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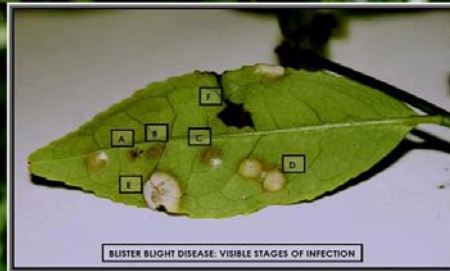
Dissecting molecular mechanisms related to blister blight resistance in tea

An EST approach

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INTRODUCTION

- Tea serves as the most popular drink for two third of the world population because of its taste, aroma and medicinal properties
- Blister blight (caused by *Exobasidium vexans*) is a destructive disease that causes substantial losses of tea in general and Darjeeling tea in particular.
- This is the first report at the molecular level where we constructed a full length cDNA library from a resistant tea cultivar of Darjeeling



METHODOLOGY FOLLOWED.....

Plant materials:
Resistant Tea clone P1258 leaves and leaves from highly susceptible but popular cultivar T78 collected during high incidence of blister blight disease from Darjeeling tea gardens.

SSH library construction:
RNA was extracted from tea leaves using Trizol reagent (Invitrogen). The library was constructed using Clontech SSH cDNA library construction kit following kit manual. Recombinant plasmids transformed in DH10B electro competent cells (Invitrogen).

Sequencing analysis:
One thousand expressed sequence tags (EST's) were analyzed using BLASTX algorithms. Putative Functional assignment of sequences was done based on homology search. Twenty Genes showed high similarity to known disease resistance genes. Validation was done by RT-PCR analysis.

BACKGROUND

- Despite its impact on tea production, information available related to the molecular mechanism involved in the regulation of host-pathogen interactions in this particular disease is scanty
- Deep insight into the molecular determinants governing host-pathogen interaction is essential to strategies for engineering durable resistance in tea

ESTs RELATED TO BLISTER BLIGHT (FUNGAL PATHOGEN) RESISTANCE

Protein Identity of Blast	Accession No.	Unigene	No. Peptides	Local
JIP and WOUND INDUCIBLE PROTEINS				
βTD	AA34492	U33024	36	CP
LAP-A	AA290496	U33027	3	CP
Tyrosin inhibitor-like protein	AA80497	U33034	4	SP
GDI/chymotrypsin inhibitor	CA20836	U33063	4	SP
YjgF family protein	BT00440	U33009	5	CP
Stress-induced LH domain protein	BT00996	U33003	3	SP
Aspartic protease inhibitor	BT00992	U33023	3	SP
GLP	CH38026	U33803	3	SP
PR PROTEINS				
P6pA (PR-7)	CA20334	U33025	2	SP
P6pA (PR-7)	CA26486	U33022	2	SP
Lipase-Binding protein-like (PR-9)	CA20927	U33006	100	SP
β-1,2-Glucanase (PR-3)	CA20872	U33035	7	SP
Endoglucanase inhibitor protein	AAN2720	U33072	3	SP
PR protein P3 (PR-4)	CA24329	U33008	3	SP
OTHER PROTEINS				
Flavonoylase	CA23338	U33060	10	CP
Malate dehydrogenase	AA10008	U33038	7	MT
Ferredoxin	BT00928	U33030	3	CP
Superoxide dismutase	AA20907	U33034	3	CP
Cytochrome b5/h-hemoglobin	AW09200	U33000	3	CP
Chlorophyll a/b-binding protein	CA24626	U33048	3	CP

ABSTRACT

Blister blight (caused by *Exobasidium vexans*) is a destructive disease of tea (*Camellia sinensis*) in tea growing countries worldwide, that causes substantial losses. Despite its impact on tea production, information available related to the molecular mechanism involved in the regulation of host-pathogen interactions in this particular disease is scanty. This is the first report at the molecular level where we constructed a full length cDNA library from a resistant tea cultivar of Darjeeling. Approximately 1000 expressed sequence tag's (EST's) were analyzed using bioinformatic tools (BLASTX algorithms) for the presence of resistance genes related to the defense mechanism of tea challenged by *Exobasidium*. More than 10% of the transcripts were specifically induced in defence and/or signal transduction including master switch genes (transcription factors, kinases), R genes (NBS-LRR), phytohormone biosynthesis pathway genes, pathogenesis related genes and genes for antifungal peptides (*chitinase, glucanase*). Resistance genes and plant defense genes are potential target for development of disease resistance specific markers. Development of EST's is a fundamental requirement for future studies in functional and comparative genomics in tea.

Causal organism: *Exobasidium vexans*

LIFE CYCLE : 11-28 DAYS

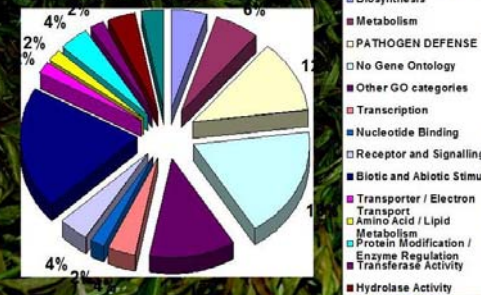
Stages of blister lesion development

- Stage 1- Occurrence of a translucent spot less than 0.5 mm in diameter
- Stage 2- Spot more than 0.5 mm and less than 1.0 mm in diameter
- Stage 3- Spot of 1.0 to 3.0 mm in diameter
- Stage 4- Well-defined lesion, 3.0 to 6.0 mm in diameter
- Stage 5- Convex-shaped lesion
- Stage 6- Incipient stage of spore formation
- Stage 7- Vigorously sporulating lesion

DISEASE

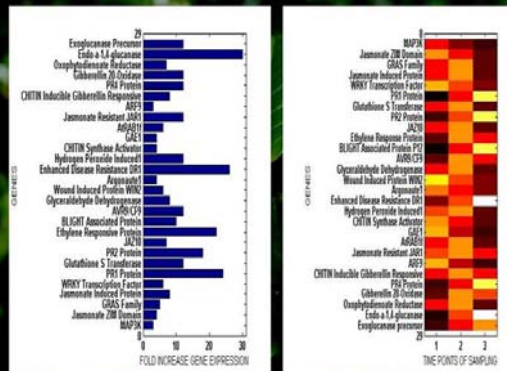


BLISTER BLIGHT (*Exobasidium*) related library characterization



FINDINGS....

- Twenty transcripts clearly showed homology with known disease resistance and defense related genes in databases
- Many important genes involved in plant metabolism, cell cycle, DNA synthesis, protein synthesis, signal transduction and energy transport were obtained



FUTURE PROSPECTS....

Resistance genes /Defense genes from tea governing host-pathogen interaction is essential to strategies for engineering durable resistance against blister blight in tea

cDNA clones can be used to establish cDNA microarrays for screening of germplasm

Further characterization of the genes reported here will help unravel the mechanisms of pathogenicity, particularly at the stage of disease progression, and the defense responses of host plants using techniques to study gene expression and for study of gene function such as development of knockout mutants

