Research Article

Journal of Plantation Crops, 2013, 41(2): 202-208

Changes in biochemical constituents and defense related enzymes in response to red spider mite incidence in tea

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(Manuscript Received: 19-03-13, Revised: 18-06-13, Accepted: 01-07-13)

Abstract

In recent years, red spider mite (RSM), *Oligonychus coffeae* (Nietner) menace challenged the crop productivity in tea. Though the reports on bush physiology are available, a compressive data on changes in biochemical constituents including enzymes is lacking. Crop shoots were collected from the field grown tea plants (UPASI-3 & UPASI-10) and segregated into healthy, moderately infested (~4 mites leaf^{-1}) and severely infested (>4 mites leaf^{-1}). The crop shoots were used for determining the biochemical constituents and quality. Stress-related enzymes such as catalase, superoxide dismutase, glutathione reductase and ascorbate peroxidase were also analysed. Irrespective of the RSM damage, UPASI-3 significantly recorded higher amount of polyphenols, catechins, amino acids, reducing sugars and carotenoids. UPASI-10 possessed higher amount of total chlorophylls than that of UPASI-3. Irrespective of the clones, polyphenols, catechins, and pigments linearly decreased with severity of RSM infestation while reducing sugars and hydrogen peroxide linearly increased. Irrespective of the RSM damage, crop shoots of UPASI-10 contained significantly lower amount of quality parameters and stress-related enzymes, except polyphenol oxidase. Though quality related enzymes enhanced due to RSM attack initially and declined when the RSM infestation was severe. All the stress related enzymes progressively increased with the increasing degree of RSM damage. Interactions between activities of enzymes and clones with respect to RSM damage were elucidated.

Keywords: Antioxidant enzymes, *Camellia* spp, quality parameters, red spider mite

Introduction

Tea (*Camellia* spp.) is one of the important plantation crops cultivated in India. As a monoculture crop, tea bushes harbor a variety of pests (caterpillars, leaf rollers, flies, bees, wasps, ants, grasshoppers, crickets, locusts, aphids, scale insects, plant bugs *etc*.) due to its perennial nature which offers a congenial microclimate (Muraleedharan and Chen, 1997; Somchowdhury *et al*., 1993; Sudhakaran, *et al*., 2000). Mites are reported to be seasonal pests and their population attains a peak during the summer months and declines below the threshold level during monsoon. Incidence of red spider mite (RSM), *Oligonychus coffeae* (Nietner) follow the identical trend in bio-ecology; but, due to their adoptability, they migrate to older leaves during unfavourable conditions. Crop loss due to RSM infestation is as high as 14 to 18 per cent, when the RSM populations traverse beyond the threshold level (Babu, 2010).

Even though, tea plants exhibit a wide range of defense responses against RSM attack, generation of reactive oxygen species (ROS) is unavoidable which causes destructive oxidative process like chlorophyll bleaching, lipid peroxidation, protein oxidation and finally the death of the cells (Mur *et al*., 2005; Lu and Finkel, 2008). Due to the generation of ROS, tea leaves turned into coppery brown colour where the RSM infestation is severe. In order to defend against the pest attack, plants synthesize certain antioxidative compounds which activate enzymes (Ni *et al*., 2001; Zhu *et al*., 2004).

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Reports are available on the physiology of tea bush with particular reference to RSM infestation (Sudhakar *et al*., 2000), and the present study was envisaged to document the changes that occur to the biochemical constituents and stress related enzymes due to RSM infestation.

Materials and methods

The experiment was conducted at UPASI Tea Research Foundation, Tea Research Institute's experimental farm between 2009 and 2010. For this purpose, two field grown tea clones, UPASI-3 and UPASI-10 representing "Assam" and "China" cultivars were selected. Each experimental block consisted of forty bushes each. Except the RSM control measures, all other cultural practices were carried out as per UPASI recommendations (Muraleedharan *et al*., 2007). Leaf samples were collected during peak RSM infestation (February/ March) period of each agricultural year. Crop shoots and mother leaves collected from individual bushes were observed under binocular microscope for presence of number of RSM and recorded. Accordingly the crop shoots were segregated in to three different group *viz*., healthy (control), moderately infested (~4 mites leaf^{-1}) and severely infested (>4 mites leaf^{-1}). Designated crop shoots were subjected to chemical analyses and enzyme assays.

Quantification of polyphenols and catechins were done following the methods reported by Dev Choudhury and Goswami (1983) and Swain and Hillis (1959), respectively, while amino acids and reducing sugars were determined following the methods Moore and Stein (1948) and Hedges and Hofreiter (1962), respectively. Results were expressed in per cent dry weight. Chlorophyll and carotenoid pigments were quantified using the method suggested by Wellburn (1994). Total H_2O_2 content was assayed by measuring formation of ferrithiocyanate complex (Thurman *et al.,* 1972).

Both quality related enzymes such as polyphenol oxidase (PPO), peroxidase (POX), phenylalanine ammonia lyase (PAL) and stress-related enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and ascorbate peroxidase (APX) were analysed adopting the standard protocols. Polyphenol oxidase was assayed following the method described by Singh and Ravindranath (1994). Peroxidase activity was determined as described by Chance and Maehly (1955) while PAL activity was assayed spectrophotometrically following the formation of transcinnamic acid (Brueske, 1980). Antioxidant enzymes, ascorbate peroxidase (Chen and Asada, 1989), glutathione reductase (Rao *et al*., 1996), superoxide dismutase (Beyer and Fridovich, 1987) and catalase (Mishra *et al*., 1993) were also assayed in response to clonal variation and degree of RSM infestation.

Statistical analysis

Factorial design statistical analysis was adopted where two factors *viz*., clones and degree of RSM infection were considered as primary and secondary variables (Gomez and Gomez, 1984). Results are presented with standard error mean, critical difference (between clones/among the degree of RSM infestation) and coefficient of variation.

Results and discussion

UPASI-3 had marginally higher amount of polyphenols than that of UPASI-10 however, both the clones exhibited significant difference in terms of catechins, irrespective of the RSM infestation (Table 1). Amino acids and reducing sugars were also significantly higher in UPASI-3 than that of UPASI-10. Being with a dark shaded leaves, UPASI-10 had significantly higher amount of chlorophylls while carotenoids found to be on par with each other, irrespective of the RSM infestation. On the other hand, UPASI-3 contained significantly higher amount of hydrogen peroxide than the UPASI-10, irrespective of RSM damage.

Healthy crop shoots contained higher amount of polyphenols/catechins followed by moderate and severe infestation of RSM (Table 1). Both polyphenols and catechins reduced significantly in response to degree of RSM infestation. Chlorophylls and carotenoids linearly declined with degree of RSM damage and followed identical pattern of decrease as observed in the case of polyphenols. On the other hand, amino acids, reducing sugars and hydrogen peroxide (free radicals) were significantly increased with increasing level of RSM damage.

Table 1. Native levels of biochemical constituents in response to varying degree of RSM damage in clonal tea crop shoots

Clone	Infestation	*PP (%)	Cat (%)	AA (%)	RS (%)	Chl	Caro	H_2O_2
UPASI-10		27.49	16.76	2.58	5.62	2.75	0.91	25.45
UPASI-3		27.96	17.28	2.80	5.85	2.62	0.95	31.07
	S.E	0.31	0.14	0.02	0.09	0.03	0.02	0.27
	CD	0.61	0.27	0.04	0.18	0.06	0.04	0.55
	Healthy (H)	28.95	18.11	2.55	5.40	2.82	1.01	29.68
	Moderately affected (M)	27.60	17.00	2.70	5.78	2.61	0.93	31.14
	Severely affected (S)	26.64	15.94	2.82	6.04	2.43	0.85	32.40
	S.E	0.65	0.29	0.04	0.19	0.06	0.04	0.56
	C.D	1.28	0.58	0.08	0.37	0.12	0.08	1.13
UPASI-10	H	28.74	17.76	2.45	5.27	2.95	0.97	26.98
	M	27.28	16.54	2.56	5.65	2.79	0.91	27.50
	S	26.46	15.98	2.72	5.95	2.51	0.84	28.65
UPASI-3	H	29.16	18.46	2.65	5.53	2.79	1.05	29.68
	M	27.92	17.46	2.83	5.90	2.67	0.95	31.14
	S	26.81	15.90	2.91	6.12	2.52	0.86	31.94
	S.E	1.37	0.62	0.09	0.40	0.12	0.08	1.10
	C.D	2.68	1.20	0.17	0.78	0.24	0.16	2.18
	C.V	8.17	4.66	7.95	4.87	6.49	2.88	7.86

*PP: polyphenols; Cat: catechins; AA: amino acids; RS: reducing sugars; Chl: chlorophyll (mg g $fr.wt.^{-1}$); Caro: carotenoids (mg g $fr.wt.^{-1}$); H_2O_2: hydrogen peroxide (µ mole g $fr.wt^{-1}$)

Interaction between clones and RSM damage exerted significant variation in terms of certain biochemical constituents. RSM infestation dramatically decreased the polyphenols and catechins by >2% irrespective of the clones. Polyphenols declined substantially due to RSM infestation but it was not statistically different at five per cent probability (Table 1). Identical trend was noticed in the case of reducing sugars as well. However, reduction in catechins and chlorophylls was significant between healthy and severely affected crop shoots collected from two different tea clones. On the other hand, RSM infestation significantly enhanced the amino acid content in both the clones. Only UPASI-3 exhibited significant reduction of carotenoids while the reduction was not statistically different in UPASI-10. In general, hydrogen peroxide production was higher in both the clones due to RSM infestation. But production of free radicals was significantly higher in UPASI-3. Even though UPASI-10 produced hydrogen peroxide in response to RSM damage, it was not statistically significant at five per cent level.

UPASI-10 exhibited marginally lower PPO activity than that of UPASI-3. Except PPO activity, all other enzyme activities were significantly higher in UPASI-3 crop shoots than the UPASI-10, irrespective of the RSM damage (Table 2). Exponential relationship existed between quality related enzymes (PPO, POX and PAL) and degree of RSM infestation. Activities of PPO and PAL attained their peak when the tea crop infested moderately and declined substantially when RSM infestation attained severe. But these enzyme activities sustained above native (control/healthy) levels. Interestingly, POX activity fell below the level of healthy shoots when RSM damage found severe. Antioxidant enzyme activities (CAT, SOD, GR and APX) progressively increased linearly with increasing level of RSM damage. Significant difference in CAT and SOD was found with respect to healthy and moderately damaged and so on. In the case of GR and APX, significant difference between healthy and severely damaged crop shoots was evident. Being a quality clone, UPASI-3 exerted higher activities of enzymes than the UPASI-10 related to quality. However, antioxidative enzyme activities were more pronounced due to RSM infestation in UPASI-3 than that of UPASI-10.

Table 2. Native levels of quality related and antioxidant enzymes in response to varying degree of RSM damage in clonal tea crop shoots

Clone	Infestation	PPO	POX	PAL	CAT	SOD	GR	APX
UPASI-10		381.14	15.30	0.501	0.202	2.26	2.98	2.88
UPASI-3		392.55	16.13	0.574	0.220	2.64	3.24	3.44
	S.E	9.10	0.18	0.012	0.004	0.06	0.06	0.09
	CD	17.80	0.35	0.025	0.008	0.11	0.12	0.18
	Healthy (H)	346.28	15.30	0.479	0.132	2.14	2.95	2.94
	Moderate (M)	417.05	16.01	0.553	0.210	2.42	3.14	3.01
	Severe (S)	380.11	14.54	0.517	0.291	2.79	3.25	3.55
	S.E	19.10	0.38	0.026	0.009	0.13	0.13	0.19
	C.D	37.45	0.74	0.051	0.017	0.25	0.25	0.37
UPASI-10	H	330.00	14.54	0.445	0.120	1.97	2.83	2.90
	M	409.09	15.12	0.509	0.203	2.19	2.99	2.60
	S	370.13	13.63	0.459	0.283	2.64	3.13	3.15
UPASI-3	H	362.56	16.05	0.550	0.145	2.30	3.07	2.97
	M	425.00	16.89	0.597	0.217	2.66	3.28	3.42
	S	390.09	15.44	0.574	0.299	2.95	3.37	3.94
	S.E	39.15	0.79	0.055	0.018	0.27	0.27	0.39
	C.D	76.78	1.56	0.108	0.035	0.52	0.52	0.77
	C.V	4.51	6.39	7.92	3.69	9.11	2.29	10.12

PPO: polyphenol oxidase (U mg protein^{-1}); POX: peroxidase (µ mole product formed mg protein^{-1} min^{-1}); PAL: phenyl alanine ammonialyase (µ mole cinnamic acid formed min^{-1} mg protein^{-1}); CAT: catalase (µmole H_2O_2 oxidised min^{-1} mg protein^{-1}); SOD: super oxide dismutase (U mg protein^{-1}); GR: glutathione reductase (µmole NADPH oxidized min^{-1} mg protein^{-1}); APX: ascorbate peroxidase (µmole product formed mg protein^{-1} min^{-1})

Field grown plants invariably affected by prevailing environmental conditions caused either by biotic or abiotic variables. Invasion of pests and pathogens impose physiological stress to the host plants. Under such conditions, plants have to make necessary metabolic and structural adjustments to cope with the stress conditions. For example, the plant cuticle is regarded as a barrier to encounter most herbivores and pathogens. Once this is breached, general strategies, such as the production of hydrogen peroxide, are used to strengthen cell walls (Lattanzio *et al*., 2006). Phenolics occur in most plants and many of these compounds likely serve some protective role (Agrawal and Heil, 2012). Most of the plants produce a wide range of secondary metabolites either as part of their growth and development processes or in response to biotic stress which may protect plants against attack by a wide range of potential pests and pathogens (Lattanzio *et al*., 2006). They also stated that there had been a positive relationship between resistance/ susceptibility characteristics and flavanols against aphids infestation. In the present study, healthy crop shoots possessed higher quantum of flavanols which has been declined substantially due to RSM infestation.

Increase or decrease in amino acid content was reported earlier in the leaves of mulberry varieties due to thrips attack (Mahadeva, 2011). RSM damage resulted in significant enhancement in amino acids and reducing sugars, irrespective of the tea clones, in the present study. It has already been demonstrated that tea plants under soil moisture stress synthesized relatively higher amount of proline (an amino acid) to over come soil moisture stress which acts as an osmoticum (Marimuthu and Raj Kumar, 2001). Being the sucking pest, RSM infestation might mimic the soil moisture stress that would have enhanced the amino acid content in the crop shoots or blockage of protein synthesis/break down of the proteins as reported earlier (Ponmurugan *et al*., 2007). Apart from the nutritional imbalances, cell wall damage will cause the increase in amino acid content and sugars in leaf apoplast (Spann and Schurmann, 2011). Alterations in quantum of reducing sugars may be due to reduction in leaf lamina and malformation of leaves due to pest infestation. Mahadeva (2011) reported mixed trend in reducing sugars in a variety of mulberry plants.

Significant reduction in leaf pigments were reported earlier due to biotic stresses in crop plants

(Ponmurugan *et al*., 2007; Mahadeva, 2011). Rajalakshmi and Ramarethinam (2000) reported reduction in contents of chlorophylls and carotenoids due to infection of leaves by blister blight. Identical results were obtained in the present study in terms of leaf pigments, particularly degradation of chlorophylls and carotenoids.

Wound-induced ROS accumulation, in particular hydrogen peroxide, is observed both locally and systemically in leaves of several plant species (Maffei *et al*., 2006). RSM damage resulted in production of hydrogen peroxide significantly and its production was linearly increased with concurrent increase in the RSM damage. As reported earlier, being the most stable form of ROS, hydrogen peroxide can move into the cell membrane and initiate oxidative damage in leaf cells, resulting in disruption of metabolic function and loss of cellular integrity (Hung *et al*., 2005). Due to oxidative damage and disruption in metabolic functions severely infected tea leaves coppery brown in colour.

In general, defensive response to wound can be regarded as activation and induction phases. Under activation phase immediate response to cellular damage is loss cell integrity wherein a variety of oxidative and hydrolytic enzymes are released from compartmentalization (Sanjayan, 2008). Plants synthesize different types of antioxidant compounds and antioxidant enzymes such as catalase and peroxidase (Ni *et al*., 2001; Zhu *et al*., 2004). Phloem-feeding alfalfa hoppers (*Spissistilus festinus* L.) increase the activities of several oxidative enzymes (Felton *et al*., 1994). In the present study, oxidative enzymes increased exponentially in response to RSM damage. As reported earlier (Sanjayan, 2008) oxidative enzymes influence the lignin formation. PPO, POX and PAL activities enhanced significantly under moderately RSM infested shoots (activation stage) while it declined with severe infestation (induction stage). As a quality related enzymes their sustenance in crop shoots are highly regarded but quality of pest infested crop shoots declined considerably. *Spodoptera litura* feeding on tomato plants resulted in enhancement of induction of peroxidase and polyphenol oxidase (Sanjayan, 2008). However, persistence of induced responses to herbivory is not clearly understood.

As mentioned earlier, an immediate response of plants to injury is the accelerated accumulation of oxidative enzymes that is required for scavenging toxic radicals. It has been reported that *Helopeltis theivora* infestation enhanced the activities of all oxidative enzymes, particularly, peroxidase, ascorbate peroxidase and polyphenol oxidase (Chakraborty and Chakraborty, 2005). In the present study, except PPO all other enzymes studied were increased significantly and in particular, the antioxidative enzymes increased linearly with increasing damage by RSM. Alterations in biochemical constituents were enhanced during initial stages of blister blight infection and considerable decline in advanced stages (Ajay, 2008).

In general, all the biochemical constituents were reduced in response to biotic stress. However, the rate of depletion was more pronounced in the susceptible clone than in the tolerant tea clone (Ponmurugan *et al*., 2007). In the present study, depletion of biochemical constituents, particularly, polyphenols and catechins were higher in UPASI-3 followed by UPASI-10. Similarly, accumulation of amino acids and free radicals were more prominent in UPASI-3 rather than UPASI-10. Native levels of oxidative and antioxidative enzymes were also considerably higher in UPASI-3 which translates its susceptible nature to biotic and abiotic stresses. In one of the experiments, Chakraborty and Chakraborty (2005) reported that total phenolic contents decreased with insect attack (*Helopeltis theivora*). The decrease was most significant in the UPASI clones which had the highest disease incidence where the authours included UPASI-3 as one of the test material.

Acknowledgements

Authors are grateful to Dr. P. Mohan Kumar, Director, UPASI Tea Research Foundation, Tea Research Institute, Valparai for his constant encouragements and facilities provided to explore the objectives. First author is thankful to CSIR-NMITLI, New Delhi for research fellowship and Mr. M. Sankara Rama Subramanian for his technical help in the present study.

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