

Abstract

Tea (*Camellia sinensis* (L.) O. Kuntze.) as a beverage is an important plantation crop. Tea has stimulating and good health effects. Like many other plants tea also affected by a number of pathogens. Differential interaction of different varieties of tea against different pathogens has been observed by several scientists. The understanding of molecular events of the host pathogen interaction leading to induction of resistance in a susceptible variety is of great importance in the present day agriculture. Hence, the present study has been taken in to consideration. The title of the present work is “Analysis of specific transcripts following induction of defense in tea against foliar fungal pathogens”. To fulfill the study the whole work has been divided into following objectives: 1) Control of disease by different abiotic inducers and disease assessment. 2) Induction of defence-related enzymes in tea by abiotic inducers. 3) Molecular identification of defense related genes of tea. 4) Analysis of selected gene specific transcript accumulation in induced tea plants and 5) *In vitro* control of pathogenic microorganisms by botanicals and biocontrol agents.

To fulfil the objectives, at the onset of the study, review of literature has been done, in the line of the present work, in a selective manner rather than in a comprehensive one.

Before initiation of the experimental work a survey was made for prevalence of the pathogens in the present study area (sub-Himalayan West Bengal). During the survey, four pathogens such as *Colletotrichum gloeosporioides*, *Curvularia eragrostidis*, *Pestalotiopsis theae* were found to attack tea plants and affect tea plants leading to loss of production. On the basis of Pathogenicity tests, two pathogens were selected for the study. Three susceptible tea varieties were also selected to study induction of resistance.

To understand the resistance induction by inducers at the time of host-pathogen interaction three different abiotic inducers (BTH, 3-ABA,

and GABA) were used. Detailed procedures of experiments and materials used for the experiments have been presented in the materials and methods section. Teenali variety was found to be most susceptible among the tested varieties.

BTH induced plants showed best induction of resistance, whereas, BABA and GABA showed moderate induction of resistance on the basis of disease index in comparison to control (untreated-inoculated) plants.

Three inducers (BTH, BABA and GABA) were further used to induce resistance in susceptible tea plants (Teenali variety). Following induction up and down regulation of three different defense related enzymes were studied against challenge inoculation by *C. gloeosporioides*, the most virulent pathogen as found from results of pathogenicity tests. The enzymes studied were β -1, 3-glucanase, phenylalanine ammonia-lyase and peroxidase.

From the results it was found that BTH treated tea plants following challenge-inoculation by *C. gloeosporioides* showed maximum phenylalanine ammonia-lyase (PAL) activity after 4 days but highest activity of PAL was found only in BTH pre-treated plants after six days. In case of β -1, 3-glucanase, highest enzyme activity was found after six days of BTH treatment following challenge-inoculation with *C. gloeosporioides*. Marginally different results were found in case of peroxidase activity. GABA pretreated and *C. gloeosporioides* inoculated plants showed maximum peroxidase activity after six days.

The phylogenetic analysis of PAL gene showed three different clusters among three different *Camellia* species (*C. sinensis*, *C. talensis* and *C. flavida*), where the present isolates clusters together with *C. sinensis*. Phylogenetic tree of CHS gene showed five different clusters with different *Camellia* species but APX gene did not show any significant clusters like PAL and CHS gene. The results of BLASTn analysis of all the three genes (PAL, CHS and APX) showed more than 90% nucleotide sequence similarity with other *Camellia sinensis* gene submitted in the GenBank. From the sequence similarity matrix it was found that PAL gene of *Camellia sinensis*

showed above 90% nucleotide identity within the species and below 60% identity between *C. sinensis* and other species of *Camellia*. CHS gene showed above 95% nucleotide identity within the species and below 75% to 95% identity between *C. sinensis* and other species of *Camellia*. In case of APX gene, no significant group was observed as PAL and CHS gene through sequence similarity matrix.

Further analysis of sequence diversity among these three defense related genes (PAL, CHS and APX) relative synonymous codon usage (RSCU) pattern was also studied. From the results it was observed that highest %GC and GC3 along with overall pyrimidine content at third codon position were higher in CHS and PAL than APX.

To understand the pattern of codon usage and probable position of a particular gene an Nc plot were prepared. The results showed that codon usage bias was mainly influenced by mutational bias along with translational selection. To interpret this result a neutrality plot analysis was carried out to further understand the effect of GC content in different codon position to influence the major factor (mutational pressure or natural selection) in overall codon usage bias through a slope of regression line. The results indicated that natural selection pressure was much higher in APX gene followed by CHS and PAL gene respectively, whereas stronger correlation was found between GC12 and GC3 content in PAL gene which indicated equal forces of mutational pressure on each codon position worked on PAL gene.

From the results of codon usage bias of 59 sense codons on the basis of RSCU values it was found that number of frequently used codons as well as optimal codons are higher in PAL genes followed by CHS and APX respectively which indicated strong bias in PAL genes than CHS and APX genes. Correlation analysis indicated that A3 and L_aa affected codon usage in tea plants irrespective of different genes. But, correlation of other factors with Nc was more in PAL followed by CHS than APX.

Relative expression of phenylalanine ammonia-lyase (PAL) gene in tea was analyzed using quantitative real-time PCR (qRT-PCR) to compare the

effect of BTH on PAL transcript level against challenge inoculation with two foliar fungal pathogens *C. gloeosporioides* (thought to be major pathogen) and *C. eragrostidis*. From the results it was observed that PAL transcript accumulation was elevated on 4th, 6th and 7th day in response to pre-treatment (by BTH) and challenge inoculation (by *C. gloeosporioides*), whereas, in BTH treated and *C. eragrostidis*-inoculated plants, elevation in PAL gene expression was observed on 1st, 5th and 7th day post treatment. During enzyme estimation also, increased level of phenylalanine ammonia lyase was observed on 4th and 6th days after BTH pretreatment and challenge inoculation (by *C. gloeosporioides*).

In vitro study of antagonistic activity of some common botanicals and four known biocontrol agents such as *Bacillus subtilis*, *B. pumilus*, *B. megaterium* and *Trichoderma harzianum* were tested to control mycelia growth of one of the most virulent pathogen *Colletotrichum gloeosporioides*. From the results it was found that 50% ethanolic extracts of *Datura metel* and *Clerodendrum viscosum* could inhibit more than 70% mycelia growth of the fungus. *Bacillus pumilus* was found to be the best antagonist and could inhibit 78% radial growth of the fungus tested. The other three bacterial antagonists also could inhibit the growth of the fungus *Colletotrichum gloeosporioides* upto a level of 60% and above.

This study also revealed certain new facts of fundamental importance. The significance of some defense related genes and their molecular characteristics have been demonstrated. Our investigations have provided an insight in to the mechanism of resistance induction in tea plants against some pathogens of tea. Differential expression of PAL gene have been studied by semi-quantitative (by RT PCR) and quantitative (by qRT PCR) methods following induction of resistance by known resistance inducer (BTH). The studies on expression of mRNA of PAL gene have extended our present knowledge of molecular expression of PAL gene in tea plants.