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BRIEF COMMUNICATION

Growth and photosynthetic and biochemical responses of tea cultivars to blister blight infection

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Abstract

Growth characteristics such as leaf area, fresh and dry mass, and shoot length, and physiological parameters such as photosynthetic and transpiration rates, stomatal conductance, and water use efficiency were reduced by blister blight significantly more in a susceptible tea clone TES-34 than in a tolerant clone SA-6. Also the contents of total sugars, nitrogen, amino acids, proteins, polyphenols, and catechin were reduced more in diseased plant leaves. However, the reduction was more prominent in susceptible than in tolerant clone. Among the different hybrids of tea, Assam hybrid UPASI-3 was highly susceptible to blister blight followed by Cambod UPASI-27 and China UPASI-9. Similarly, tea seedling cv. Caline was highly susceptible to blister blight when compared to tea clone UPASI-3. Susceptibility of tea cultivars to blister blight infection is connected with many physical barriers including leaf area, shoot length, moisture contents, and other physiological and biochemical parameters.

Additional key words: *Camellia* species; chlorophyll; clone differences; *Exobasidium vexans*; fresh and dry masses; hybrids; net photosynthetic rate; stomatal conductance; stomatal index; transpiration.

The tea plants that give 'cup that cheers' belong to the family Camelliaceae that contains 82 species of the genus *Camellia*. Taxonomists attribute the heterogeneous origin of the present day commercial tea plants to three distinct taxa, namely 'China' *Camellia sinensis* (L.) O. Kuntze, 'Assam' *C. assamica* (Masters) Wight, and 'Cambod' *C. assamica* ssp. *lasiocalyx* (Planex *ex* Watt) Wight. Among the biotic factors that hinder tea production, blister blight caused by a biotrophic fungus *Exobasidium vexans* Massee is an economically important disease (Muraleedharan and Chen 1997). In a study in Sri Lanka, a crop loss of 33 % was recorded in unprotected areas compared to fields which were sprayed with chemicals (de Silva *et al*. 1977). The pathogen is an obligate parasite without any alternate host and it completes its life cycle in a short span of 11–28 d (Venkataram 1961). Many generations of the fungus are completed when weather conditions are favourable. The fungus infects only the tender leaves and stem, from which tea is manufactured; shoots that are severely affected produce tea of very poor quality (Baby *et al*. 1998).

In southern India, more than 50 tea clones and 15 seedlings are available including 5 bi-clonal seed stocks. Certain clones of tea plants manifest resistance to blister blight. Tea seedlings are prone to attack by blister blight very severely (Hajra 2001). Debnath and Paul (1994) tried to correlate some anatomical and morphological characters of 17 clones with disease severity, but they could not find any significant correlation. Even low levels of resistance are valuable since the need for chemical control measures can be limited. Effective infection of the host plant by the pathogen follows a complex phenomenon involving a series of events that enable or deter the pathogen to effectively cause infection. Pre- and post-infection biochemical and physical changes in the host plants are vital as they influence the events that impart resistance to the disease (Jayaramraja *et al*. 2005).

The objective of the present study was to investigate growth, and physiological and biochemical changes due to blister blight infection in the leaves of susceptible

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(TES-34) and tolerant (SA-6) clones evaluated by Premkumar (2002). We used also tea clones UPASI-3 (belongs to 'Assam'), UPASI-9 (belongs to 'China'), and UPASI-27 (belongs to 'Cambod') hybrids. In addition, we compared the physiological and biochemical changes in leaves due to infection in different tea hybrids and seedlings. Third leaves of crop shoots were selected from the field and analysed afresh. Triplicates were maintained in all experiments and repeated twice.

Growth parameters, *viz*. leaf area, shoot length, and fresh (FM) and dry (DM) masses, were recorded. Leaf area was measured by plotting leaves on graph paper. Shoot length between leaves were measured by using a metric scale. FM and DM of infected and healthy leaves were recorded with suitable procedures. The sequence of lesion development in leaves such as oil spot, developing lesion, sporulating lesion, and necrotised lesion were selected from TES-34 and SA-6 cultivars to blister blight.

Net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and water use efficiency (WUE, P_N/E) were determined in the field using portable infra-red gas analyzer with a broad-leaf chamber model *LCA-3* (*Analytical Development*, UK). Stomatal index (SI) was determined by taking epidermal peelings of the respective leaves according to Baby *et al*. (2000). Diseased (I) and healthy (H) leaves were then ground in 5 cm^3 of hot 80 % ethanol. The supernatant after centrifugation was taken for the analysis of contents of total carotenoids, Car (Harborne 1973), sugars (Dubois *et al*. 1956), nitrogen (AOAC 1990), proteins (Lowry *et al*. 1951), amino acids (Moore and Stein 1948), polyphenols (Bray and Thorpe 1954), and catechin (Swain and Hillis 1959). Relative chlorophyll (Chl) content was measured by Chl analyzer (*Minolta*, Singapore). Analysis of variance was carried out and the significance of variation was determined (Gomez and Gomez 1984).

Generally, the lesions did not appear in the tolerant clone, SA-6. Under favourable environmental conditions, the lesions enlarged fast in the susceptible clone TES-34, while they were restricted in the tolerant ones. SA-6 was the most resistant clone to blister blight infection while the clone TES-34 was susceptible to blister blight (Table 1). We found a significant reduction in P_N, E, g_s, SI, and WUE parallel with the development of lesions on the leaves. The maximum reduction was noticed in the leaves having necrotised lesions. The pathogen usually infects the leaves and causes tiny translucent spots within 3–10 d of infection, which later enlarges and become depressed on the upper surface of the leaves. Simultaneously, the underside of the leaf becomes convex to form the typical blister lesion. These blisters turn necrotic and lead to dieback (Fig. 1). In addition to all these morphological changes several physiological alterations also take place in response to infection (Rajalakshmi and Ramarethinam 2000). It produces enzymes that degrade the polysaccharides of cell wall and thereby enable entry into the cell (Albersheim *et al*. 1969).

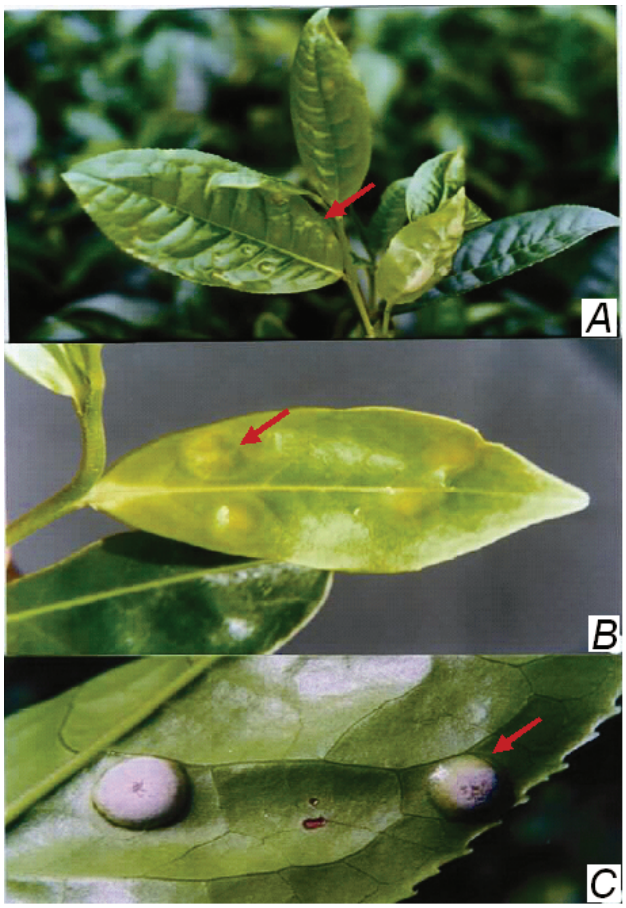

Fig. 1. Appearance of the blister blight lesions in the leaves of susceptible tea clone (TES-34). *A*: tiny translucent spots; *B*: lesions on upper surface of the leaf; *C*: matured sporulating lesions on lower surface of the leaf. Arrows indicate single lesions.

Reduction in physiological and biochemical parameters was more prominent in TES-34 than in SA-6. A reduction in P_N, E, g_s, and shoot water potential was also observed in infected plants of coconut palm (Dhillon *et al*. 1992), alfalfa (Packer *et al*. 1990), and arecanut (Chowdappa and Balasimha 1992). Reduction in g_s can be attributed to the water stress induced by the pathogen (Packer *et al*. 1990). The reduction in P_N in diseased leaves was in accordance with the reduction in Chl and Car contents. A similar result was reported by Rajalakshmi and Ramarethinam (2000). The reduction in P_N may also be due to less efficiency in CO_2 fixation in the chloroplasts which in turn reduces dry matter content (Dhillon *et al*. 1992). According to Jayaramraja *et al*. (2005), thickness of the upper and lower cuticle in SA-6 was the highest. The susceptible clone TES-34 had only fewer trichomes and a less thickened epidermis and cuticle when compared to the resistant clone. The SI was also higher in TES-34 than in the other clones examined.

Due to *E. vexans* infection, contents of sugars, nitrogen, amino acids, proteins, polyphenols, catechin,

Table 1. Net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}], transpiration rate, E [μmol(H_2O evolved) m^{-2} s^{-1}], stomatal index, SI [number per cm^2], stomatal conductance, g_s [mm s^{-1}], water use efficiency, WUE (P_N/E), contents of total chlorophyll (Chl) and carotenoids (Car) [g kg^{-1}(FM)] of blister blight infected tea leaves of susceptible (TES) and tolerant (SA-6) clones.

Clone	Age of lesion	P_N	WUE	g_s	SI	E	Chl	Car
TES	Healthy tissue	4.78	0.45	0.35	95.66	2.88	3.35	1.14
	Oil spot	4.18	0.24	0.33	95.06	2.46	2.83	1.00
	Developing lesion	3.80	0.10	0.30	89.00	2.13	2.53	0.88
	Sporulating lesion	2.38	0.07	0.26	75.23	2.00	1.35	0.64
	Nectrotised lesion	2.02	0.07	0.21	71.56	1.00	1.05	0.41
SA-6	Healthy tissue	6.24	0.95	0.85	98.47	3.88	5.05	1.38
	Oil spot	5.62	0.44	0.73	96.23	3.66	3.83	1.19
	Developing lesion	4.88	0.21	0.39	92.80	2.85	3.66	1.02
	Sporulating lesion	4.08	0.18	0.32	83.26	2.16	2.03	0.80
	Nectrotised lesion	3.62	0.10	0.28	75.00	1.11	1.98	0.66
	CD at p=0.05	0.36	0.05	0.08	5.25	0.57	0.78	0.13

Table 2. Leaf area, LA [cm^2], shoot length, SL [cm], fresh mass, FM [g], dry mass, DM [g], net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}], transpiration rate, E [μmol(H_2O evolved) m^{-2} s^{-1}], stomatal index, SI [number per cm^2], stomatal conductance, g_s [mm s^{-1}], water use efficiency, WUE (P_N/E), contents of total chlorophyll, Chl and carotenoids, Car [g kg^{-1}(FM)], and other biochemical [%] components of blister blight infected (I) and healthy (H) tea leaves of different tea hybrids. CD at p=0.05; Caline clone is compared with UPASI-3 clone in the last column.

	Assam UPASI-3		China UPASI-9		Cambod UPASI-27			Caline		
	I	H	I	H	I	H	CD	I	H	CD
LA	25.18	25.23	18.16	18.56	22.24	22.47	2.56	22.00	22.03	1.08
SL	34.26	34.36	22.00	22.03	29.07	29.20	3.50	30.04	30.33	2.54
FM	43.62	40.18	22.08	22.00	33.12	31.11	5.12	39.47	37.42	3.47
DM	8.02	11.56	5.03	7.50	5.00	8.51	2.04	7.00	10.62	2.00
P_N	3.06	4.88	3.85	5.50	3.21	4.50	1.09	3.14	4.90	0.02
E	1.87	2.00	1.89	2.12	1.80	2.52	0.56	2.00	2.08	0.06
SI	92.14	95.43	95.56	98.80	93.21	95.00	2.00	90.12	92.21	1.42
g_s	0.60	0.64	0.74	0.81	0.69	0.74	0.19	0.60	0.64	0.11
WUE	0.77	0.88	0.65	0.70	0.70	0.79	0.12	0.77	0.88	0.09
Chl	4.78	5.96	5.89	7.98	4.97	7.98	0.82	3.21	4.96	0.08
Car	1.05	1.35	1.14	1.45	1.11	1.38	0.02	1.00	1.35	0.04
Sugars	4.23	7.12	5.89	8.92	5.45	8.34	0.88	4.54	7.12	1.08
Nitrogen	3.88	4.55	3.84	4.63	3.44	4.44	0.89	3.28	4.00	0.92
Protein	3.45	4.07	3.89	4.55	3.84	4.60	0.56	3.09	4.12	0.88
Amino acids	3.45	5.23	3.55	5.58	3.41	5.55	1.26	3.42	5.18	1.22
Polyphenols	11.08	14.58	12.87	17.17	12.00	16.88	2.15	10.89	14.00	3.55
Catechin	11.00	13.89	13.56	16.89	13.45	16.00	2.11	8.89	11.00	2.41

and other constituents were depleted significantly in the leaves. The depletion was more prominent in Assam followed by Cambod and least in China hybrids (Table 2). It was due to leaf area, internodal length, and FM and DM. The reduction in biochemical constituents may be attributed to the secretion of certain metabolites that degrade them or are utilized by the pathogen. Similar observations were reported in other pathosystems (Naqvi 1987, Kaur and Mehrotra 1990). On the other hand, reports on the accumulation of certain biochemical constituents due to pathogen infection in the leaves are also available (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 the blockage of protein synthesis or degradation of protein in the host plants. Sugars are precursors of phenolics and the depletion of sugars in diseased leaves would result in the depletion of phenolic compounds (Hegde and Anahosur 2000).

UPASI-3 belonging to Assam jats was less susceptible to blister blight infection than Caline seedlings belonging to the same jats. But UPASI-3 was more susceptible to the same disease when compared to Cambod followed by China hybrids under natural conditions. The third leaf and mother leaf supply adequate amounts of sugars to sinks. The third leaf is in transient stage, and

hence it neither imports nor transports sugars (Raj Kumar *et al*. 1998).

Prominent reduction in all constituents in the susceptible clone is due to the susceptibility nature to the disease. Similar observations were also made in tea plants infected by collar canker pathogen, *Phomopsis theae* (Ponmurugan *et al*. 2002, 2007). Pius *et al*. (1998) found that the thickness of the cuticle, content of epicuticular wax, stomatal frequency, and other biochemical parameters were responsible for tolerance in SA-6. Jayaramraja *et al.* (2005) studied the role of certain factors, physical barriers, and chitinase enzyme associated with blister blight resistance in tea cultivars. According to them, the blister blight resistance of the tea clone SA-6 was due to higher amount of epicuticular wax, increased thickness of cuticle/epidermal layer, size of vascular bundles, and SI, and functioning as physical barrier to hyphal penetration of *E. vexans*.

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