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Molecular analysis and expression profiling of blister blight defense-related genes in tea

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Abstract

Fungal diseases are one of the biggest threats for plant growth, development and productivity. *Exobasidium vexans* is an obligate biotrophic fungal pathogen of tea that causes blister blight, a devastating disease resulting in significant crop losses. A study was carried out to do a comprehensive transcriptome profiling utilizing cDNA-AFLP and SSH along with qRT-PCR based expression profiling, with the objective of identification of blister blight inducible defense-related transcripts in tea, particularly in a resistant cultivar. This could potentially be utilized for development of molecular markers associated with resistance blister blight. Candidate transcripts were screened using cDNA-AFLF method, which are differentially expressed between a resistant and a susceptible cultivar under blister blight stress. Further, SSH libraries were constructed for identification of blister blight induced ESTs in the resistant cultivar. *In silico* analysis, functional annotation and characterization showed that *E. vexans* challenge rapidly modulates the expression of a large repertoire of genes associated with an array of biochemical processes, particularly defense. A high level of *E. vexans*- dependent induction was detected for most of the selected genes through qRT-PCR-based expression profiling of selected ESTs. This entire collection of blister blight induced ESTs includes genes involved not only in defense, but in metabolism, energy, transport, protein modification, cell wall fortification, oxidative stress response and signal transduction, which have either direct or indirect implications in conferring plant defense or resistance responses. A number of novel genes were also identified, which need to be investigated further for their probable roles in defense progression. The data provide potential candidates for improving resistance to blister blight either by marker assisted breeding or genetic engineering and sequence information for marker development (SNPs and SSRs), microarray construction, and genome annotation and the information accrued is expected to accelerate molecular characterization of genes of interest.

Key words: Blister blight, transcriptome profiling, cDNA-AFLP, suppression subtractive hybridization, defense.

Introduction

Blister blight, caused by the biotropic fungus *Exobasidium vexans* Massee is one of the most serious foliar diseases of tea *Camellia sinensis*, it is capable of causing enormous crop loss throughout the tea growing regions of Asia, including India. The disease attacks young succulent and harvestable tender shoots of the plant thereby seriously affecting the quality and quantity of the harvested leaves. The yield losses due to blister blight may be as high as 43%. In North-East India, the disease occurs particularly in the hilly regions of Darjeeling and Assam, inflicting a crop loss of upto 24% depending on the severity and duration of the disease. Apart from causing severe crop losses blister blight also results into marked quality deterioration of tea. Diseased leaves if included in processing, affect the quality of made tea as several biochemical characteristics are changed resulting in reduced quality [1, 2]. Though, fungicides have more or less proven effective in controlling the disease, large scale application of chemicals results in environmental pollution, besides causing various health hazards. Host plant resistance appears to be the most practical, effective and economical approach to control the devastating disease. However, the nature and basis of resistance to blister blight in tea cultivars is not completely understood. Identifying host genes that are differentially expressed in tea cultivars resistant to the disease, in response to infection by the fungal pathogen, could be a faster and more efficient approach towards monitoring the tea defense transcriptome.

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Transcriptome studies have provided a better understanding of plant responses to stress and numerous novel stress-responsive genes have been discovered [3]. High-throughput analysis of differential gene expression is a powerful tool for discovering novel genes or for gaining additional information about certain biological processes on a genomic scale [4]. Transcript profiling approaches together with quantitative Real-Time PCR (qRT-PCR) technology, allow the simultaneous analysis of thousands of distinct and well-defined genes representing a comprehensive coverage of the whole transcriptome. Techniques such as Suppression Subtractive Hybridization (SSH), cDNA-Amplified Fragment Length Polymorphism (cDNA-AFLP), Serial Analysis of Gene Expression (SAGE), Massively Parallel Signature Sequencing (MPSS) and differential display are useful in identifying previously unknown transcripts that are differentially regulated. Among these, SSH [5] and cDNA-AFLP [6] have emerged as appropriate tools to study plant disease development in both resistant and susceptible interactions, as well as time-course based analysis to detect early and subsequent molecular events. Large-scale transcriptional profiling studies have investigated plant defense responses to biotic stresses and revealed novel aspects in compatible and incompatible interactions between plants and their pathogens [7-15].

Exploring the tea plant's defense transcriptome in response to blister blight infection as well as identifying and understanding the functions and regulation of candidate defense-related (or resistance-response) genes, would not only unravel the mechanisms associated with resistance development but also contribute towards breeding for improved disease resistant varieties. Further, in order to devise a complete and effective control strategy for the disease, it is essential to understand the molecular mechanisms underlying tea- *E. vexans* interaction. Our goal in the present study was to identify tea genes that are differentially expressed during resistance response to the fungal pathogen *E. vexans*, in a cultivar resistant to blister blight utilizing transcript profiling approaches. We complemented the study by validating gene expression levels, and obtained a more thorough understanding of relative transcript accumulation of defense-related genes by expression analysis of selected genes induced during tea- *E. vexans* interaction, using qRT-PCR. The transcript profiles from these studies were examined to describe the molecular events of the tea plant's responses to blister blight for an incompatible interaction when host resistance is established.

Materials and methods

Plant materials and pathogen inoculation

For the present study, two Darjeeling tea cultivars were selected based on their resistance or susceptibility to blister blight disease, *viz*., P-1258 (resistant) and T-78 (susceptible). Three year old plantlets of each cultivar, which were grown and maintained in polyethylene sleeves, were chosen for the artificial pathogen inoculation experiment. For inoculation, basidiospores were collected (6 hrs after sporulation) from sporulating blister lesions in naturally infected plants from the field and spore suspensions were prepared in sterile distilled water containing 10^6 spores ml^{-1} [2]. The leaves were surface sterilized by wiping with 90% ethanol and the spore suspension was dropped on to the 1st, 2nd and 3rd leaves of the plant materials. The plantlets were maintained at 100% RH by covering with polyethylene-bags for 72 hours in shade (avoiding direct sunlight) after which they were transferred to glasshouse. Samples were collected at two different timepoints *i.e*., at 72 hours post inoculation (hpi) and 240 hpi. Uninfected leaves were collected from water-treated plants for use as control.

Isolation of RNA from leaf tissues

Extraction of total RNA was done from 1 gm of leaf tissue from each of the plant material using RNAqueous kit (Ambion). From the high quality total RNA, mRNA was isolated using PolyA-Tract mRNA Isolation Systems III (Promega, USA), following manufacturer's instructions. The quality and concentration of mRNA was checked using a spectrophotometer.

cDNA-AFLP analysis

cDNA-AFLP analysis was carried out as described by Bachem *et al*. [6] with some modifications. Restriction enzyme digestion and adapter ligation were carried out in the same reaction for 600ng of ds cDNA. For restriction digestion, two restriction enzymes, *BstYI* and *MseI* (New England Biolabs, Beverly, Mass) were used and for ligation, adapters for the two restriction enzymes were used. The reaction mix (10U *BstYI*, 10U *MseI*, 10X Ligase buffer, 5U/µl T4 DNA Ligase, 10X BSA, 0.5 M NaCl and sterile distilled water in a total volume of 20 µl) was incubated at 37°C overnight. The product was incubated at 65 °C for 20 minutes (min) to inactivate the enzymes and subsequently diluted in sterile distilled water in 1:1 ratio. Pre-amplification was done using Bst (5'-GAC TGC GTA GTG ATC-3') and Mse (5'-GAT GAG TCC TGA GTA A-3') primers complementary to the

adapter ligated sites. The Mse primer carried no selective nucleotides but the Bst primer had a selective nucleotide 'C' at the 3' end. The Mse primer was end labelled with radioactive ^{32}P. Selective amplification was carried out using Bst and Mse primers with 2 or 3 additional selective nucleotides, following the cycling profile: 1 hold at 94 °C followed by 10 cycles of 30 s at 94°C, 30 s at 65°C and 1 min at 72°C; followed by 23 touch-down cycles at 0.7°C/cycle (30 s at 94°C, 30 s at 56°C and 1 min at 72°C). Electrophoresis was carried out on a 6% polyacrylamide gel in a Sequi-Gen GT Sequencing Cell (Bio-Rad Laboratories, Hercules). The gel was transferred to a 3mm Whatman paper and dried in a Bio-Rad gel dryer at 80°C for 1 hour. For visualization of bands, the dried gel was exposed to an X-Ray film for 15 hours. Polymorphic transcript derived fragments (TDFs) exhibiting a differential banding pattern between the resistant and susceptible plant materials were identified and eluted using the GenElute Gel Extraction Kit (Sigma-Aldrich, Switzerland). The isolated fragments were reamplified, cloned into pGEM-T easy vector (Promega, Madison, USA) and transformed into DH10β chemical competent cells. The transformed colonies were selected through blue-white screening on LB-agar plates containing Ampicillin (100 µg/ml) selection. Subsequently, sequencing was performed by using the BigDye Terminator v3.1 Cycle Sequencing Kit in the 3130xl Genetic Analyzer (Applied Biosystems, California, USA). The TDF sequences were compared against all available sequences in the non-redundant (nr) databases using the blastx algorithm of NCBI (National Center for Biotechnology Information).

Subtractive cDNA library construction

For the present investigation, forward and reverse SSH libraries (SSH1 and SSH2) were prepared using the artificially infected P-1258 and T-78 materials (designated as P-1258i and T-78i). For the forward library (SSH1), P1258i was used as the tester and T-78i as the driver; and vice-versa for the reverse library (SSH2). Construction of SSH libraries was performed using the PCR-Select cDNA Subtraction kit (Clontech, Palo Alto, USA) following manufacturer's instructions. The cDNA populations of the tester and the driver were digested with the restriction enzyme *RsaI* (at 37°C overnight) to obtain short blunt-ended fragments. The tester pool was then divided into two populations, of which the first was ligated to adaptor 1 and the second to adaptor 2R, provided with the kit, by incubating at 16°C overnight. Then, an excess of the driver cDNA was mixed with each tester pool, which were subsequently resuspended in hybridization buffer, denatured, and then allowed to hybridize for 10 hours at 68°C. After this first hybridization, the two samples were combined, and then a fresh portion of heat-denatured driver in hybridization buffer was added. The samples were allowed to hybridize at 68°C overnight. For each subtraction, two PCR amplifications were performed (primary and secondary PCR), according to manufacturer's recommendations.

Cloning and sequencing of subtracted cDNA.

The subtracted cDNA fragments (secondary PCR product) were ligated into pGemT Easy vector (Promega, Madison, USA) and electroporated into ElectroMax DH10β *E. coli* cells (Invitrogen, USA). The transformation mixture was plated onto Luria-Bertani (LB)- Agar plates containing Ampicillin (100 µg/ml), IPTG (125mM), X-Gal (40µg/ml) and were incubated at 37°C overnight to select for transformed colonies through alpha-complementation. White colonies containing putative recombinant plasmids were randomly picked and cultured in LB broth containing Ampicillin (100 µg/ml) at 37°C for 16 to 18 hours under continuous shaking at 220 rpm. Plasmid DNA isolation was done by using HiPura miniprep plasmid purification kit (Himedia). The extracted plasmid DNA was digested with *Eco*RI to check for the presence of inserts. Automated sequencing was performed in the GA3130xl Genetic Analyzer (Applied Biosystems, California, USA) using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to manufacturer's recommendations. The EST sequences obtained after sequencing were trimmed of vector, adapter, and low-quality sequences by using the software Sequence Scanner v1.0 (Applied Biosystems). Only high-quality ESTs that were greater than 100 bp in length were further subjected to assembly analysis and functional analysis. Sequence similarity search was carried out with the blastx program against the non-redundant (nr) protein database of NCBI, using an E-value cut-off of 1.0 E^{-10} [16].

Quantitative RT-PCR analysis of gene expression

The qRT-PCR reactions were carried out on total RNA derived from two independent biological experiments. First-strand cDNA was synthesized from DNase-treated total RNA using Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany). Suitable primers for qRT-PCR were designed on the basis of specific sequences of the selected ESTs using Primer3 software. The reference genes for C. sinensis 18S and 26S rRNA expression levels were used to normalize expression data between samples. Experiments were

carried out using the LightCycler 480 SYBR Green I Master kit (Roche) on a LightCycler 480 II System (Roche) according to the manufacturer's instructions. Each biological replicate was analyzed in three technical replicates. Amplification was carried out following the cycling profile: Pre incubation at 95ºC for 5 min, followed by 45 cycles of amplification, denaturation at 95^{o}C for 10sec, annealing for 5-20 sec (primer dependent temperature) and extension at 72^{o}C for 30 sec. Then a cycle of melting curve analysis was performed to check specificity of the amplified products, followed by one cycle of final cooling at 40^{o}C. Expression data were analyzed using software GeNorm v3.5 (http://medgen.ugent.be/genorm/) and the normalization factor obtained for each of the treated sample was used to normalize the expression of the genes. A hierarchical cluster analysis for the normalized expression values of the different blister blight induced genes, differentially modulated in the cultivars, was performed by the software GenePattern [17] and the result was represented with the help of a heat map.

Results and discussion

cDNA-AFLP and SSH based defense transcriptome profiling in tea

Vigorous infection in terms of growth and development of blister lesions was observed in case of the susceptible cultivar T-78, while in the resistant cultivar P-1258, infection was restricted by localized cell death at the site of infection. The cultivar P-1258 is thus capable of rapidly deploying a wide variety of defense responses leading to a hypersensitive response that prevents pathogen colonization. In contrast, a T-78 exhibits much weaker and slower responses that fail to restrict growth or spread of the pathogen.

Through cDNA-AFLP, we could screen for candidate transcripts which are differentially expressed between P-1258 and T-78 under blister blight stress. A total of 287 bands were found to present a differential banding pattern of which 176 were present in P-1258 but were absent from or markedly under-expressed in T-78. A total of 162 TDFs were recovered from the dried gels, of which only 104 TDFs with valid sequences ranging from 100 bp to more than 700 bp were obtained after sequencing. Sequence comparison of the TDFs against the nr database revealed that the defense transcriptome coverage was relatively low, with only 6.8% of the transcripts implicated in direct defense responses. However, transcripts indirectly associated with defense progression were also detected. To verify the results generated from the cDNA-AFLP analysis, an expression analysis was performed for selected TDFs by qRT-PCR. The TDFs showing homology with acyl-CoA binding protein, zinc finger family protein, ubiquitin and proline-rich protein respectively, were considerably upregulated after infection and revealed a positive correlation between cDNA-AFLP and qRT-PCR expression patterns of the genotypes under control and disease stress. In this study we identified that, among the TDFs with known functions, a vast majority were involved in metabolism. Others were implicated in transport processes, response to biotic/abiotic stresses, nuclear organization, transcriptional regulation, photosynthesis etc. Most of the TDFs sequenced could not be assigned putative functions as they did not show any homology to the genes/proteins in the databases. Furthermore, a good number of transcripts detected and isolated were of very short sequence length and could not be analyzed further. Nevertheless, the study has provided a preview of the genes associated with tea- *E. vexans* interaction, which could potentially be converted to molecular markers through studying their association with the trait of interest. Besides, the novel ESTs generated from this study could be investigated further for their probable roles in resistance mechanisms.

A comprehensive transcriptome analysis was further performed using the SSH technique to encompass the entire tea defense transcriptome during blister blight attack, with the objective of identification of a large repertoire of blister blight inducible transcripts in tea, particularly in the resistant cultivar P-1258. From a total of 843 ESTs generated from the SSH libraries, we obtained 259 unigenes after assembly analysis. After processing the sequences, the SSH1 library was found to have 443 ESTs while SSH2 contained 400 ESTs. In SSH1, the size of the inserts ranged from 102 bp to 692 bp with an average size of 313 bp and, in case of SSH2 it ranged from 135 bp 731 bp, the average insert size being 344 bp. The percentage of unique sequences in each library was 30.5% while the rate of redundancy was 69.5%, approximately. While carrying out the assembly analysis, it was observed that the high rate of redundancy seen in the SSH libraries, was mainly caused by a few but highly expressed genes. After sequence assembly, 55 contigs and 82 singlets were generated from library SSH1 and, 36 contigs and 86 singlets were generated from SSH2. Thus, the ESTs from SSH1 and SSH2 libraries resulted in a total of 137 and 122 unigenes respectively. Among the most highly represented sequences in the P-1258i material were

the contigs showing sequence similarity to proteins such as ubiquitin family protein, endo-alpha-1,4-glucanase, short chain dehydrogenase, wound induced protein WIN2, iron-sulfur cluster scaffold protein, ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit and thioredoxin m. Further, analysis of the ESTs and their functional characterization indicated that the tea plant responds to *E. vexans* challenge with rapid modulation of the expression of a large repertoire of genes associated with a vast array of biochemical processes. Blastx analysis of the unigenes showed that approximately 47% of sequences were found to be showing positive homology with the nr protein database and putative functions could be assigned to them. Most of the sequences from the forward library could be identified to be directly associated with defense/ resistance response mechanisms and defense-related signal transduction in plants. A majority of them could be associated with cell wall fortification and oxidative stress response which were found to prevent or suppress entry, growth or multiplication of the pathogen, thus, contributing towards resistance as a first line of defense. Several others were found to be associated with metabolism, transcriptional regulation, protein modification, transport and photosynthesis. These may not be specific to pathogen-induced resistance responses but were found to play a role in initiating defense responses in several plants.

A significant group of genes with homology to known sequences representing pathogenesis-related (PR) and defense-response transcripts were differentially expressed in the resistant cultivar post-infection. These include chitinase, endo-glucanase, beta-glucosidase, wound induced protein, protease inhibitor, thaumatin-like protein, cystatin, a blight associated protein p12 and aspartic proteinase. As expected, a substantial number of transcripts encoding proteins having functions in defense related signal transduction pathways were also found to be highly induced in the forward library. Such proteins include serine/threonine-protein kinase, leucine-rich repeat transmembrane protein kinase, oxo-phytodienoic acid reductase, mitogen-activated protein kinase kinase, calcium ion binding or calmodulin-related protein, salicylic acid-binding protein chitin-inducible gibberellin-responsive protein and calreticulin. The considerable induction of the PR-genes in this investigation implies that their induction has led to an increased and durable resistance towards blister blight through SAR (systemic acquired resistance). Moreover, a number of differentially expressed transcripts have been found to code for proteins or enzymes having a function in reducing oxidative stress. A hypersensitive induced response protein, hydrogen peroxide-induced protein, thioredoxin, pheophorbide A oxygenase, glutathione S-transferase, a catalase and an *Arabidopsis thaliana* Nudix hydrolase protein belong to this category. Expression of plant transcription factors such as NAC domain protein and chitin-inducible gibberellin-responsive protein has been well documented in the forward library, suggesting their potential roles in defense response against blister blight in tea.

The SSH technique eliminates the abundantly and constitutively expressed housekeeping genes and genes expressed in both control and treated plants, thereby normalizing expressed cDNA profiles during library construction. As a result, it significantly enhances the chances of cloning differentially expressed genes. This is particularly important because many PR protein genes are expressed at low levels, and can be limited to a particular tissue or cell type [19]. This study constitutes the first genome-wide effort to understand the molecular basis of an interaction between the host *C. sinensis* and the blister blight pathogen. It has led us towards a better understanding of the molecular mechanism involved in host defense and resistance responses to *E. vexans* infection. The study also resulted in the discovery of a large number of novel ESTs as a vast majority of the sequences are not currently represented in the available databases. These sequences need to be investigated further for their potential roles in resistance mechanisms.

qRT-PCR based gene expression analysis

Based on their putative roles in mediating resistance responses and the level of transcript accumulation as observed through SSH analysis, qRT-PCR was employed to verify and profile the differential expression patterns of 14 selected ESTs. Among the differentially expressed ESTs selected for expression analysis were those with sequence homology to genes that encode proteins involved in metabolism (β-D-glucosidase), transcriptional regulation (chitin-inducible gibberellin-responsive protein), energy processes (ATP binding protein), transport (ABC transporter family protein), cell wall fortification (hydroxyproline rich glycoprotein), oxidative stress response (glutathione S-transferase, hypersensitive induced response protein), signal transduction (CBL-interacting serine/threonine-protein kinase, calmodulin-related protein) and defense (wound-induced protein WIN2, endo-1,4-glucanase, chitinase IV, cystatin, blight-associated protein p12). These ESTs

were chosen as they represented almost all the different functional categories identified, with a preference for defense-related genes possibly involved in basal protection, or in the development of HR and SAR. Based on their upregulation across the two timepoints, the genes were identified as early- or late-responsive to blister blight. In order to group together the genes and the associated genotypes with similar patterns of expression, hierarchical cluster analysis was carried out for the normalized gene expression values of the control and infected samples from the cultivars under study, using the software GenePattern. The result was visualized with the help of a heat map (Fig. 1) where relationships among the genes (or the genotypes) are represented by a tree whose branch lengths reflect the degree of similarity between them, as assessed by a pairwise similarity function. In this case, pairwise complete-linkage method of hierarchical clustering (HierarchicalClustering, version 5) was followed using a function of the Pearson correlation coefficient. The heat map indicates that the genes representing similar expression patterns are clustered next to, or in the immediate vicinity of, each other.

The expression of the selected genes was in good agreement with the results obtained, except for a few. Most of the genes under study showed significant upregulation in expression upon infection by *E. vexans* by two-fold or more. For most of the transcripts, strong changes in gene expression between infected and control tissues were detected. Induction of gene expression upon infection was found to be highly modulated in P-1258, which is evident from the considerable upregulation in expression, as recorded for most of the genes under study. On the other hand, significant upregulation in expression was not observed in T-78 post-infection, as was expected, except in a few cases. The genes were identified to be expressing at

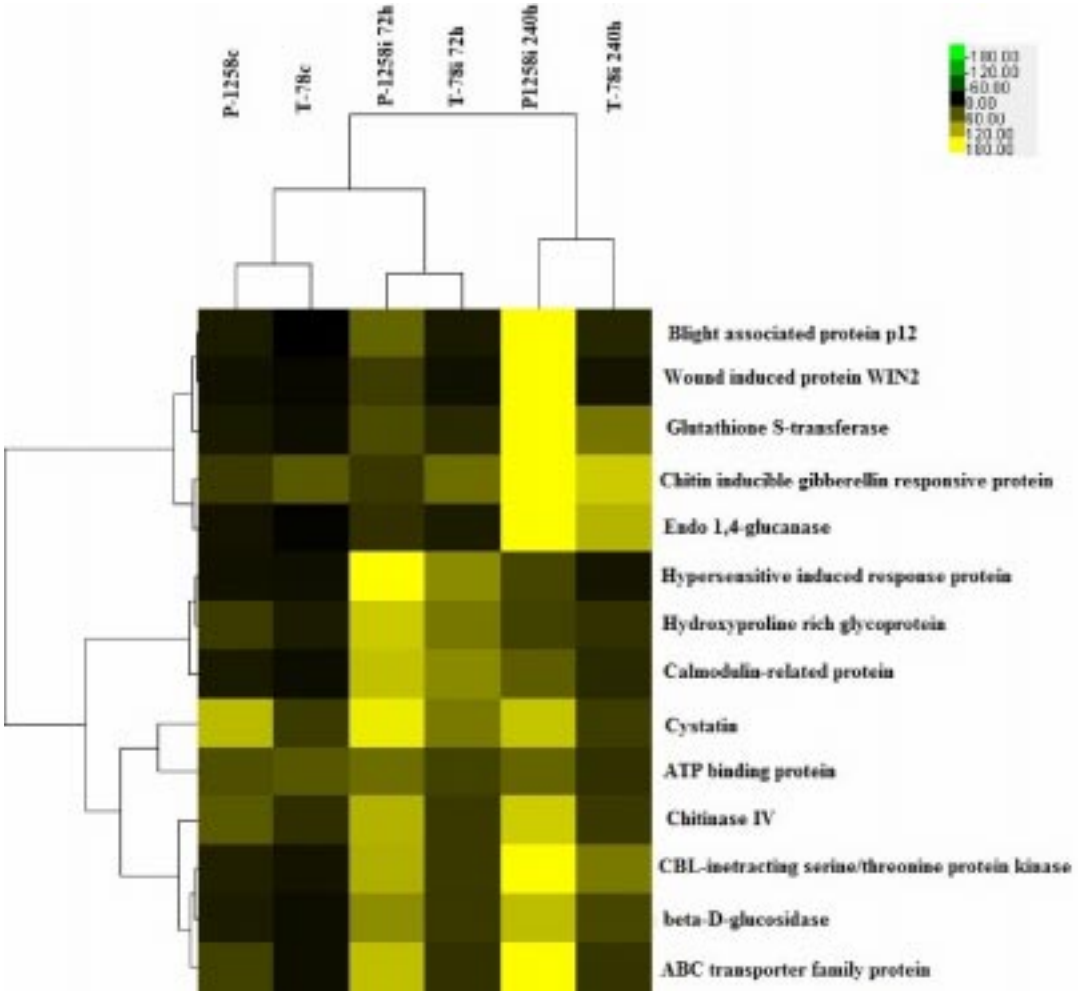

Fig. 1. Heat map generated by hierarchical clustering of relative differential gene expression levels in control and infected tea cultivars, P-1258 and T-78, at two different time points: 72 hpi and 240 hpi.

basal levels before *E. vexans* infection and were found to be upregulated temporally upon infection at two different stages of infection. Some of the genes were early-responsive to blister blight attack and were induced and upregulated within 72 hours of infection. Expression of such genes was either lowered or maintained at high levels at the later stage of infection. Then again, some genes were detected to be late-responsive to infection. These showed minor upregulation during the early stage but were observed to be highly induced during the later stage of infection (240 hpi). It, therefore, seems that the expression of the late-responsive genes gradually increases with the progression of the disease. Infection resulted in significant induction in expression of genes coding for hypersensitive induced response protein (HIR), blight-associated protein p12, wound induced protein WIN2, chitin inducible gibberellin responsive protein (CIGR), endo-1,4-glucanase, CBL-interacting serine/threonine-protein kinase (CIPK), glutathione S-transferase (GST) and ABC transporter family protein in P-1258, either at 72 hpi or 240 hpi. Among the defense-related transcripts, it is worth noting the substantial induction in transcript levels of *WIN2* with ~18-fold increase in accumulation after infection in P-1258. About 17-fold increase in expression was documented for *blight-associated protein p12* as well as *endo-1, 4-glucanase* in P-1258. Significant induction was also documented in the expression of *HIR* (~18-fold increase) and *GST* (~13-fold increase), which are implicated in oxidative stress response, in the cultivar P-1258. Genes implicated in defense signalling such as, *calmodulin-related protein* and *CIPK* were also found to be considerably upregulated, showing approximately 7 to 9-fold increase in transcript levels in P-1258. In the cultivar T-78, increased modulation was observed for genes particularly involved in defense signalling such as *calmodulin-related protein* at 72 hpi. An upregulation was also recorded for blight-associated protein p12 at the early stage of infection in T-78. At 240 hpi, gene for *endo-1,4-glucanase* was found to show considerably enhanced expression in T-78 as compared to P-1258. Thus, the results also indicate that not only the resistant but also the susceptible genotype has a defense response mechanism, through upregulation of an identical array of transcripts against pathogen attack.

When plants are infected by pathogens, early local defense reactions and delayed systemic responses are activated in order to counteract the pathogen attack. This defense strategy, which is particularly successful against biotrophic fungi, is based on pathogen recognition and cell-to-cell communication in the tissue adjacent to the site of infection [19]. Later on, the plant can develop SAR leading to resistance throughout the whole plant. In case of SAR, the signal is transmitted from the infected tissue into the whole plant for induction of overall defense gene expression. This demonstrates that the development of plant resistance to pathogens entails early and late events, with initial pathogen recognition and signal transduction to initiate further defense responses such as HR, followed by expression of defense-related genes, leading to SAR. In this study, we have seen that the transcription of almost all the selected genes is upregulated after *E. vexans* infection. The present investigation also revealed that the expression levels of genes involved in disease signalling and oxidative stress response were higher than those of other types of genes. However, genes related to metabolism, energy and transcription factors were also found to participate in the development of resistance responses. Expression profiling further demonstrated that, genes *HIR*, *WIN2*, *GST*, *CBL-CIPK* and *CIGR*, are greatly involved in contributing towards development of most of the resistance responses in tea against the fungal pathogen. The progression of resistance development, therefore, entails many aspects of systemic modulation and coordination of various physiological and biochemical pathways within the plant system. Thus, the mechanism of blister blight resistance in tea involves not only an identification process during pathogenesis and the activation of disease resistance-related genes, but also the stimulation of signal transduction and associated modulation of other biochemical pathways.

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