

## BRIEF COMMUNICATION

**Growth, photosynthetic and biochemical responses of tea cultivars infected with various diseases**

P. PONMURUGAN\*, U.I. BABY, and R. RAJKUMAR

UPASI Tea Research Foundation, Tea Research Institute, Valparai-642 127, Coimbatore District, TN, India

**Abstract**

Under natural and greenhouse conditions we found a significant reduction in the physiological and biochemical constituents in leaves of five disease types when compared to healthy ones. The growth characteristics such as height, dry mass, photosynthetic and transpiration rates, stomatal conductance, and water use efficiency were reduced significantly more in susceptible cv. TRI-2024 than in tolerant cv. TRI-2025. Also contents of total sugars, nitrogen, amino acids, proteins, polyphenols, and catechin were reduced in diseased plant leaves. However, the reduction was more prominent in susceptible than tolerant cultivar. Canker size and barker moisture content were larger in the susceptible cultivar than in the tolerant cultivar.

*Additional key words:* *Camellia sinensis*; dry mass; growth; photosynthesis; polyphenols; stomatal conductance; sugars; transpiration.

Tea is the most popular and inexpensive beverage produced from young leaves of the commercially cultivated tea plant [*Camellia sinensis* (L.) O. Kuntze]. Being a monocultural crop, tea 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 (Hajra 2001). A large number of pathogenic organisms from different parts of the plant are available in this ecological niche. The crop loss in tea due to pests and diseases is around 10–20 % in southern India (Sathyanarayana and Barua 1983). Chen and Chen (1990) described nearly 400 tea pathogens. Leaf diseases are more important due to the obvious reason that tea plant is cultivated for its young succulent leaves (Muraleedharan and Chen 1997). These diseases affect the crop by their indirect effect on bush health, but if both young and mature leaves are attacked, the quantity of harvest is reduced (Baby 2001). Leaf blight, leaf spot, leaf rot, and leaf rust are the common leaf diseases of the tea bush.

Tea leaves affected by blister blight (caused by *Exobasidium vexans* Masee), grey blight (by *Pestalotia theae* (Sawada) Steyaert), brown blight (by *Colletotrichum camelliae* Masee), sooty mould (by *Capnodium*

*theae* Boedijn), and red rust (by *Cephaleuros parasiticus* Karst) diseases were studied at UPASI Tea Research Institute's garden, Valparai, located in the western Ghats of southern India (10°30'N, 77°O'E, altitude 1 050 m a.s.l.). The diseased and healthy leaves of clone UPASI-3 from the same bush canopy of fourth year fields (after pruning) planted in the year 1972 were studied.

Further an isolate designated as MP (*Phomopsis theae*, IMI No. 384005) obtained from diseased tea stem was tested using two-year-old susceptible TRI-2024 and tolerant TRI-2025 tea cultivars. These plants were grown in earthen pots and maintained under greenhouse condition. The pathogen inoculum was raised in autoclaved tea stem bits (10-d-old) and used for artificial infection of the plants by soil infestation method (Ponmurugan *et al.* 2002). Soil was infested with the pathogen by placing the inoculum at 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 humidity for the establishment of pathogen. The plants were watered regularly for three weeks and thereafter the surface soil was kept dry, by watering the plants to the root zone through the PVC pipe kept into the pot to a depth of 20 cm. Observations were made on the

Received 29 May 2006, accepted 11 July 2006.

\*Corresponding author; address: School of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode – 637 209, Namakkal District, TN, India, fax: +91 4288 274745, e-mail: drponmurugan@rediffmail.com

**Acknowledgement:** We thank Dr. N. Muraleedharan, Director, and Dr. R. Premkumar, Head of Plant Pathology Division, UPASI Tea Research Institute, Valparai, for providing necessary facilities and constant encouragement to carry out this study.

Table 1. Net photosynthetic rate ( $P_N$ ) [ $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ ], transpiration rate [ $\mu\text{mol}(\text{H}_2\text{O}) \text{ m}^{-2} \text{ s}^{-1}$ ], water use efficiency (WUE), stomatal conductance ( $g_s$ ) [ $\text{mm s}^{-1}$ ], and contents of total chlorophyll (Chl) [ $\text{g kg}^{-1}(\text{FM})$ ] and other biochemical components [%] of diseased (D) and healthy (H) leaves of tea clone UPASI-3 affected by five diseases.

Parameter		Disease	Blister blight	Grey blight	Brown blight	Sooty mould	Red rust	SE	CD at $p=0.05$
$P_N$	H	4.45	4.36	4.44	4.38	4.50	0.47	0.88	
	D	2.12	3.58	3.98	4.15	3.88	0.32	0.44	
$E$	H	2.33	2.32	2.35	2.46	2.38	0.12	0.25	
	D	1.08	1.44	1.50	1.62	1.19	0.12	0.18	
WUE	H	3.65	3.57	3.56	3.69	3.69	0.42	0.72	
	D	2.00	2.02	2.33	2.69	2.47	0.32	0.52	
$g_s$	H	0.09	0.08	0.07	0.07	0.09	0.01	0.03	
	D	0.04	0.05	0.02	0.06	0.07	0.01	0.02	
Chl	H	3.42	3.22	3.22	3.38	3.42	0.05	0.14	
	D	1.15	1.86	2.11	2.74	2.26	0.25	0.62	
Sugars	H	10.12	10.12	9.97	9.88	10.03	2.22	9.66	
	D	3.52	6.44	6.15	5.72	5.45	1.33	5.02	
N	H	4.88	4.86	4.22	4.80	4.58	0.77	4.00	
	D	2.52	3.28	3.56	4.11	2.97	0.58	4.79	
Proteins	H	5.27	5.28	4.88	4.23	5.77	1.68	4.01	
	D	3.22	2.87	2.69	3.29	3.56	1.32	4.65	
Amino acids	H	4.58	5.03	4.96	5.58	4.99	0.66	4.00	
	D	2.52	2.54	2.14	3.25	3.56	1.32	4.65	
Polyphenols	H	16.66	17.23	16.06	18.00	17.77	0.07	11.67	
	D	12.34	12.54	12.28	11.27	13.08	0.43	7.05	
Catechins	H	13.85	13.00	12.44	12.84	13.18	3.15	11.34	
	D	11.00	10.84	10.52	10.87	12.97	2.24	8.15	

development of canker as well as on the responses of the plants due to infection after two years. A control was maintained with inoculation of the pathogen and without collar injury.

Growth response was studied in terms of plant height, dry matter (DM), and plant strength expressed as total DM of the plant and height of the plant as described by Maskina *et al.* (1984) and represented as DM per unit area. Canker size (length and width) was measured using a metric scale. The plant bark moisture content was estimated following the method of Bier (1959). Five-mm disc of bark was removed from the plant collar portion with the help of a sharp cork borer and recorded as fresh matter (FM). The discs were soaked in distilled water till the tissues attained constant matter. Finally bark moisture content was calculated.

Net photosynthetic rate ( $P_N$ ), transpiration rate ( $E$ ), stomatal conductance ( $g_s$ ), and water use efficiency (WUE,  $P_N/E$ ) were measured using portable infrared gas analyzer with a broad-leaf chamber model LCA-3 (Analytical Development Co., UK). Diseased and healthy leaves were then ground in 5 cm<sup>3</sup> of hot 80 % ethanol. The supernatant after centrifugation was taken for the analysis of total sugars (Dubois *et al.* 1956), nitrogen (AOAC 1990), proteins (Lowry *et al.* 1951), amino acids

(Moore and Stein 1948), lipids (Folch *et al.* 1957), polyphenols (Bray and Thorpe 1954), and catechin (Swain and Hillis 1959). Total chlorophyll (Chl) content was measured by using Chl analyzer (Minolta, Singapore). Root saccharide contents were estimated following the method of Mc Cready *et al.* (1950).

We found a significant reduction in  $P_N$ ,  $E$ ,  $g_s$ , and WUE in diseased leaves when compared to healthy ones (Table 1). The reduction in  $P_N$  in diseased leaves was in accordance with the reduction in Chl contents (Table 1). Rajalakshmi and Ramarethinam (2000) found reduction in contents of Chl and carotenoids due to infection of leaves by blister blight. Inhibition in the synthesis of pigments reduces the efficacy of CO<sub>2</sub> fixation in the chloroplasts (Dhillon *et al.* 1992), which in turn reduces DM content (Michael 1978). Harvest index of tea is related to the retention and allocation of photosynthates (Barman and Saikia 2005). Reduction in  $P_N$ ,  $E$ ,  $g_s$ , and shoot water potential due to pathogen infection has been reported in coconut palm (Michael 1978) and area nut (Chowdappa and Balasimha 1992).

Due to pathogen infection, contents of sugars, nitrogen, amino acids, proteins, polyphenols, and catechin were depleted in the leaves (Table 1). The reduction may be attributed to the secretion of certain metabolites to

Table 2. Plant height [cm], dry matter (DM) [g], DM per unit area, bark moisture [%], canker size [cm], net photosynthetic rate ( $P_N$ ) [ $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ ], transpiration rate [ $\mu\text{mol}(\text{H}_2\text{O}) \text{ m}^{-2} \text{ s}^{-1}$ ], water use efficiency (WUE), stomatal conductance ( $g_s$ ) [ $\text{mm s}^{-1}$ ], and contents of chlorophyll (Chl) [ $\text{g kg}^{-1}(\text{FM})$ ] and other biochemical components [%] of tea cultivars susceptible (TRI-2024) and tolerant (TRI-2025) to *P. theae* infection.

Parameter	TRI-2024		TRI-2025		CD at $p=0.05$ %
	Healthy	Infected	Healthy	Infected	
Canker size	-	2.6×1.4	-	0.8×0.2	-
DM per unit area	0.93	0.61	0.98	0.86	0.17
Plant height	38.33	35.53	39.33	38.03	2.24
DM	3.82	2.72	2.89	2.07	0.59
Bark moisture	80.50	76.81	95.76	92.94	5.84
$P_N$	5.73	4.13	6.73	5.93	0.63
$g_s$	0.32	0.27	0.34	0.31	0.06
$E$	1.93	1.43	2.03	1.83	0.34
WUE	3.23	2.57	3.87	3.33	1.12
Chl	3.27	2.17	3.73	3.43	0.21
Sugars	5.80	3.09	4.74	4.04	0.74
N	1.58	1.08	1.24	1.08	0.58
Polyphenols	2.72	1.18	3.37	2.53	0.32
Catechins	2.60	1.79	3.21	2.29	1.34
Proteins	0.94	0.83	0.83	0.73	0.21
Amino acids	0.82	0.93	0.60	0.76	0.28
Lipids	0.86	0.54	0.82	0.67	0.23
Root saccharides	17.62	12.12	23.15	21.74	8.59

degrade them or utilization by the respective pathogen. Similar observations were reported in other pathosystems (Naqvi 1987, Kaur and Mehrotra 1990). On the other hand, due to pathogen infection, biochemical constituents may be accumulated (Prasad *et al.* 1989, Dhillon *et al.* 1992). The reduction of sugar content in diseased leaves might be due to the increase in the rate of utilization by the pathogen as respiratory substrate during pathogenesis (Ponmurugan 2002). The reduction in protein content might be due to blockage of protein synthesis or degradation of protein in the host plants. Sugars are precursors of phenolics and the depletion of sugars in the diseased leaves would result in the depletion of phenolic compounds (Hegde and Anahosur 2000).

The disease incidence was more severe in TRI-2024 than in TRI-2025, which coincided with the susceptibility of the cultivar (Table 2). No canker was developed in the control plants, which confirms the earlier reports that *P. theae* is a wound pathogen (Venkata Ram 1973). Requirement of wound as a pre-requisite for infection and canker development is known for many species of *Phomopsis* such as *P. occulta* on blue spruce (Igoe *et al.* 1995), *P. subordinaria* on *Plantago* (Nooji and Vender 1987), *P. vaccinii* on blueberry (Parker and Ramsdell 1977), and *P. asparagi* on asparagus (Zheng *et al.* 1983).

Plant growth parameters such as plant strength, height, and DM were reduced significantly in infected plants of both cultivars (Table 2). The pathogen effect was mild in TRI-2025 due to its susceptibility. In alfalfa plants infected with *Verticillium albo-atrum*, Packer *et al.* (1990) observed a reduction in plant strength, height, and

DM. These alterations in plant growth might be due to the energy demands of the host-parasite interaction (Igoe *et al.* 1995) and low DM production due to inefficient  $\text{CO}_2$  assimilation (Michael 1978).

Bark moisture content in both healthy and infected plants was higher in TRI-2024 than in TRI-2025, and in the infected/susceptible plants fell in the same range (Table 2). Bier (1964) advocated that bark moisture below 80 % predisposes the plants to pathogen infection. A prominent reduction in physiological parameters in TRI-2024 was noticed when compared to TRI-2025 (Table 2). Due to pathogen infection, all the biochemical parameters were also depleted in both TRI-2024 and TRI-2025. However, the depletion was more pronounced in the susceptible clone than in the tolerant one (Table 2). The higher content of total sugars in susceptible cultivars can be correlated with the susceptibility of the plants to disease (Sindham *et al.* 1987). On the other hand, higher amounts of polyphenols and catechin can be correlated with the tolerance of the plants to the disease. The reduction of biochemical constituents in diseased plants might be due to the increase in the rate of respiration by the pathogen as respiratory substrate during pathogenesis (Kaur and Mehrotra 1990), poor protein synthesis (Michael 1978), and involvement of defence mechanisms (Chowdappa and Balasimha 1992). In infected plants we found a significant reduction in the root saccharide reserves.

We conclude that due to pathogen infection all the biometrical, physiological, and biochemical parameters were reduced which in turn adversely affected plant health and vitality.

## References

- AOAC: Official Methods of Analysis of the Association of Official Analytical Chemists. Vol. 1 & 2. 15<sup>th</sup> Ed. – AOAC, Gaithersburg 1990.
- Baby, U.I.: Diseases of tea and their management – a review. – In: Trivedi, P.C. (ed.): Plant Pathology. Pp. 315-327. Pointer Publication, Jaipur 2001.
- Barman, T.S., Saikia, J.K.: Retention and allocation of <sup>14</sup>C assimilates by maintenance leaves and harvest index of tea (*Camellia sinensis* L.). – *Photosynthetica* **43**: 283-287, 2005.
- Bier, J.E.: The relation of bark moisture to the development of canker diseases caused by native, facultative parasites: I. *Cryptodiaporthe* canker on willow. – *Can. J. Bot.* **37**: 229-238, 1959.
- Bier, J.E.: The relation of some bark factors to canker susceptibility. – *Phytopathology* **54**: 250-275, 1964.
- Bray, H.C., Thorpe, W.V.: Analysis of phenolic compounds of interests in metabolism. – *Meth. biochem. Analysis* **1**: 27-52, 1954.
- Chen, Z.M., Chen, X.F.: [The Diagnosis of Tea Diseases and Their Control.] – Shanghai sci. tech. Publ., Shanghai 1990. [In Chinese.]
- Chowdappa, P., Balasimha, D.: Non-stomatal inhibition of photosynthesis in areca nut palms affected with yellow leaf disease. – *Indian Phytopath.* **45**: 312-315, 1992.
- Dhillon, W.S., Bindra, A.S., Kapoor, S.P.: Some biochemical changes induced in powdery mildew infected grape vine leaves. – *Plant Disase Rep.* **7**: 248-250, 1992.
- Dubois, M., Gills, K.A., Hamilton, J.K., Rebers, P.A., Smith, F.: Sugar estimation by phenol-sulphuric acid method. – *Anal. Chem.* **26**: 350, 1956.
- Folch, J., Less, M., Sloane, S.G.H.: A simple method for isolation and purification of total lipids from animal tissues. – *J. biol. Chem.* **226**: 497-509, 1957.
- Hajra, G.N.: Tea Cultivation. Comprehensive Treatise. – International Book Distributing Company, Lucknow 2001.
- Hegde, Y.R., Anahosur, K.H.: Effect of false smut of rice as yield compounds and growth parameters. – *Indian Phytopathol.* **53**: 356-184, 2000.
- Igoe, M.I., Peterson, N.C., Roberts, D.L.: A *Phomopsis* canker associated with branch die-back of Colorado blue spruce in Michigan. – *Plant Disease* **79**: 202-205, 1995.
- Kaur, G., Mehrotra, R.S.: Biochemical studies in pigeon pea varieties resistant and susceptible to *Phytophthora* blight. – *Plant Disease Res.* **5**: 122-125, 1990.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent. – *J. biol. Chem.* **193**: 265-275, 1951.
- Maskina, M.S., Meelu, O.P., Roberts, D.L.: Effect of organic and inorganic manuring on rice nurseries. – *Inst. Rice Res. Newslett.* **9**: 23-24, 1984.
- Mc Cready, R.M., Guggolz, J., Silveira, V., Owens, H.S.: Determination of starch and amylose in vegetables. – *Anal. Chem.* **22**: 1156-1158, 1950.
- Michael, K.J.: Rate of photosynthesis in coconut palm affected by root wilt disease. – *Placrosym* **1**: 372, 1978.
- Moore, S., Stein, W.H.: Photometric method for use in the chromatography of amino acids. – *J. biol. Chem.* **170**: 367-388, 1948.
- Muraleedharan, N., Chen, Z.M.: Pests and diseases of tea and their management. – *J. Plantation Crops* **25**: 15-43, 1997.
- Naqvi, S.A.M.H.: Possible role phenolics and reducing sugars in stem gall of coriander. – *Plant Disease Res.* **2**: 73-76, 1987.
- Nooji, M.P., Vender, H.A.: *Phomopsis subordinoria* and associated stalk disease in natural populations of *Plantago lanceolata*. – *Can. J. Bot.* **65**: 2318-2325, 1987.
- Packer, B.W.P., Leach, K.T., Hill, R.R.: Growth and physiological response of resistant alfalfa clones infected by *Verticillium albo-atrum*. – *Phytopathology* **80**: 1247-1253, 1990.
- Parker, P.E., Ramsdell, D.C.: Epidemiology and chemical control of *Phomopsis* canker of highbush blueberry. – *Phytopathology* **67**: 1481-1484, 1977.
- Ponmurugan, P.: Studies on *Phomopsis theae* Petch Infecting Tea (*Camellia sinensis* (L.) O. Kuntze) in South India. – Ph.D. Thesis. Bharathiar University, Coimbatore 2002.
- Ponmurugan, P., Baby, U.I., Premkumar, R.: Physiological response of tea plants infected with *Phomopsis theae*, the collar canker pathogen. – In: Rethinam, P., Khan, H.H., Reddy, V.M., Mandal, P.K., Suresh, K. (ed.): Plantation Crops Research and Development in the New Millennium. Pp. 592-594. Coconut Development Board Publ., Kochi 2002.
- Prasad, B.K., Singh, R.N., Narayan, N.: Biochemical changes in nitrogen and carbohydrates in coriander infected with *Protoomyces macrosporos*. – *Indian Phytopathol.* **42**: 426-430, 1989.
- Rajalakshmi, N., Ramarethinam, S.: The role of *Exobasidium vexans* Massee in flavanoid synthesis by *Camellia assamica* Shneider. – *J. Plantation Crops* **28**: 19-29, 2000.
- Sathyanarayana, G., Barua, G.C.S.: Leaf and stem diseases of tea in north east India with reference to recent advances in control measures. – *J. Plantation Crops* **11**: 27-31, 1983.
- Sindham, G.S., Jaglan, B.S., Parashar, R.D.: Changes in phenols and carbohydrates in resistant and susceptible cultivars of groundnut in relation to tikka disease. – *Plant Disease Res.* **2**: 100-101, 1987.
- Swain, T., Hillis, W.E.: The phenolic constituents of *Prunus domestica* I. The quantitative analysis of phenolic constituents. – *J. Sci. Food Agr.* **10**: 63-68, 1959.
- Venkata Ram, C.S.: Influence of genetic factors and cultural practices on *Phomopsis* canker disease of tea. – *UPASI Bull.* **30**: 5-17, 1973.
- Zheng, W.Y., Xun, L.Y., Oiming, Z.H.Y.: Screening of fungicides to *Phomopsis asparagi*. – *Acta agr. zhejiangensis* **7**: 457-463, 1983.