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Morphological, Physiological and Biochemical Changes in Resistant and Susceptible Cultivars of Tea in Relation to Phomopsis Disease

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Abstract: An experiment was conducted under greenhouse condition to study the morphological, physiological and biochemical changes in tea plants due to *Phomopsis* infection. Physiological responses of tea plants to infection in term of photosynthetic rate, transpiration rate, stomatal conductance, water use efficiency and total chlorophyll content were studied in susceptible TRI-2024 and tolerant TRI-2025 cultivars. In addition, growth characteristics such as height, dry weight and plant strength and biochemical parameters such as total sugar, nitrogen, amino acids, protein, polyphenols and catechins of infected and healthy plants were also studied. The results revealed that all the growth characteristics, physiological and biochemical parameters were reduced significantly in infected plants rather than healthy plants. However, the reduction was more prominent in susceptible cultivar than in tolerant ones. Clonal susceptibility of a few tea cultivars was tested by inoculating the pathogen onto susceptible and tolerant clones. A clear variation in the size of the canker was noticed in the susceptible TRI-2024 and tolerant TRI-2025 cultivars.

Key words: Tea, *Camellia*, *Phomopsis*, canker, photosynthesis

INTRODUCTION

Tea (*Camellia sinensis* (L.) O. Kuntz) is a perennial woody plant having a single main stem from which numerous branches are developed to a crown of leaves to get a busy appearance. Being a monocultural crop, it provides a stable microclimate for a number of pests and diseases. Perennial habit of the tea plant, peculiar cultural conditions and warm humid climate of the tea growing areas are highly conducive for disease development (Baby, 2001).

The majority of diseases in tea are of fungal origin and bacteria and one each of virus and algae. In a recent monograph on tea diseases, Chen and Chen (1990) described nearly 400 pathogens. Irrespective of the pathogen and the parts affected, the disease symptoms manifest as debilitation, defoliation and sometimes death of bushes. Among the stem diseases of tea, *Phomopsis* canker disease caused by the fungus *Phomopsis theae* Petch is the most common stem disease in young tea (Ponmurugan and Baby, 2006). This disease is a serious problem in all tea growing areas of the world leading to replanting debacle (Shanmuganathan, 1965; Venkata Ram, 1973; Rattan, 1986). The disease has great economic importance as the area under replanting and new clearings with clonal tea (Ponmurugan *et al*., 2006). Crop loss due to this disease is substantial as it leads to capital losses. In general, due to pathogen attack on host plants, the plant metabolism is getting altered obviously. However, the change of plant metabolism is varied significantly between susceptible and tolerant plants. No information is available on the response of tea plants to infection by *Phomopsis* canker pathogen. In this context, present problem was taken up and attempts were made to study the morphological, physiological and biochemical response of susceptible and tolerant tea cultivars to infection.

MATERIALS AND METHODS

The present study was carried out at Division of Plant Pathology, UPASI Tea Research Institute, Valparai-642 127, Coimbatore District, TN, India, for a period of five years (1999-2003). *Phomopsis theae* isolated from diseased tea stem (IMI No. 384005) was used for the present study. Two year old susceptible TRI-2024 and tolerant TRI-2025 cultivars of tea were selected for the present study. These plants were grown in earthen pots and maintained under greenhouse condition. The pathogen was grown in autoclaved tea stem bits (10 days old) and used as inoculum. These plants were infected with the pathogen by means of soil infestation method. Soil infestation of the pathogen was done by placing the inoculum 5 cm below the soil level around the plants. Wound was made at the collar with a sterile scalpel. The pots were covered with polythene sheets to maintain the

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humidity for the establishment of pathogen. The plants were watered regularly for three weeks and thereafter the surface soil kept dry, by watering the plants to the root zone through the PVC pipe kept into the soil to a depth of 20 cm. Observations were made on the development of canker and other responses due to infection in the plants after one year. Simultaneously, cankered portion was cut and incubated in moist chamber for re-isolation of the pathogen. A control was maintained with inoculation without collar injury.

Physiological parameters such as photosynthetic rate (Pn rate), Transpiration rate (Tr), Stomatal conductance (Sc) and Water Use Efficiency (WUE) were measured in the leaves of diseased and healthy plants of both susceptible and tolerant plants. Pn. Tr, Sc and WUE were recorded by using portable infra-red gas analyzer fitted with a Parkinson leaf chamber (Analytical Development company, UK, Model LCA-3). WUE was computed with the data on evaporation rate and Pn rate.

For estimation of biochemical constituents, canker portion was cut and ground with 5 mL of hot 80% ethanol. The supernatant after centrifugation was taken for the analysis of total sugar (Dubois *et al*., 1956), nitrogen (AOAC, 1990), protein (Lowry *et al*., 1951), lipid (Folch *et al*., 1957), amino acids (Moore and Stein, 1948), polyphenols (Bray and Thorpe, 1954) and catechin (Swain and Hillis, 1959). Total chlorophyll content was measured in the healthy and diseased plant leaves by using a chlorophyll analyzer (Minolta, Singapore). Root carbohydrate level was estimated following the method of Mc Cready *et al*. (1950). Growth response of the plants was studied in term of plant height, dry weight and plant strength. Plant strength was calculated as based on the total dry weight of the plant (g) and height of the plant (cm) described by Maskina *et al*. (1984) and represented as dry matter per unit area.

Canker size (length and width) was measured using metric scale. The bark moisture content of plants was estimated following the method of Bier (1959). Five millimeter discs of barks were removed from the plant collar portion with the help of a sharp cork borer and recorded fresh weight. The discs were soaked in distilled water till the tissues attain constant weights. Finally bark moisture was calculated based on fresh weight of the bark disc-final weight × 100.

RESULTS AND DISCUSSION

The results showed that infection of the pathogen was more severe in TRI-2024 than in TRI-2025, which coincided with the susceptibility of the cultivar (Table 1). The canker size in TRI-2024 was 2.6 × 1.4 cm and it was 0.8 × 0.2 cm in TRI-2025. No canker was developed in the control plants which confirm the earlier reports that *P. theae* is an wound pathogen (Venkataram, 1973). Requirement of wound as a pre-requisite for infection and canker development is known for many species of *Phomopsis* like *P. occulta* on blue Spruce (Igoe *et al*., 1995), *P. subordinoria* on Plantago (Nooji and Vender, 1987), *P. vaccinii* on Blueberry (Parker and Ramsdell, 1977) and *P. asparagi* on Asparagus (Zheng *et al*., 1983).

Table 1: Biometric parameters, bark moisture and canker size of susceptible and tolerant tea cultivars to *P. theae* infection

	Cultivars				
	TRI-2024		TRI-2025		
Parameters	Healthy	Infected	Healthy	Infected	CD at p=0.05%
Plant height (cm)	38.33	35.53	39.33	38.03	2.24
Dry weight (g)	3.82	2.72	2.89	2.07	0.59
Plant strength (Dry matter/unit area)	0.93	0.61	0.98	0.86	0.17
Bark moisture (%)	80.50	76.81	95.76	92.94	5.84
Canker size (cm)	-	2.6 × 1.4	-	0.8 × 0.2	-

Growth response of the plants such as height, dry weight and plant strength were reduced significantly in infected plants (Table 1). The plant strength of uninfected TRI-2024 was 0.93, which was reduced into 0.61 dry matter/unit area due to *Phomopsis* canker. On the other hand, it was very mild in TRI-2025. It was due to the susceptibility of the cultivar. In Alfalfa plants infected with *Verticillium albo-atrum*, Packer *et al*. (1990) observed a reduction in growth parameters like height, dry weight and plant strength of the plant. These alterations in growth might be due to the energy demands of the host-parasite interaction (Igoe *et al*., 1995) and low dry matter production due to inefficient CO_2 assimilation (Michael, 1978). The results of the estimation of bark moisture content indicated that the difference in bark moisture content of healthy and infected plants was highest with TRI-2024 and least with TRI-2025. Bier (1964) advocated that bark moisture below 80% predispose the plants to pathogen infection. The bark moisture of infected TRI-2024 clone was 76.81% and it was 92.94% in TRI-2025 clone. In the present study, the bark moisture of the infected/ susceptible plants fell in the same range. This confirms the observations of Bier (1964).

Estimation of the physiological parameters such as photosynthetic rate, transpiration rate, stomatal conductance, water use efficiency and total chlorophyll content showed a reduction in diseased plant leaves compared to healthy ones (Table 2). Reduction on these parameters was more prominent in susceptible clones than in tolerant ones. In healthy plant leaves, there was no significant difference in physiological parameters such as Pn rate, Tr rate, WUE and Sc. While a significant

Table 2: Physiological response of susceptible and tolerant tea cultivars to *P. theae* infection

	Cultivars				
	TRI-2024		TRI-2025		CD at
Parameters	Healthy	Infected	Healthy	Infected	p = 0.05%
Photosynthetic rate (μ mol CO_2 $m^{-2}sec^{-1}$)	5.73	4.13	6.73	5.93	0.63
Stomatal conductance (mm sec^{-1})	0.32	0.27	0.34	0.31	0.06
Water use efficiency (Ratio of Pn/Tr rate)	3.23	2.57	3.87	3.33	1.12
Transpiration rate (μ mol H_2O evolved $m^{-2}sec^{-1}$)	1.93	1.43	2.03	1.83	0.34
Total chlorophyll (mg g m^{-1} fresh wt.)	3.27	2.17	3.73	3.43	0.21

Table 3: Biochemical changes* of susceptible and tolerant tea cultivars to *P. theae* infection

	Cultivars				
	TRI-2024		TRI-2025		CD at
Parameters	Healthy	Infected	Healthy	Infected	p = 0.05%
Total sugar	5.80	3.09	4.74	4.04	0.74
Total nitrogen	1.58	1.08	1.24	1.08	0.58
Total polyphenols	2.72	1.18	3.37	2.53	0.32
Total catechin	2.60	1.79	3.21	2.29	1.34
Total protein	0.94	0.83	0.83	0.73	0.21
Total amino acids	0.82	0.93	0.60	0.76	0.28
Total lipid	0.86	0.54	0.82	0.67	0.23
Total root Carbohydrate	17.62	12.12	23.15	21.74	8.59

* % of dry matter content

reduction in Sc was noted only in susceptible clone TRI-2024 and transpiration rate in tolerant clone TRI-2025. Reduction in photosynthetic efficiency of cankered plants may be due to reduction in chlorophyll content. Reduction in Pn rate, Tr rate, Sc and shoot water potential due to pathogen infection has been reported from other pathosystems such as Coconut palm (Michael, 1978) and Area nut (Chowdappa and Balasimha, 1992). The reduction in stomatal conductance in diseased leaves has been attributed to the water stress induced by the pathogen (Packer *et al*., 1990). Further, in infected plants there was significant reduction in the root carbohydrate reserves. This may be attributed to the decreased photosynthetic efficiency of the plant and impaired conducting system.

Due to pathogen infection, biochemical parameters such as total sugar, nitrogen, lipid, amino acids, protein, polyphenols and catechin were depleted in the plants (Table 2). The depletion was more pronounced in susceptible clone than in tolerant one. The depletion may be attributed to the secretion of certain metabolites to degrade them or utilized by the respective pathogen. Similar observations were reported in many pathosystems (Nooji and Vender, 1987; Kaur and Mehrotra, 1990). On the other hand, due to pathogen infection, biochemical constituents were found to be accumulated in the plants (Prasad *et al*., 1989; Dhillon *et al*., 1992).

From the Table 3 observed that the healthy susceptible clone had higher (8.50%) sugar content than tolerant clone (4.74%) and the reduction of sugar content was more (46%) with TRI-2024 and less (15%) with TRI-2025. The higher level of total sugars in susceptible cultivars can be correlated with the susceptibility of the plants to disease (Sindham *et al*., 1987). The reduction of sugar content in diseased plants might be due to the increase in the rate of respiration or utilization by the pathogen as respiratory substrate during pathogenesis (Kaur and Mehrotra, 1990). The reduction in protein content might be due to blockage of protein synthesis or degradation of protein in the host plants.

Sugars are phenolics and the depletion of sugars in the diseased plant parts would result in the depletion of phenolic compounds (Hegde and Anahosur, 2000). In the present study also reduced sugar content was noted in diseased tissues compared to healthy tissues which supported this view.

From the study, it may be concluded that due to pathogen infection, all the morphological, physiological and biochemical parameters were depleted to some extend in the plants which ultimately affect the tea quality either directly or indirectly. Moreover, the plant health may also affect directly, which in turn affect the yield potential.

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