7
S-449	11.8 ± 0.5	11.2 ± 0.3	08.8 ± 0.5	06.4 ± 0.1	25.0 ± 0.8	39.0 ± 0.2	14.3 ± 0.2	10.2 ± 0.6
TV-29	195 ± 0.1	22.5 ± 0.2	32.5 ± 0.3	16.3 ± 0.6	09.7 ± 0.5	39.5 ± 0.1	07.4 ± 0.1	19.3 ± 0.8
TV-26	41.8 ± 0.5	41.2 ± 0.7	28.2 ± 0.5	37.3 ± 0.3	44.5 ± 0.7	47.4 ± 0.2	22.3 ± 0.1	10.1 ± 0.5
UP-3	13.8 ± 0.7	15.2 ± 0.3	20.2 ± 0.1	58.4 ± 0.4	35.2 ± 0.5	46.1 ± 0.3	24.3 ± 0.7	13.1 ± 0.7
CP-1	09.2 ± 0.2	10.1 ± 0.5	07.3 ± 0.4	41.9 ± 0.6	26.8 ± 0.5	14.6 ± 0.1	12.5 ± 0.6	09.2 ± 0.4
TV-22	19.1 ± 0.3	21.2 ± 0.1	24.6 ± 0.8	60.5 ± 0.9	34.3 ± 0.8	22.5 ± 0.3	11.7 ± 0.2	23.4 ± 0.3
UP-9	29.7 ± 0.9	28.24 ± 0.6	20.0 ± 0.4	60.0 ± 0.5	45.3 ± 0.7	52.7 ± 0.2	37.8 ± 0.3	30.7 ± 0.8

H — Healthy ; I — Infected
Means ±SE, n = 3

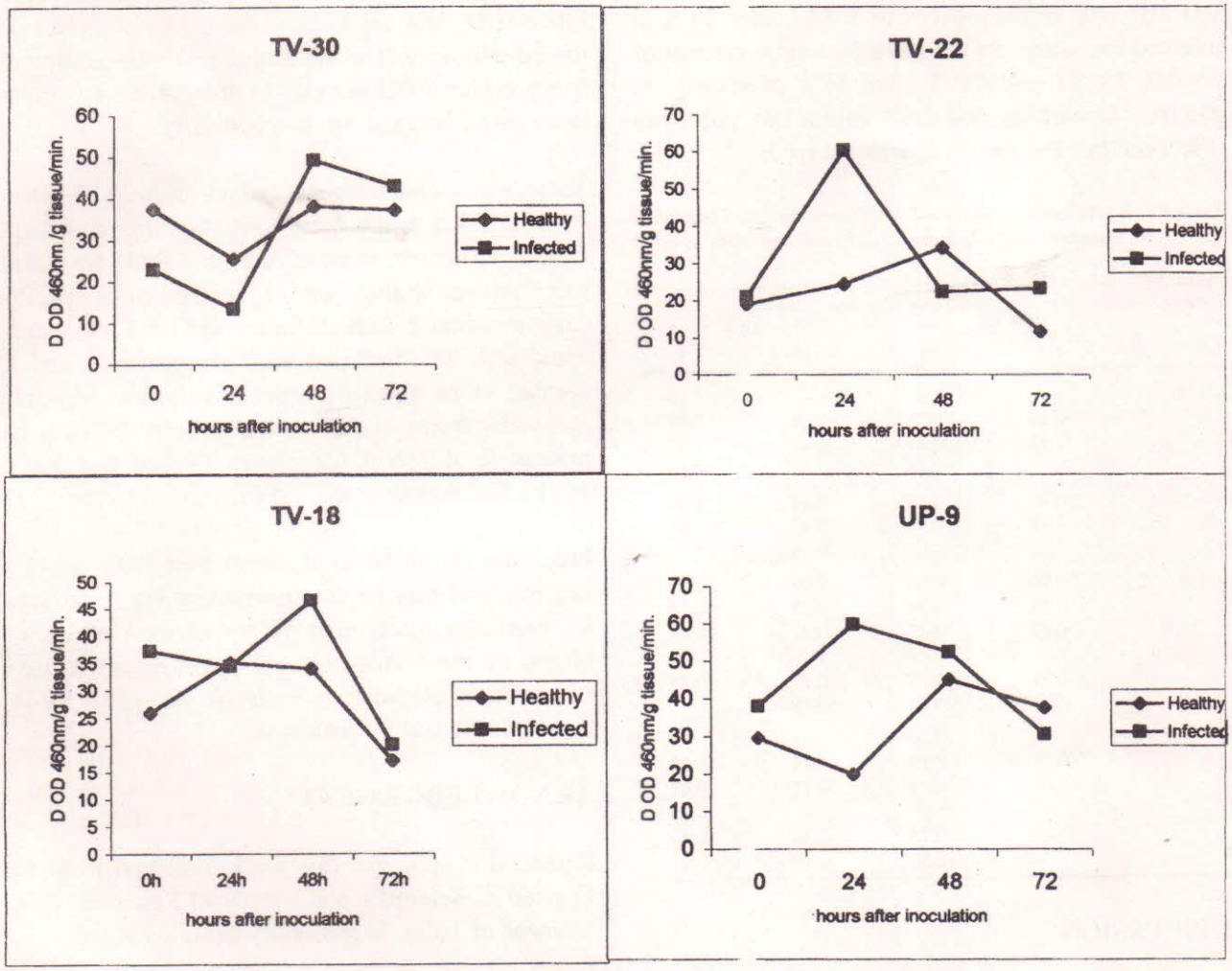

Fig. 1 : Peroxidase activity of tea varieties at different time intervals after inoculation with *G. cingulata.*

showed a response similar to that of inoculated samples, with a prominent reduction in activity 24 h after wounding. However, the peak activity in control was always less than in the infected leaves.

POD isozyme analysis

In total, 6 anodic were detected (Table3), isozyme R_m = 0.28 (I_2) and R_m = 0.47 (I_3), were present throughout the study in all the varieties. The isozyme R_m = 0.66 (I_4) was found to be induced in resistant reactions (TV-30 and S-449) 24 h after inoculation in infected leaves. Only a faint band of I_4 was detected in UP-3 after 48 h and it was totally absent in TV-22. R_m = 0.06 (I_1) was present throughout in S-449, induced in UP-3 after 24 h and in TV-30 after 48 h in inoculated leaves. R_m = 0.75 (I_5) and R_m = 0.85 (I_6) appeared after 24 h in S-449 and UP-3, but I_6 disappeared after 48 h in infected leaves Isozyme I_5 was only weakly present in TV-22. All isozymes, except I_4 were detected in control sets also. All 6 are present in S-449 after 24 h in infected set, while only 2 were present in control of TV-30, TV-22 and UP-3 after 24 h of setting. In general, scratching (control) mimicked pathogen infection, but the response was delayed.

Table 3 : *R_m (relative mobility) values of isoperoxidases in healthy (wounded) and CG-a inoculated leaves of some selected tea varieties.*

Variety	Time after inoculation			
	24 h		48 h	
	H	I	H	I
TV-30	-	-	-	0.06
	0.28	0.28	0.28	0.28
	0.47	0.47	0.47	0.47
	-	0.66	0.66	0.66
TV-22	0.28	0.28	0.28	0.28
	0.47	0.47	0.47	0.47
	-	-	0.75	0.75
S-449	0.06	0.06	0.06	0.06
	0.28	0.28	0.28	0.28
	0.47	0.47	0.47	0.47
	-	0.66	-	0.66
	0.75	0.75	0.75	0.75
	0.85	0.85	0.85	-
UP-3	-	0.06	-	-
	0.28	0.28	0.28	0.28
	0.47	0.47	0.47	0.47
	-	-	-	0.66
	-	0.75	0.75	0.75
	-	0.85	0.85	-

DISCUSSION

Artificial infection with *G. cingulata* showed an interesting switching on and off of different isoperoxidases with sharp fluctuations in activity. POD activity increased by 2-fold or in some cases, almost by 4-fold (S-449) after challenge with the pathogen. Chakraborty *et al.*, (2002a) reported increase in peroxidase activity following infection with blister infection. However, in the present study, increased activity could not be always correlated with induction of new isozymes, showing importance of qualitative analysis. Similar result was reported in case of response of tea plants to water stress by Chakraborty *et al.* (2002c).

Higher POD activity was more associated with susceptibility than resistance, which confirmed the earlier reports by Gupta *et al* (1990) in *Alternaria* leaf blight and Gupta *et al.* (1992) in groundnut leaf spot. POD was needed to neutralize the peroxide radical formed during oxidative burst on infection. Since H_2O_2 was reported to have a toxic effect on fungal pathogen (Lu and Higgins, 1999), comparatively higher POD activity in compatible combinations could account for susceptibility.

There was a characteristic reduction in POD activity after 24 h in the more tolerant varieties except BSS-2. However, most of the new POD isozymes were induced at that point. I_4 seemed to be specifically associated with tolerance to GC. I_1, I_5 and I_6 could also be correlated with resistance. I_2 and I_3 seemed to be general purpose isozymes. Multiple molecular forms of POD had also been shown to be present in different tea clones (Takeo and Kato, 1971 ; Gunasekar *et al.*, 1996).

From the above study it seems that POD plays a key role and may be acting as a regulatory enzyme in resistance mechanism in tea in case of brown blight. Its mechanism of action and induction may be useful in elucidating Systemic Acquired Resistance (SAR) and its pathways.

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REFERENCES

Barbora, B. C. (1988). Latest concept on management of young tea. *In : Field management of tea* (Eds. S. K. Sarkar, H. P. Bezbaruah) pp. 56-62 ; Tocklai Experimental Sta-

tion, Jorhat, Assam.

Chakraborty, B. N., Dutta, S. and Chakraborty, U. (2002a) Biochemical responses of tea plants induced by foliar infection with *Exobasidium vexans. Indian Phytopath.* **55**(1) : 8–13.

Chakraborty, U. ; Das, R. ; Basu, P.; Guha, S. and Chakraborty, B. N. (2002b) Serological cross reactivity between *Glomerella cingulata* and *Camellia sinensis. Indian Phytopath,* **55**(1) : 1–7(2002)

Chakraborty, U.; Dutta S. and Chakraborty, B. N. (2002c) Response of tea plants to water stress. *Biologia Plantarum.* **45**(4) : 557–562.

Davis, B. J. (1967). Disc electrophoresis methods and application to human serum protein. *Annl. N. Y. Acad. Sci.,* **121** : 404.

Dickens, J. S. and Cook, R.T.A. (1989). *Glomerella cingulata* on *Camellia. Plant Pathology* **38** : 147–168

Gunasekhar, M.; Marimuthu, S., and Ramasamy, V. (1996). Substrate specificity of isoperoxidases in the shoots of tea (*Camellia* spp. clones. *J. Plantation Crops.* **24** : 560–568.

Gupta, S. K.; Gupta, P. P.; Kaushik, C. D. and Chawla, H. K. L. (1992). Metabolic changes in groundnut leaf due to infection by leaf spot pathogens. Indian Phytopath, **45**(4) : 434–438.

Gupta, S. K.; P. P.; Yadava, T. P. and Kausik, C. D. (1990). Metabolic changes in mustard due to *Alternaria* leaf blight. *Indian Phytopath.,* **43** : 64-69

Lu, H. and Higgins, V. J. (1999). The effect of hydrogen peroxide on the viability of tomato cells and of the fungal pathogen *Cladosporium fulvum. Physiol. and Mol. Pl. Path.* **54** : 131 – 143

Takeo, T., and Kato, Y. (1971). Tea leaf peroxidase : Isolation and Partial purification. *Plant and Cell Physiol.* **12** : 217–223.

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