

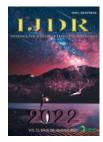
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RELATIVE DISTRIBUTION OF TEA CATECHINS AS INFLUENCED BY BLISTER BLIGHT PATHOGEN

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ABSTRACT

Blister blight pathogen is not only affect the crop productivity,but also impart quality deterioration of processed teas. Among the biochemical constituents, polyphenols and catechins declined considerably due to blister blight infection. This study deals with impact of blister blight infection in relation to relative distribution of catechins of popular, quality tea clones. Results obtained in the present study revealed that irrespective of the cultivars, processed green tea obtained from healthy crop shoots contained significantly higher amount of total polyphenols and catechins. Though, the tea clones selected in the present study were characterized as quality clones, they exhibit variations in terms of total polyphenols and catechins. Irrespective of the clones, blister infected teas registered significantly lesser quantum of total polyphenols and catechins. As it was evident from colorimetric analysis of total catechins, HPLC analysis confirms the reduction in contents of catechin fractions. Generated results on catechin fractions in relation to blister blight pathogen infection are presented and discussed.

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INTRODUCTION

Tea is one of the most popular beverages and widely used from dawn to dusk in the world. As peninsular India is one of the tea growing nations, it contributed substantial quantum of processed teas and earning foreign exchequer, considerably. Several factors influenced the crop productivity; primarily heterozygous origin of tea plants, edaphic and environmental variables, agronomic practices and biotic variables like pest and diseases (Raj Kumar and Mohan Kumar, 2010). Among the biotic variables, disease incidence accounts considerable crop loss. Blister blight is caused by a fungus Exobasidium vexans is one of the most serious foliar diseases of tea and capable of causing enormous crop loss, especially during monsoon period. A comprehensive account of blister blight disease, and various control measures are elaborated by Baby and Premkumar (2000) and Baby (2002). As a plant protection measure contact and systemic fungicides are recommended to protect the plants from blister blight pathogens. Crop loss blister blight disease estimated higher than 37% if uncontrolled (Premkumar and Baby, 1996). Besides the crop loss, tea produced with infected crop shoots deteriorated the quality constituents of processed teas.

From the time of its discovery, tea was used as herbal medicine and chronically evolved to a healthy beverage. Tea has many medicinal properties owing to having no additional calories and the presence of many organic constituents. Nutritional and therapeutic importance of tea arises from its unique combinations of large number of biochemical constituents and it may help in prevent a wide range of diseases due to the presence of polyphenols and catechins (Cabrera *et al.*, 2003; Karori*et al.*, 2007; McKay and Blumber, 2002). Reports are available on blister incidence in relation to quality constituents of made tea (Baby *et al.*, 1998). However, no reports available on fate of catechin fractions due to blister blight infection. This article deals with catechin fractions of quality tea clones as influenced by blister blight infection.

MATERIALS AND METHODS

Irrespective of taxonomic classification, UPASI clones (UPASI-9, UPASI-20, TRF-1 & TRF-4), estate selection (CR 6017) and tea clone released by Tata Tea Limited (TTL-2) representing "Assam", "China" and "Cambod" cultivars were selected for the study. All the tea clones are known for their inherent quality and productivity characteristics and widely used in planting and replanting programs.

These clones are grown in R & D Experimental Farm of Kanan Devan Hills Plantations Company Limited (formerly known as Tata Tea Limited), Munnar, Idukki District, Kerala which is located at an altitude of 1500 m above mean sea level. All the tea plants brought into regular cultural operations in accordance with recommendations of UPASI (United Planters' Association of Southern India) Tea Research Foundation coupled with estate practices (Durairajet al., 2015). The experiments was conducted during monsoon period, where the blister blight infection is highly pronounced. Healthy- and blister-infected (about to sporulation) crop shoots were collected individually and subjected green tea preparation. Collected tea samples were subjected to green tea manufacture (Ramamoorthy and Sathish, 2011) followed by biochemical estimations. Individually collected crop shoots were subjected to steaming process in order to inactivate the enzyme activity in the harvested samples and green tea samples prepared as described by Ramamoorthy and Sathish (2011). The method described by the International Organization for standardization (ISO: 14502-1, 2005) was followed for sample extraction; quantification of total polyphenols was carried out by adopting Folin-Ciocalteu phenol reagent method where gallic acid used as standard and the polyphenol content of the samples expressed as gallic acid equivalents. Total catechins was estimated by the method described by Swain and Hillis (1959) using (+) catechins as standard. Catechin fraction quantification was done following ISO 14502-2 (2005) method. Technical standards (epigallocatechin, (+) catechin, epicatechin, epigallocatechingallate and epicatechingallate) procured from Sigma Aldrich, USA were used for preparation of calibration curve. Individual catechin fractions were computed and presented as per cent corresponding to catechin fraction/g dry weight. Generated data were statistically analyzed (factorial design) and the differences that existed among the results were compared with critical difference (C.D.) at five per cent probability.

RESULTS AND DISCUSSION

As expected green tea manufactured from healthy crop shoots contained significantly higher quantum of polyphenols and catechins, irrespective of tea clones (Table 1).

Table 1. Differential expression of polyphenols and catechins as
influenced by blister blight pathogen infection and clonal
variation

Particular	Clone	Total	Total	
i articular	Cione	polyphenols	catechins	
		(%)	(%)	
Tea made from healthy shoots		25.76	16.30	
Tea made from infected shoots		19.45	12.24	
S.E.		0.038	0.031	
C.D.		0.038	0.031	
C.D.		0.074	0.061	
Clone	TRF – 1	21.73	14.09	
Cione		22.29		
	TRF - 4		14.16	
	CR 6017	23.55	14.91	
	UPASI - 20	22.83	13.98	
	TTL – 2	23.30	14.52	
	UPASI - 9	22.54	13.92	
S.E.		0.082	0.057	
C.D.		0.160	0.111	
Tea made from healthy shoots	TRF – 1	24.58	15.98	
	TRF-4	24.70	16.06	
	CR 6017	27.58	17.40	
	UPASI - 20	24.74	15.91	
	TTL – 2	26.48	16.62	
	UPASI - 9	26.49	15.80	
Tea made from infected shoots	TRF – 1	18.88	12.20	
	TRF-4	19.98	12.25	
	CR 6017	20.12	12.42	
	UPASI - 20	18.92	12.05	
	TTL – 2	20.12	12.45	
	UPASI - 9	18.79	12.04	
S.E.		0.164	0.122	
C.D. P = 0.05:		0.321	0.235	
C.V. (%)	İ	4.18	7.29	

On the other hand, teas produced from blister infected crop shoots registered significant fall in total polyphenols and catechins, where total polyphenols declined by 6.31% and catechins dropped by 4.06%. Tea clones selected in the present study are distinctly defined as high quality clones. However, they exhibit significant variation among them in relation to total poly phenols and catechins. Among the tea clones, CR 6017 recorded significantly higher amount of polyphenols and catechins followed by TTL-2, irrespective of teas produced from healthy or blister blight infected samples. Per cent reduction in polyphenol content due to blister blight infection was more pronounced. Tea clone, UPASI-9 registered as high as 7.70% reduction in polyphenols while TRF-4 recorded 4.72% reduction. When considering the reduction in total catechins, it ranges between 3.76% (UPASI-9) and 4.98% (UPASI-20) in response to blister blight infection. The values obtained in this study with particular reference to primary (healthy/infected), secondary (tea clones) factors and their interactions are significantly different at five per cent probability.

Relative distribution of catechins revealed that among the catechin fractions epigallocatechingallate (EGCG) is predominant followed by epicatechingallate (ECG) and epigallocatechins (EGC). Distinct reduction in individual catechin fractions was noticed in response to blister blight pathogen infection (Table 2). As evident from the Table 1, here again, clonal variation and blister blight infection exhibited variations in terms of individual catechins. Since the HPLC method was advocated in determination of catechin fractions, replicate data were not generated and hence statistical analysis is not possible. However, individual catechin fractions declined in response to blister blight infection. So also, clones were also exhibited variations in catechin fractions. Present study is in conformity with earlier reports with respect to deterioration in biochemical constituents in response to blister blight infection. However, the study was carried out to enumerate the fate of catechin fractions with special reference to blister blight infection that too in quality clones. The disease caused a reduction in the contents of polyphenols, catechins and enzymes present in green leaf which are known to be important precursors determining the quality of black tea (Baby et al., 1998). A corresponding decrease in the quality constituents like theaflavins and quality parameters such as total liquor colour and water soluble solids were also observed in made tea. The disease caused an increase in palmatic acid content and decrease in unsaturated long chain fatty acids and the flavor index value showed a decline upon infection. Analytical data obtained were further confirmed with tasters' evaluation (Baby et al., 1998; Shalini, 2012). Numerous reports are available on the accumulation of phenolic compounds in plant tissues in response to infection. They are often claimed to confer resistance to infection by microbes, presumably by inactivation of fungal enzymes. These compounds accumulate around the sites of injury caused by microbial penetration which are sufficient to inhibit the proliferation of pathogens. In general, tolerant species had higher concentrations of phenolic compounds than susceptible ones and the depletion of phenols due to infection was higher in susceptible plants (Shalini 2012). This may be one of the reason for reduction in phenolic compounds in the present study. In fact, blister infection modified the composition of biochemical constituents which tilted the enzyme to substrate ratio dramatically which ultimately affecting the quality of final produce (Raj Kumar and Shalini, 2010).

Teas produced from diseased leaves affects the quality characteristics. Blister blight pathogen caused a reduction in the bio-constituents like polyphenols, catechins and enzymes present in green leaves which are known to be important precursors determining the quality of processed teas (Baby *et al.*, 1998). Results generated in the present study substantiated the earlier findings. It may be noted that high degree of severity of infection is related with quality deterioration besides the decline in flavor index (Baby *et al.*, 1998). There was an alteration in biochemical constituents of crop shoots due to blister blight pathogen infection. Polyphenols and catechins and amino acids were declined significantly due to blister blight infection except reducing sugars irrespective of susceptible and resistant clones. Similar kind of results were reported by Ajay (2008).

Healthy/BB infected	Catechin Fraction * (%)	TRF-1	TRF-4	CR 6017	UPASI-20	TTL-2	UPASI-9
Healthy	EGC	1.85	1.54	1.73	1.54	1.99	1.58
	С	0.44	0.84	0.99	1.04	1.15	1.06
	EC	0.35	0.77	1.21	0.54	1.12	0.87
	EGCG	7.12	6.56	7.85	6.41	7.06	5.89
	ECG	1.52	1.79	1.84	1.06	1.59	1.49
	Total	11.28	11.50	13.62	10.69	12.91	10.89
BB infected	EGC	1.41	1.11	1.25	1.01	1.49	0.92
	С	0.35	0.71	0.82	0.61	0.86	0.52
	EC	0.29	0.53	0.73	0.41	0.84	0.65
EGCG ECG Total	EGCG	5.12	4.98	5.39	4.81	5.29	4.82
	ECG	1.14	1.17	1.38	0.79	1.19	1.12
	Total	8.31	8.50	9.57	7.63	9.67	8.03

Table 2. Reduction in catechin fractions as influenced by blister blight pathogen infection

It has also been reported that the initial increase in the biochemical constituents as defense mechanisms and its subsequent decline was attributed to the secretion of certain toxins produced by the pathogen to degrade them or the same were utilized by the pathogen (Sanjay and Baby, 2007).

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