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Journal of Tea Science Research, 2022, Vol.12, No.1, 1-12
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Research Article Open Access

Gene Expression Profiling Analysis of Resistant and Susceptible Tea Cultivars in Response to Tea Blister Blight (*Exobosidium vexans* Massee)

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Journal of Tea Science Research, 2022, Vol.12, No.1 doi: 10.5376/jtsr.2022.12.0001
Received: 01 Jun., 2022
Accepted: 12 Jun., 2022
Published: 25 Jun., 2022

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Preferred citation for this article:
Long Y.Q., Ran L.X., Xia L.F., Qu H., Tian Y.P., Chen L.B., and Liang M.Z., 2022, Gene expression profiling analysis between resistant and susceptible tea cultivars (*Exobosidium vexan*s Massee) in response to tea blister blight, Journal of Tea Science Research, 12(1): 1-12 (doi: 10.5376/jtsr.2022.12.0001)

Abstract The resistant and susceptible tea cultivars were selected for revealing the defense response mechanism induced by tea blister blight. The DEGs before and after infection by tea blister blight were analyzed based on RNA sequencing and digital expression spectrum. The results showed a total of 974 DEGs were identified, of which 122 DEGs were co-expressed genes in both resistant and susceptible cultivars, 364 DEGs were specific genes only in blister disease-resistant cultivar, and 488 DEGs were specific genes only in blight disease-susceptible cultivar. The infection of tea blister blight mainly affected the expression levels of key genes involved in metabolic pathways, protein processing in endoplasmic reticulum, biosynthesis of secondary metabolites, plant-pathogen interaction, plant hormone signal transduction, starch and sucrose metabolism, phenylalanine, tyrosine and tryptophan biosynthesis and so on. These DEGs included disease resistance protein gene, hydrolase gene, cell wall reinforcement genes, transcription factor genes, plant hormones and their signal transduction genes, secondary metabolism and oxidase genes, transporter gene and so on. The differential expressions of 6 genes were verified by Real-time quantitative PCR, which showed a general consistency consistent with the results of transcriptome sequencing. This study preliminarily clarified the influence of tea blister blight infection on gene transcription levels in tea plants with this study, which laid a theoretical foundation for researching the molecular mechanism of disease resistance in tea plants.

Keywords Tea plant (*Camellia sinensis*); Tea blister blight; Transcriptome sequencing; Resistance gene

Tea is a traditional health beverage in China, and it is also an important export commodity. Tea production plays an important role in China’s agricultural economy, rural development and the construction of a new socialist countryside. It is an important industry for agricultural efficiency and farmers’ income. Tea plant diseases have always been one of the important limiting factors for high yield, high quality and high efficiency of Chinese tea (Zhao et al., 2018; Zhi et al., 2018). The diseases in tea plants and agricultural residues in the process of prevention and control are important factors restricting the export of China’s tea products (Zheng and Gao, 2015). The EU has repeatedly improved the detection standards of agricultural residues in tea imports, and the particularity of tea as a beverage restricts the use of chemical pesticides and exogenous resistance genes, which brings greater difficulties to the prevention and control of diseases. Therefore, new breakthroughs should be made in the theory of disease resistance breeding technology, resistance gene resources, the exploration of endogenous resistance agents and comprehensive control. The research on tea disease defense mechanism and the screening of new endogenous resistance genes has become the key to solve this problem.

Exobosidium vexans Massee, also known as tea blister blight, is a fungus of Basidiomycotina, which mainly damages young leaves and shoots. It is a major bud leaf disease on tea plants. It not only affects the yield, but also makes tea from diseased leaves fragile and dry tea tastes bitter, resulting in a significant decline in the quality of tea and a direct impact on economic benefits (Saravanakumar et al., 2007; Tan et al., 2015). Tea blister blight is distributed in almost all tea areas in China, especially in tea gardens in southwest mountainous areas (Ran et al., 2017; Zhi et al., 2018). At present, the prevention and treatment of tea blister blight is mostly through chemical pesticides, which brings the problem of pesticide residues to tea. Therefore, in this study, RNA-seq technology was used to sequence the transcriptome of tea varieties resistant and susceptible to tea blister blight before and

after the induction of tea blister blight, and the genes related to the defense mechanism induced by tea blister blight were screened out, so as to provide a basis for further research on the molecular mechanism of tea plant defense induced by tea blister blight and the breeding of disease resistant tea varieties in the future.

1 Results and Analysis

1.1 Phenotypic differences between tea varieties resistant and susceptible to tea blister blight

The variety resistant to tea blister blight, Yuncha 1 (YCKC), has thick mesophyll, many and long hairs, short vein spacing, few stomata and small opening; The variety susceptible to tea blister blight, Yunkang 22 (YCGB), has relatively thin mesophyll, few and short hairs, long vein spacing, many stomata and large opening (Figure 1). According to field investigation and indoor inoculation, the spores germinated in the leaves of the resistant variety, Yuncha 1 (YCKC) for a long time, and the average daily expansion rate of disease spots was relatively slow; While the susceptible variety, Yunkang 22 (YCGB), showed early spore germination, short infection time and rapid expansion of disease spots.

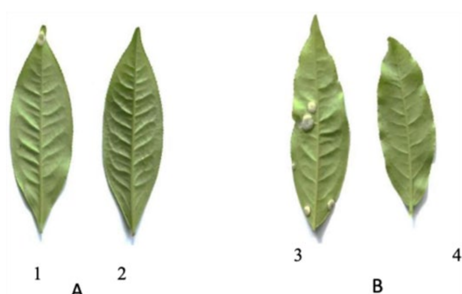

Figure 1 Leaves of tea cultivars

Note: A: Resistant tea cultivars; B: Susceptible tea cultivars; 1,3: Infected leaf; 2,4: Healthy leaf

1.2 Transcriptome sequencing and tea genome comparison

Illumina HiSeq^{TM} 2500 high-throughput sequencing technology was used to analyze the transcriptome of the variety resistant to tea blister blight, Yuncha 1 (YCKC), and the variety susceptible to tea blister blight, Yunkang 22 (YCGB), respectively. Each sample was repeated for 3 times, all detection parameters of the sample met the quality requirements, and the filtered clean reads exceeded 94% of the original data (Table 1).

The obtained tea plant clean reads sequence was performed positioning analysis on the tea plant genome website (http://www.plantkingdomgdb.com/tea_tree/) by HISAT software (Table 2). The results showed that the percentage of clean reads of 12 samples located on the tea plant genome was higher than 70%, and the percentage of multiple mapped reads or fragments in the total was lower than 10%, indicating that the comparison result of the reference genome was good.

Table 1 The transcriptome sequencing of 12 samples

Sample name	Raw reads	Clean reads	Clean bases	Q20 (%)	Q30 (%)	GC content (%)
YCKC_1	56 726 908	53 496 366 (94.31%)	8.02G	95.98	90.36	44.18
YCKC_2	58 005 280	54 841 974 (94.55%)	8.23G	95.86	90.13	44.25
YCKC_3	51 779 382	48 914 308 (94.47%)	7.34G	95.83	90.08	44.21
YCKB_1	56 176 868	53 026 752 (94.39%)	7.95G	96.38	91.10	44.17
YCKB_2	60 845 490	57 507 414 (94.51%)	8.63G	96.21	90.77	44.19
YCKB_3	52 947 280	49 792 940 (94.04%)	7.47G	96.21	90.76	44.62
YCGC_1	44 370 370	42 824 172 (96.52%)	6.42G	97.02	92.59	44.05
YCGC_2	51 324 278	48 314 180 (94.14%)	7.25G	95.89	90.21	44.75
YCGC_3	59 055 512	55 723 796 (94.36%)	8.36G	96.10	90.64	44.02
YCGB_1	60 497 396	57 214 740 (94.57%)	8.58G	95.82	90.04	44.21
YCGB_2	53 930 040	51 063 032 (94.68%)	7.66G	95.94	90.30	44.22
YCGB_3	52 266 686	49 474 026 (94.66%)	7.42G	95.91	90.22	44.21

Table 2 The statistics of reads mapped to reference genome with 12 samples

Sample name	Total reads	Total mapped reads	Sequencing with multiple alignment locations on the reference sequence	Uniquely mapped	Reads map to '+'	Reads map to '-'	Non-splice reads	Splice reads
YCKC_1	53 496 366	42 275 158 (79.02%)	1 380 914 (2.58%)	40 894 244 (76.44%)	20 402 015 (38.14%)	20 492 229 (38.31%)	26 439 293 (49.42%)	14 454 951 (27.02%)
YCKC_2	54 841 974	43 301 629 (78.96%)	1 430 968 (2.61%)	41 870 661 (76.35%)	20 889 254 (38.09%)	20 981 407 (38.26%)	26 950 063 (49.14%)	14 920 598 (27.21%)
YCKC_3	48 914 308	38 467 495 (78.64%)	1 260 778 (2.58%)	37 206 717 (76.07%)	18 564 975 (37.95%)	18 641 742 (38.11%)	23 879 568 (48.82%)	13 327 149 (27.25%)
YCKB_1	53 026 752	42 428 446 (80.01%)	1 408 804 (2.66%)	41 019 642 (77.36%)	20 491 260 (38.64%)	20 528 382 (38.71%)	26 253 367 (49.51%)	14 766 275 (27.85%)
YCKB_2	57 507 414	45 584 370 (79.27%)	1 508 788 (2.62%)	44 075 582 (76.64%)	22 012 155 (38.28%)	22 063 427 (38.37%)	28 247 874 (49.12%)	15 827 708 (27.52%)
YCKB_3	49 792 940	39 176 835 (78.68%)	1 311 058 (2.63%)	37 865 777 (76.05%)	18 918 727 (37.99%)	18 947 050 (38.05%)	24 314 757 (48.83%)	13 551 020 (27.21%)
YCGC_1	42 824 172	35 053 797 (81.86%)	1 088 840 (2.54%)	33 964 957 (79.31%)	16 961 278 (39.61%)	17 003 679 (39.71%)	21 961 476 (51.28%)	12 003 481 (28.03%)
YCGC_2	48 314 180	38 734 835 (80.17%)	1 205 215 (2.49%)	37 529 620 (77.68%)	18 718 263 (38.74%)	18 811 357 (38.94%)	24 042 651 (49.76%)	13 486 969 (27.92%)
YCGC_3	55 723 796	44 651 835 (80.13%)	1 415 224 (2.54%)	43 236 611 (77.59%)	21 561 025 (38.69%)	21 675 586 (38.9%)	27 976 975 (50.21%)	15 259 636 (27.38%)
YCGB_1	57 214 740	45 896 173 (80.22%)	1 437 532 (2.51%)	44 458 641 (77.7%)	22 177 725(38.76%)	22 280 916 (38.94%)	28 531 225 (49.87%)	15 927 416 (27.84%)
YCGB_2	51063032	40 859 664 (80.02%)	1 286 281 (2.52%)	39 573 383 (77.5%)	19 734 424 (38.65%)	19 838 959 (38.85%)	25 510 968 (49.96%)	14 062 415 (27.54%)
YCGB_3	49474026	39 633 817 (80.11%)	1 228 165 (2.48%)	38 405 652 (77.63%)	19 155 708 (38.72%)	19 249 944 (38.91%)	24 766 620 (50.06%)	13 639 032 (27.57%)

1.3 Gene expression level analysis and data evaluation

This time, HTSeq software was used to analyze the gene expression level of each sample, and the used model was union. The gene expression levels under different experimental conditions were compared by FPKM distribution map and Violin map of all genes. For repeated samples under the same experimental conditions, the final FPKM was the average of all repeated data (Figure 2). In order to prove that the biological test operation involved could be repeated and had little variation, and ensure that the subsequent differential gene analysis can obtain more reliable results, the Pearson correlation coefficient analysis was performed on the gene expression level between samples, and R^2 between samples was greater than 0.8 (Figure 3). It showed that the similarity of expression patterns between samples was good.

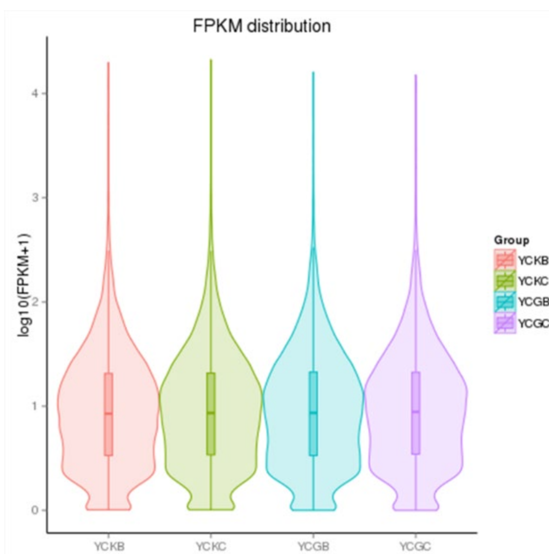

Figure 2 Violin plot of FPKM

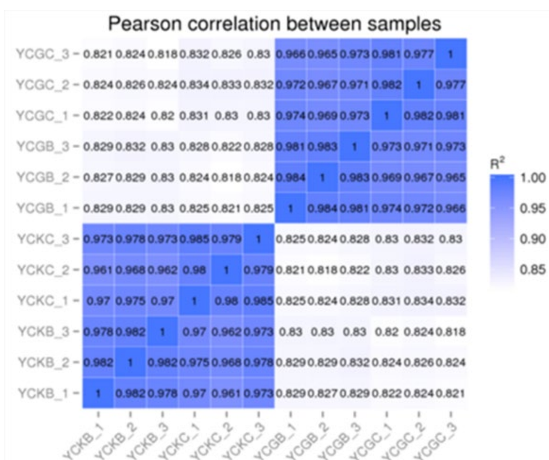

Figure 3 Correlation analysis for 12 samples

1.4 Screening of differential genes

In this experiment, DESeq (Anders and Huber, 2010) method was used to screen differential genes according to the conditions meeting the standard of $p<0.05$ and $|\log2FoldChange|\geq0.5$. After the susceptible varieties were harmed by tea blister blight, 610 differential genes were obtained (Figure 4A), including 399 up-regulated and 211 down-regulated genes. While 486 differential genes were obtained from resistant varieties (Figure 4B), of which 253 were up-regulated and 233 were down-regulated genes. There were 122 differential genes in the both varieties

(Figure 4C). These results showed that both resistant and susceptible tea plants have the same reaction mechanism and their own unique reaction mechanism in the defense of tea blister blight.

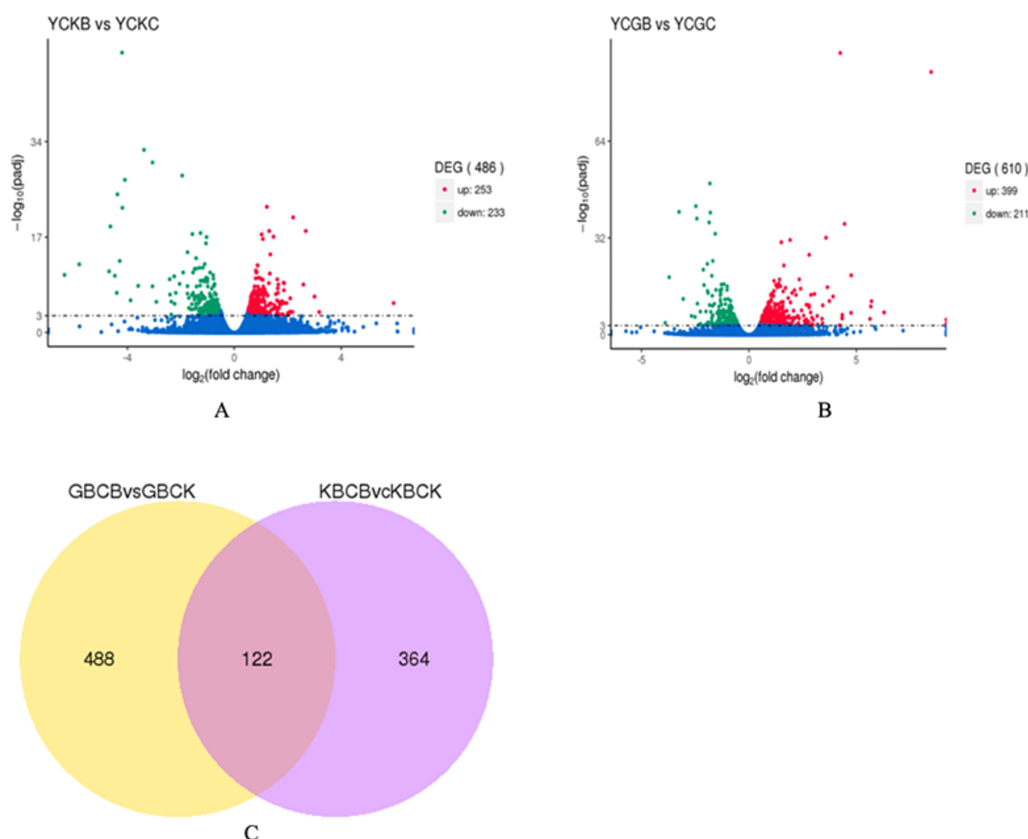

Figure 4 Volcano plot and Venn diagram of differential genes of susceptible cultivar and resistant cultivar
Note: A: Volcano plot of DEG in susceptible tea plants; B: Volcano plot of DEG in resistant tea plants; C: Venn diagram of differential genes of resistant cultivar and susceptible cultivar before infection and after infection by tea blister blight

1.5 Functional analysis of differential genes

Through GO enrichment analysis of 974 differential genes, 20 GO terms with the most significant enrichment were selected and displayed in the table, of which the molecular function was the most, followed by biological process, and cell components was less. Significant enrichment included phosphorylation, protein phosphorylation, phosphorus metabolism, chitin metabolism and compound metabolism containing glucosamine; Catalytic activity, protein kinase activity, chitin binding, phosphotransferase activity and alcohol base were as receptors (Table 3).

In order to identify the biological functions of differential genes in different metabolic pathways and further understand the relationship between genes, KEGG analysis was carried out. The pathways enriched in the top 15 included metabolic pathways (59), protein processing in endoplasmic reticulum (40), biosynthesis of secondary metabolites (38), plant-pathogen interaction (17), plant hormone signal transduction (17), starch and sucrose metabolism (13), phenylpropanol biosynthesis (9), carbon metabolism (8), nitrogen metabolism (7), flavonoid biosynthesis (7), etc.

According to the GO annotation analysis results of 974 differential genes, disease resistance related genes were screened. The up-regulated genes mainly included disease resistance gene protein (R protein), hydrolase gene, cell wall reinforcement gene, transcription factor gene, plant hormone and its signal transduction factors, secondary metabolites, oxidases and transporters; The down-regulated genes mainly included chloroplast formation related genes, heat shock proteins, aquaporins and some transcription factors, including bHLH123, ERF025, ERF1, etc.

(Table 4). These showed that the induction mechanism of tea plant in preventing the harm of tea blister blight was complex.

Table 3 Enriched KEGG pathways of DEGs

Pathway	Number of genes
Metabolic pathways	91
Biosynthesis of secondary metabolites	67
Protein processing in endoplasmic reticulum	51
Biosynthesis of amino acids	18
Starch and sucrose metabolism	15
Plant-pathogen interaction	14
Amino sugar and nucleotide sugar metabolism	10
Carbon metabolism	10
Plant hormone signal transduction	10
Phenylalanine metabolism	9
Endocytosis	8
Flavonoid biosynthesis	8
Spliceosome	7
Glycolysis / Gluconeogenesis	7
Phenylalanine, tyrosine and tryptophan biosynthesis	7

1.6 qRT-PCR verification of differential genes

6 differential genes CSA010816, Novel08377, CSA012876, CSA034045, CSA023709 and CSA028450, which were up-regulated in both resistant and susceptible varieties, were randomly screened for qRT-PCR verification. The total RNA of susceptible leaves (YCKB), control leaves (YCKC) of resistant variety Yuncha 1 and susceptible leaves (YCGB) and control leaves (YCGC) of susceptible variety Yunkang 22 were extracted respectively for quantitative PCR verification after reverse transcription. The relative expression of the 6 differential genes in resistant and susceptible varieties was consistent with the change trend of expression profile analysis, indicating that the analysis result of gene expression profile was reliable (Figure 5).

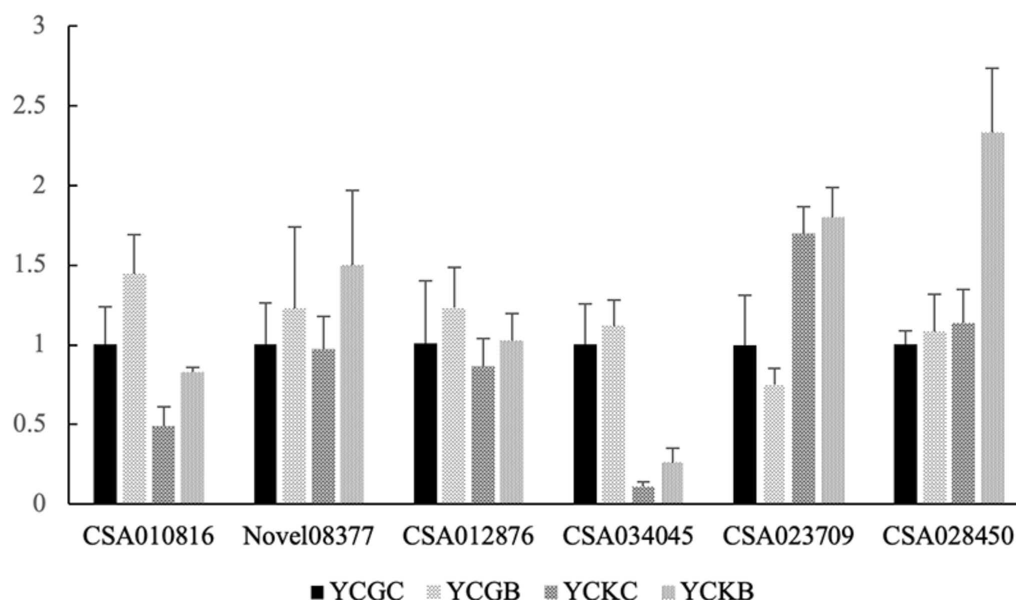

Figure 5 qRT- PCR analysis of selected DEGs

Table 4 Partial DEGs of tea plant in response to blister blight

Functional classification	Gene ID	Gene annotation	Expression pattern	
			KB	GB
Resistance gene	Novel11524	TMV resistance protein N-like	Up	Up
	CSA036702	TMV resistance protein N	Up	Up
	CSA014110	TMV resistance protein N	Up	Up
	CSA006743	TMV resistance protein N	-	Up
	CSA000007	TMV resistance protein N	Up	Up
	CSA008324	TMV resistance protein N	Up	Up
	CSA035925	TMV resistance protein N	Up	Up
	CSA017636	disease resistance protein	Up	-
	Novel07148	disease resistance protein	-	Up
	Novel02360	disease resistance gene NBS-LRR family protein	Up	-
	Novel01686	probable disease resistance RPP8-like protein 2	Up	-
	CSA000901	Probable disease resistance protein	Up	-
	CSA008325	Disease resistance protein RPP5	Up	Up
	Novel06987	Disease resistance family protein/LRR family protein, putative	-	Up
	CSA000360	Putative late blight resistance protein homolog R1B-17	Up	-
	CSA028034	Hypersensitive-induced response protein 2	-	Up
	CSA028036	Hypersensitive-induced response protein 1	Up	Up
Hydrolases	CSA012225	Endochitinase B	Up	-
	CSA018884	Endochitinase B	Up	Up
	CSA011958	Galactose oxidase	Up	-
	CSA003124	Glucan 1,3-beta-glucosidase A	Up	Up
	CSA014169	Beta-galactosidase	Up	-
	CSA012186	Probable beta-D-xylosidase 2	Up	Up
	CSA029765	Probable glucan 1,3-beta-glucosidase A	-	Up
	CSA030401	Alpha-amylase	Up	-
	CSA029747	Beta-amylase	-	Up
Cytoderm reinforcement gene	CSA017670	Basic endochitinase	-	Up
	Novel11173	basic chitinase 2-2	-	Up
	CSA000501	Caffeoyl-CoA O-methyltransferase	Up	Up
	CSA034169	Caffeoyl-CoA O-methyltransferase	-	Up
	CSA009706	Caffeoyl-CoA O-methyltransferase	Up	-
	CSA015962	Caffeic acid 3-O-methyltransferase	Up	-
	CSA028429	Caffeic acid 3-O-methyltransferase	Up	Up
	CSA035990	Caffeic acid 3-O-methyltransferase	Up	-
	CSA014974	Caffeic acid 3-O-methyltransferase 1	Up	Up
	CSA012015	Long-chain-fatty-acid--AMP ligase	-	Up
Oxidases	Novel07528	Carbonic anhydrase 2-like	Up	-
	CSA014862	Cationic peroxidase 2	Up	Up
	CSA028229	Lon protease homolog 2, peroxisomal	Up	-
	CSA032576	Glutathione reductase, cytosolic	-	Up
	CSA013247	Laccase-7	-	Up
	Novel01345	Laccase-14-like	Up	-
Secondary metabolites	CSA000829	Leucoanthocyanidin dioxygenase	-	Up
	CSA035727	Dihydroflavonol-4-reductase	-	Up
	CSA028450	Phenylalanine ammonia-lyase	Up	Up
	CSA013010	(+)-neomenthol dehydrogenase	-	Up
	CSA013009	(+)-neomenthol dehydrogenase	-	Up
	CSA001659	(-)-alpha-terpineol synthase	Up	-

Continued Table 4

Functional classification	Gene ID	Gene annotation	Expression pattern	
			KB	GB
Secondary metabolites	CSA008212	(-)-alpha-terpineol synthase	Up	-
	CSA030297	Geraniol 8-hydroxylase	Up	-
	CSA024355	Geraniol 8-hydroxylase	Up	-
	CSA006319	Geraniol 8-hydroxylase	Up	-
	CSA002616	Geraniol 8-hydroxylase	Up	Up
	CSA031137	Hyoscyamine 6-dioxygenase	-	Up
	CSA028450	Phenylalanine ammonia-lyase	Up	Up
	CSA022025	1 Phenylalanine ammonia-lyase 1	Up	Up
Plant hormones	CSA024317	1-aminocyclopropane-1-carboxylate oxidase 2	Up	Up
	CSA012876	1-aminocyclopropane-1-carboxylate oxidase 3	Up	Up
	Novel08377	abscisic acid receptor PYL4-like	Up	Up
	CSA004349	IAA-amino acid hydrolase ILR1-like 6	-	Up
	CSA028002	IAA-amino acid hydrolase ILR1-like 3	-	Up
	CSA013605	Probable indole-3-acetic acid-amido synthetase GH3.1	Up	-
Chloroplast	CSA014503	1-hydroxy-tetrahydrodipicolinate synthase, chloroplastic	Down	Down
	CSA014505	4-hydroxy-tetrahydrodipicolinate synthase, chloroplastic	Down	Down
	CSA003272	AS1 3-oxoacyl-[acyl-carrier-protein] synthase I, chloroplasticAS1	Down	Down
	CSA008681	FabG 3-oxoacyl-[acyl-carrier-protein] reductase FabG	Down	Down
	CSA010419	Beta-amylase 3, chloroplastic	Down	Down
	CSA010420	Beta-amylase 3, chloroplastic	Down	Down
	CSA033012	Beta-amylase 1, chloroplastic	Down	Down
	CSA007941	Biotin carboxylase 1, chloroplastic	Down	-
	CSA012866	Glucose-1-phosphate adenylyl transferase large subunit, chloroplastic/amyloplastic	Down	Down
Transcription factors	CSA029390	WRKY transcription factor 40	-	Up
	CSA010816	WRKY transcription factor WRKY24-like	Up	Up
	Novel01053	Transcription factor TCP8 [Vitis vinifera]	Up	-
	CSA020213	Transcription factor MYB86	Up	-
	CSA005995	Transcription factor MYB44	-	Up
	CSA026034	Transcription factor MYB1R1	Up	-
	CSA032197	Transcription factor bHLH123	Down	Down
	CSA020917	Transcription factor AS1	Down	Down
	CSA023709	NAC domain-containing protein 14	Up	Up
	CSA019024	Ethylene-responsive transcription factor ERF025	Down	-
	Novel10290	Ethylene response factor 4	Up	-
	Novel03435	ERF transcription factor	Down	-
	CSA035201	Ethylene-responsive transcription factor ERF109	Down	-
Heat shock proteins	CSA020590	18.2 kD class I heat shock protein	Down	-
	CSA014883	18.2 kD class I heat shock protein	Down	Down
	CSA001908	18.2 kD class I heat shock protein	Down	-
	CSA025136	18.2 kD class I heat shock protein	Down	-
	CSA022334	18.2 kD class I heat shock protein	Down	-
	Novel02695	18.1 kD class I heat shock protein-like	Down	-
	CSA012121	18.1 kD class I heat shock protein	Down	-
	CSA017988	18.1 kD class I heat shock protein	Down	-
	CSA035105	18.1 kD class I heat shock protein	Down	Down
	CSA022078	18.1 kD class I heat shock protein (Fragment)	Down	Down
	CSA016145	17.9 kD class II heat shock protein	Down	-

Continued Table 4

Functional classification	Gene ID	Gene annotation	Expression pattern	
			KB	GB
Heat shock proteins	CSA010838	17.9 kD class II heat shock protein	Down	-
	CSA005991	17.9 kD class II heat shock protein	Down	Down
	CSA032421	17.6 kD class I heat shock protein 3	Down	-
	CSA012981	17.3 kD class I heat shock protein	Down	Down
Aquaporins	CSA011041	Aquaporin TIP2-1	Down	Down
	CSA030470	Aquaporin TIP2-1	Down	-
	CSA018029	Aquaporin PIP2-7	Down	-
	CSA017501	Aquaporin PIP2-4	Down	Down
	CSA015939	Aquaporin PIP2-4	Down	Down
ABC transporters	CSA013191	ABC transporter I family member 17	Down	-
	CSA024835	ABC transporter I family member 17	Down	Down
	CSA023084	ABC transporter G family member 36	Up	Up
	CSA003570	ABC transporter G family member 32	-	Down
	CSA034470	ABC transporter G family member 22	Down	Down
	CSA026737	ABC transporter B family member 11	Up	Up
	CSA026738	ABC transporter B family member 11	Up	Up
	CSA007951	ABC transporter A family member 7	Up	Up
	CSA026373	ABC transporter A family member 2	-	Up
Ion transporters	CSA004440	Zinc transporter 3	-	Up
	CSA003672	Zinc transporter 1	Up	Up
	CSA011639	Putative glycerol-3-phosphate transporter 1	-	Up
	CSA011131	Probable inorganic phosphate transporter 1-9	-	Up
	CSA018957	Phosphate transporter PHO1 homolog 1	-	Up
	CSA014753	Oligopeptide transporter 3	Up	-
	CSA020400	Lysine histidine transporter 1	Up	Up
	CSA004895	Inorganic phosphate transporter 1-4	Up	Up
	CSA034045	High affinity nitrate transporter 2.5	Up	Up
	CSA004739	Cationic amino acid transporter 1	Up	-

2 Discussion

With the maturity and popularization of second-generation sequencing technology, high-throughput sequencing is used to analyze the transcriptome analysis of the interaction between plants and pathogens, explore the molecular mechanism of the interaction between plants and pathogens, and learn the more detailed molecular mechanism in the process of their interaction, so as to lay a foundation for explaining the mechanism of disease resistance (Qi, 2018; Song, 2018). In this study, high-throughput sequencing technology was used to establish the expression profiles of tea leaves of resistant and susceptible tea plants after tea blister blight infection. After being induced by tea blister blight, it is of great guiding significance to explore the differences between susceptible and resistant varieties at the transcriptome level, which is of great guiding significance to further explore the defense response mechanism of tea plants against tea blister blight.

The analysis of differential genes in tea leaves of resistant and susceptible varieties infected by tea blister blight showed that after being infected by tea blister blight, the expression of many genes changed, in which the number of up-regulated genes was slightly higher than the number of down-regulated genes, and the number of differential genes and significantly enriched GO entries in susceptible varieties were significantly more, which fully showed that the controlled genes and biological metabolism of tea trees under the stress of tea blister blight were more complex. Through the comparison of highly significantly enriched GO items, it was found that the expression of related genes such as phosphorylation (action), protein phosphorylation, protein kinase activity and

chitin metabolism was significantly up-regulated. At the same time, most genes were enriched in protein binding and catalytic activity in molecular functional ontology and participate in cellular and metabolic processes, which is similar to research result of Kuang et al. (2018) that when plants are stimulated by the external environment, they will produce a series of signal transduction to respond to various stimuli. Plant endogenous hormones play an important role in plant immune response. Salicylic acid, jasmonic acid and ethylene are important regulators in plant disease resistance signal transduction pathway (Grant et al., 2006; Vlot et al., 2009). Salicylic acid can inhibit the enzyme activity of degrading plant cell wall secreted by plant pathogens, induce the expression of genes encoding glucanase and chitinase, and inhibit the growth and reproduction of fungi by hydrolyzing the cell wall of pathogens (Shah et al., 2003). Phenylalanine ammonia lyase can catalyze the synthesis of phenylalanine to produce salicylic acid. Increasing the activity of defense enzymes such as phenylalanine enzyme in tea plants can enhance the resistance to tea blister blight and control the harm of tea blister blight (Saravanakumar et al., 2007). Singh et al. (2015) believed that the invasion of tea blister blight is related to the chitinase of tea plants. The defense response up-regulated genes obtained in this study include phenylalanine ammonia lyase involved in salicylic acid synthesis and glucan 1,3-β-glucosidase degrading cell wall, endochitinase, endo-1,4-β-xylanase, etc., which was consistent with the previous research results.

Jasmonic acid mediated defense response is related to the synthesis of phenylpropane secondary metabolites, the expression of disease course related genes and the improvement of the activity of related defense enzymes (Wu and Zhong, 2002; Meng et al., 2018). In this study, after the infection of tea blister blight, some disease course related proteins, oxidase and some secondary metabolism related enzymes were up-regulated, which was basically consistent with the research results in cucumber (Liu, 2017) and maize (Li et al., 2019). Plants infected by pathogens can induce ethylene production by inducing the synthesis of 1-aminocyclopropane-1-carboxylate (ACC) synthase at the transcriptional level (Helliwell et al., 2013; Liu et al., 2017). Ethylene mediated resistance can strengthen cell wall by promoting lignin synthesis and promote the synthesis of related enzymes and disease resistant proteins (Guo et al., 2016). In the immune response, ethylene and jasmonic acid are usually synergistically involved in inducing plant resistance to vegetative pathogens (Derksen et al., 2013). In this experiment, 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) gene involved in ethylene synthesis was induced to up regulate its expression. Jayaswall et al. (2016) also confirmed that tea blister blight infection can activate jasmonic acid and salicylic acid signal pathways and up regulate defense substances such as defense enzymes in tea plants. It can be seen that after tea plants were infected by tea blister blight, they can improve their resistance to pathogens and prevent the infection of pathogens by enhancing primary metabolism and secondary metabolism. Therefore, it was speculated that salicylic acid and jasmonic acid signaling pathways were jointly involved in the defense response of tea plants to tea blister blight.

In addition, the differential genes obtained in this study also include WRKY transcription factor, bHLH transcription factor, ERF transcription factor, ABC transporter, ion transporter, chloroplast formation related genes, heat shock protein, aquaporin and so on. WRKY transcription factor plays an important role in SA mediated SAR resistance (Vanverk et al., 2011). It can positively regulate the expression of PR gene by combining with the W-box (C/TTGACC/T) in the promoter region of downstream PR gene (Hu et al., 2012). It can also negatively regulate the disease resistance response mediated by jasmonic acid (Ren et al., 2008; Dang et al., 2014). Heat shock protein is a universal protective protein with stress resistance. It mainly protects protein homeostasis and repairs denatured proteins by combining with other proteins in the form of molecular chaperones, so as to maintain the stability of plant internal environment and play an important role in plant growth and stress regulation (Li et al., 2016). ERF transcription factors are a class of ethylene response factors, which can also mediate the interaction between ethylene signaling pathway and jasmonic acid signaling pathway (Nakano et al., 2006). These genes are related to disease resistance response and can become an important source for subsequent gene mining and research related to the disease.

3 Materials and Methods

3.1 Test Materials

The susceptible variety Yunkang 22 (YCG) and resistant variety Yuncha 1 (YCK) were selected from the Experimental Base of Tea Research Institute of Yunnan Academy of Agricultural Sciences.

3.2 Isolation and infection treatment of pathogenic bacteria of tea blister blight

Fresh diseased leaves susceptible to tea blister blight were collected in the field and screened and cultured for many times until pure strains were obtained. In the Experimental Base of Tea Research Institute of Yunnan Academy of Agricultural Sciences, Yunkang 22, a tea plant variety susceptible to tea blister blight, and Yuncha 1, a tea plant variety resistant to tea blister blight, were selected from the same plot, and the tea field was divided into two small areas, namely treatment area and control area. Inoculate 10×10 mesh spore liquid with 50~60 spores in treatment area to make it sick, and make the control area free from disease.

3.3 RNA extraction

The diseased leaves infected with tea blister blight and the leaves at the same part of tea trees not infected with tea blister blight in the control area were picked respectively (Figure 5). The diseased spots were removed, the same part of the control leaves was removed, and quickly fix the sample with liquid nitrogen. According to the method of Chen et al. (2017), extract the total RNA of diseased leaves and the control and detect the RNA quality.

3.4 Transcriptome sequencing and data analysis

After passing the inspection, they were used as sequencing samples and were sent to Novogene Biotech Co., Ltd. for mRNA enrichment and cDNA library construction. Transcriptome sequencing was performed by Illumina HiSeq™ 2500 platform. According to the sequencing results, the N-part sequence and linker in raw data were excised by cutadapt software to obtain clean data, and then the filtered clean data sequence was analyzed by HISAT software.

3.5 The analysis of gene expression level

The expression level of the gene was estimated by analyzing the count of sequenced sequences (Reads) located in the genome region or gene exon region. In order to make the estimated gene expression levels of different genes and different experiments comparable, FPKM gene expression level estimation method of Trapnell et al. (2010) and Pearson correlation coefficient were used to analyze the Pearson correlation coefficient of gene expression levels among samples.

3.6 Differential gene screening and function analysis

After DESeq standardization, differential analysis was carried out, and differential genes were screened according to the conditions of padj<0.05 and |log2FoldChange|≥0.5. For the screened differential genes, Go enrichment analysis was carried out by GOseq software method (Young et al., 2010), and KOBAS2.0 was used to carry out KEGG metabolic pathway enrichment analysis of DEGs on (http://kobas.cbi.pku.edu.cn/home.do) website.

3.7 Detection of real-time fluorescence quantitative PCR

From the differential genes obtained by sequencing, 6 genes up-regulated in both susceptible and resistant tea plants were screened for real-time fluorescence quantitative PCR. *GAPDH* in tea plants was used as the internal reference gene (Table 5). KAPA SYBR® FAST(KAPA) quantitative kit was used for fluorescence quantitative PCR. The reaction conditions were as follows: pre-denaturation at 95℃ for 1 min; Denaturation at 95℃ for 10 s, annealing at 60℃ for 20 s, signal detection at 72℃ for 5 s, and for 40 cycles; Analyze the dissolution curve at 60℃~95℃. Fluorescence quantitative detection was carried out with the help of qTOWER 2.2 fluorescence quantitative PCR instrument of Germany Analytik Jena AG. Three biological replicates were set for each sample, and the gene expression was calculated according to $2^{(-ddCT)}$ method.

Table 5 Primers for qRT-PCR

Gene ID	Forward primer (5′–3′)	Reverse primer (5′–3′)
CSA010816	TCCGCTAACTCCTACCCCAA	GTGGCAGTGGAGGACATGAA
Novel08377	CGGGAGGTCCACGTCATCT	ATAATTCGCGAGGCGGTGAT
CSA012876	TCCAGGCAATGATGCAGTGA	GGCTCTTTGGCCTGGAACTT
CSA034045	GCTGATCATGCCTCTGGTGT	AGACGGTTTGCATGAGAGCA
CSA023709	GTACTGGAAAGTGACGGGCA	CCTTTACCAGAACGCCCAGT
CSA028450	ACTCCATGCTTGCCTCTACG	CCGGCCAGTTAAGACTCCAG
GAPDH	TTGGCATCGTTGAGGGTCT	CAGTGGGAACACGGAAAGC

Authors' Contributions

LYQ and CLB were the executors of the experimental design and research of this study; RLX, XLF and TYP completed the data analysis and wrote of the first draft of the manuscript; QH participated in the experimental design and analyzed the experimental results; CLB and LMZ were the designers and principals of the project, guiding the experimental design, data analysis, manuscript writing and modification. All authors read and approved the final manuscript.

Acknowledgments

This study was jointly funded by National Natural Science Foundation of China (No.31660224), National Key Research and Development Project of China (2016YFD0200903), National Tea Industry Technology System of China (CARS-19) and Yunnan Talent Training Program (2015HB105).

References

Anders S., and Huber W., 2010, Differential expression analysis for sequence count data, Genome Biol., 11(10): 106
https://doi.org/10.1186/gb-2010-11-10-r106
PMid:20979621 PMCid:PMC3218662

Chen L.B., Xiao L.F., Tian Y.P., Li M., Song W.X., Liang M.Z., and Jiang C.J., 2017, Exploring sterility gene from tea plant flower based on digital gene expression profiling, Zuowu Xuebao (Acta Agronomica Sinica), 43(2): 210-217
https://doi.org/10.3724/SP.J.1006.2017.00210

Ran L.X., Yu X.S., Tian Y.P., Chen C.L., Chen L.B., Zhao Y.X., and Liang M.Z., 2017, Study on the resistance of different tea varieties to *Exobasidium vexans* massee, Liaoning Nongye Kexue (Liaoning Agricultural Sciences), (1): 69-70

Saravanakumar D., Vijayakumar C., Kumar N., and Samiyappan R., 2007, PGPR-induced defense responses in the tea plant against blister blight disease, Crop Prot., 26(4): 556-565
https://doi.org/10.1016/j.cropro.2006.05.007

Tan R.R., Mao Y.X., and Gong Z.M., 2015, Studies on the occurrence law of tea blister blight and biological characteristics of *Exobasidium vexans* massee, Hubei Nongye Kexue (Hubei Agricultural Sciences), 54(20): 5027-5030

Trapnell C., Williams B.A., Pertea G., Mortazavi A., Kwan G., van Baren M.J., Salzberg S.L., Wold B.J., and Pachter L., 2010, Transcript assembly and quantification by RNA-seq reveals unannotated transcripts and isoform switching during cell differentiation, Nat. biotechnol., 28(5): 511-515
https://doi.org/10.1038/nbt.1621
PMid:20436464 PMCid:PMC3146043

Young M.D., and Wakefield M.J., Smyth G.K., and Oshlack A., 2010, Gene ontology analysis for RNA-seq: accounting for selection bias, Genome Biol., 11(R): 14
https://doi.org/10.1186/gb-2010-11-2-r14
PMid:20132535 PMCid:PMC2872874

Zhang J., Tian Y.P., Xu P.Z., and Zhang H., 2008, Breeding of high quality and disease resistance Yunnan big-leaf tea cultivar "Yun cha 1", Chaye (Journal of Tea), 34(1): 39-41

Zhao X.Z., Wang Y., Ren Y.F., Li D.X., and Chen Z., 2018, The morphology observation of infection process for the pathogen *Exobasidium vexans* of tea blister blight against tea leaf, Zhongguo Nongxue Tongbao (Chinese Agricultural Science Bulletin), 34(5): 117-122

Zheng G.J., and Gao H.Y., 2015, The status analysis of tea quality safety in China, Shipin Anquan Zhiliang Jiance Xuebao (Journal of Food Safety and Quality), 6(7): 2869-2872

Zhi Y.N., Chen L.J., Shi H.Z., Wang G.J., and Guo S.B., 2018, Research advances in integrated management of tea blister blight, Xinyang Nonglin Xueyuan Xuebao (Journal of Xinyang Agriculture College), 28(1): 98-100