XXI International workshop on Bunt and Smut Disease

# Transcriptome analysis of wheat spikes in response to Tilletia controversa Kühn which cause wheat dwarf bunt

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# Significance of wheat dwarf bunt

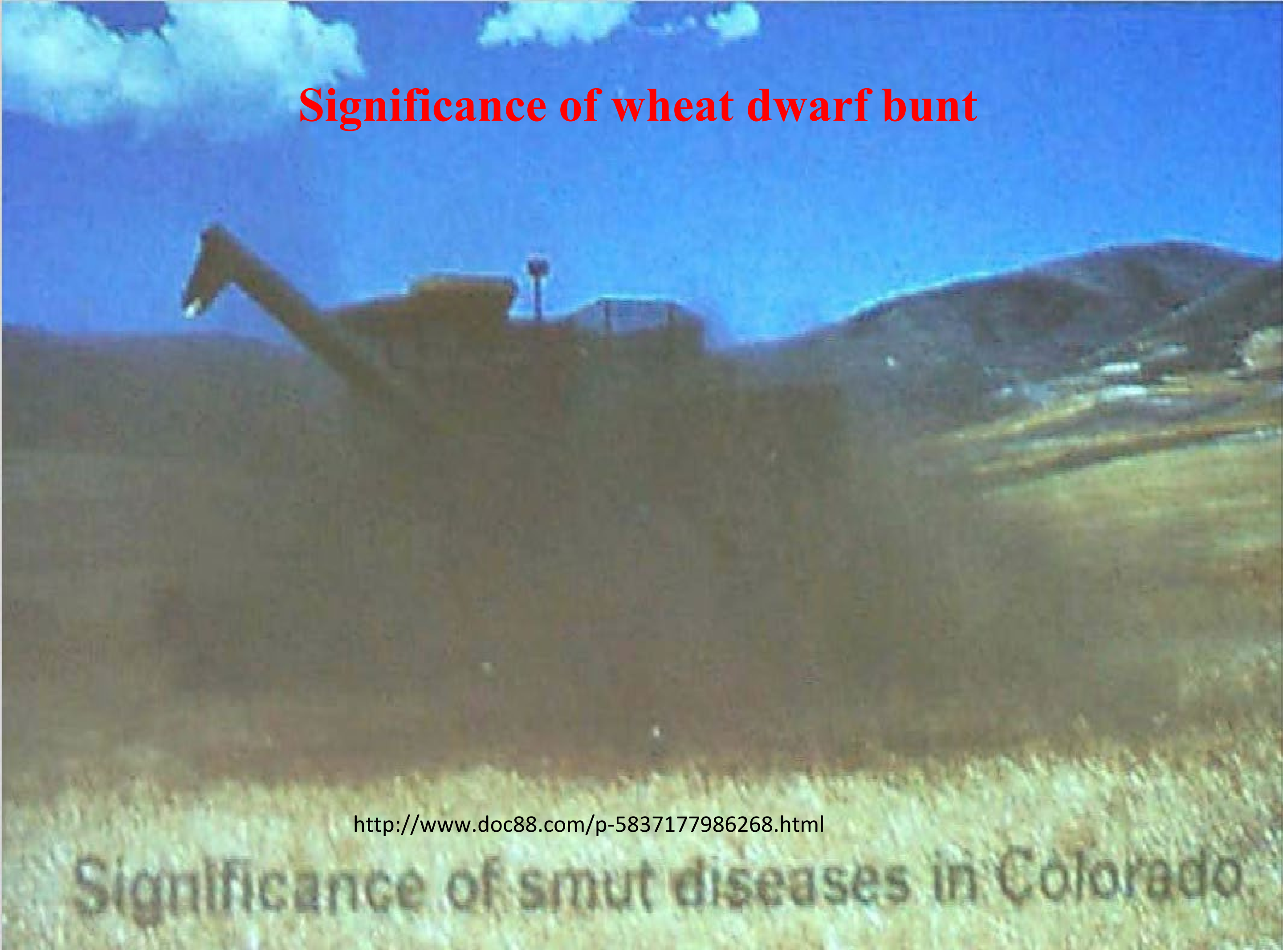

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**Fig 1. Healthy and bunted (*T. controversa*).**
**（B.J. Goates）**

**Fig 2. Close-up of wheat ear smutted by *T. controversa*,**
**showing smutted grains removed and broken open (Priekule, 2007)**

# Significance of wheat dwarf bunt

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# Significance of wheat dwarf bunt

- Tilletia controversa Kühn (TCK) is the causal organism of wheat dwarf bunt (Duran and Fischer, 1961)

- Wheat yield reduction due to bunt are nearly equal to the percentage of infected spikes, and can reach 75% during severe disease epidemics (Goates, 1996)
- Wheat crops are affected by many fungal diseases, among which dwarf bunt of wheat (DB) caused by Tilletia controversa Kühn is considered to be very dangerous in wheat-cultivating regions worldwide. However, few studies have investigated the molecular mechanism governing the interactions of wheat and T. controversa.

- In this study, RNA-Seq was performed to analyse the changes in gene expression and signal transduction in response to T. controversa infection.

- Differentially expressed genes (DEGs) involved in resistance to DB were investigated after successful infection with T. controversa.

- This approach has led to a greater understanding of the cellular and complex molecular events associated with DB and provided a basis for further studies on biotechnology and breeding for resistance to DB disease.

# Materials and Methods

- Inoculation of wheat plants with *T. controversa.*
- Extraction and purification of RNA.
- Library preparation for RNA-Seq and sequencing.
- Data analysis.
- Validation of RNA-Seq results by quantitative real-time PCR analysis.

# Inoculation of wheat plants with T. controversa

- Dongxuan 3, the susceptible wheat cultivar was used in this study.

- At the early boot stage, the spikes were injected with 1 ml inoculum suspensions of *T. controversa* . Inoculation was repeated 3 times with a one-day interval.

- The samples (spikes measuring 6.0 ± 0.5 cm in length) were collected from both *T. controversa*- and mock-infected plants, with three biological replicates for each treatment. Six samples were collected and stored at -80 °C for further use.

**Spikes whth mock-infection**

**Spike with *T. controversa*-infection**

# Extraction and purification of RNA

- Total RNA was extracted based on the protocol of the mirVana miRNA Isolation Kit (Ambion, TX, USA).
- The samples that exhibited an A260/A280 of 1.8 to 2.1 and an A260/A230 > 2.0 were chosen for further analysis.
- Furthermore, the integrity of each sample was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

# Library preparation for RNA-Seq and sequencing

Total RNA (1 μg) of each sample of mock- and T. controversa-infected plants was analysed for library construction.

The constructed cDNA library was validated by using the Qubit RNA Assay Kit in Qubit 3.0 for initial quantification。

The clustering of every sample was performed on Generation systems (Illumina, USA) following a previously described protocol.

The prepared library was loaded onto an Illumina HiSeq X Ten platform with 150-bp paired-end technology.

# Quality control and mapping

- The reads containing adapter sequences and reads with low quality (those in which more than 50% of bases presented quality of ≤ 10) and poly-N (unrecognized bases) were removed to obtain clean reads. Every downstream analysis was performed based on clear data with significantly high quality.

- The clean reads were mapped to the reference genome (https ://www.ebi.ac.uk/ena/ data/view/GCA_900519105.1) using hisat2 with the parameters set by the system.

# Data analysis

- Gene-level quantification and identification of DEGs: the $FDR < 0.05$, and at least a two-fold change ($> 1$ or $< -1$ in log2 ratio value) was set as the threshold for DEGs.
- KEGG (https ://www.kegg.jp/kegg/kegg1.html) pathway analysis was performed by using GPSeq
- GO enrichment analysis was performed, with $FDR < 0.05$ representing the significantly expressed genes.

# Validation of RNA-Seq results by quantitative real-time PCR

- Eight transcripts with various expression levels demonstrated by RNA sequencing were randomly selected for proof by qRT-PCR.

- Actin was used as an internal control in this experiment.

- Three technical replicates were employed for every gene. The 2− ΔΔCt method was employed to calculate the expression level of every gene.

# The primers were listed for validation of genes in this experiment

| Genes anotation | Primer | Sequence (5'to3') |
|---|---|---|
| Pathogenesis-related protein-1 | F | GCCAGCTACTACTCTCTCCG |
| Pathogenesis-related protein-1 | R | AGGTATCCCATGCACGACTC |
| Chitinase 1 | F | CTACACGTACGACGCCTTCA |
| Chitinase 1 | R | GACGTGGCCTTGCTTATCTC |
| Chitinase 2 | F | CACCCGGCAAGCAGTACTAT |
| Chitinase 2 | R | ACCATATCGCCGTCTTGAAC |
| Chitinase 4 | F | TTCTGGTTCTGGATGACCAAC |
| Chitinase 4 | R | ACTGCTTGCAGTACTCCGTGT |
| WRKY22 | F | CAAATGGCCGACGATTGGGATCTC |
| WRKY22 | R | CTAGTCCCCCGCGAATCATA |
| WRKY24 | F | TTGATGAAACCCTAATGATGATGC |
| WRKY24 | R | AGATGTTGGGTAGCGGGTTTGACT |
| Lipase | F | ACTGGGTATTCGTCTGTCAGC |
| Lipase | R | CACAAAATATCGACCCACCAC |
| Endo-1,4-beta-glucanase | F | CCTTGCCTCTTTGTATGCTGA |
| Endo-1,4-beta-glucanase | R | TCATCTTTTGTGGGTTCTTGC |
| Actin | F | CACTGGAATGGTCAAGGCTG |
| Actin | R | CTCCATGTCATCCCAGTTG |

# Results

- Confirmation of *T. controversa* infection in wheat plants.
- Transcriptomic analysis of RNA-Seq data.
- Identification of differentially expressed genes (DEGs).
- Gene ontology (GO) enrichment analysis of DEGs.
- KEGG enrichment analysis of DEGs.
- Differential expression of pathogenesis-related genes after *T. controversa* infection.
- Differential expression of WRKY transcription factors after *T. controversa* infection.

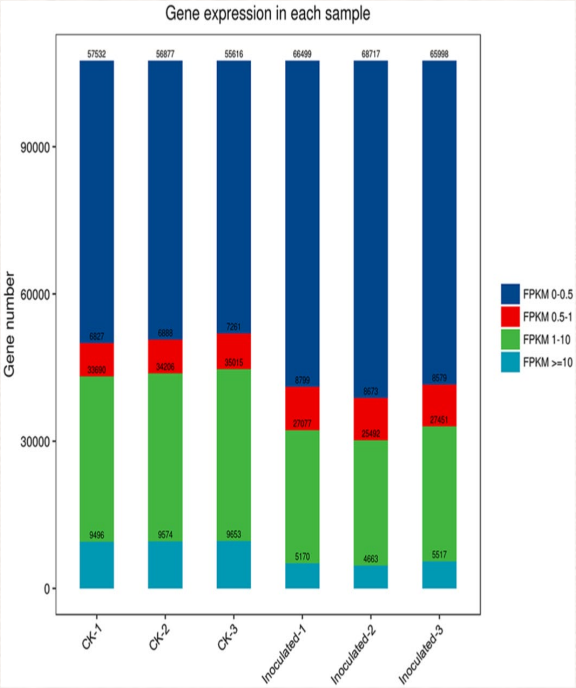
- Differential expression of protein kinase genes after *T. controversa* infection.
- Quantitative real-time PCR.

# Transcriptomic analysis of RNA-Seq data

Based on RNA-Seq, we identified alterations in wheat genes when the spike was infected by *T. controversa*. Six cDNA libraries (three *T. controversa*-infected and three mock-infected) were sequenced.

| **Type** | **CK-1** | **CK-2** | **CK-3** | **Inoculated-1** | **Inoculated-2** | **Inoculated-3** |
|---|---|---|---|---|---|---|
| Raw reads | 55.95 M | 50.30 M | 52.45 M | 58.54 M | 58.50 M | 53.72 M |
| Clean reads | 54.55 M | 49.23 M | 51.11 M | 55.72 M | 55.55 M | 51.04 M |
| Multiple mapped | 3,846,328 (7.05%) | 3,561,802 (7.23%) | 3,703,289 (7.25%) | 6,329,075 (11.36%) | 6,553,508 (11.80%) | 5,539,728 (10.85%) |
| Unique mapped | 47,120,475 (86.38%) | 42,857,444 (87.05%) | 44,404,427 (86.87%) | 44,779,099 (80.37%) | 44,195,418 (79.56%) | 41,369,664 (81.06%) |
| Q30 | 95.81% | 96.02% | 95.73% | 94.35% | 94.25% | 94.15% |
| GC | 50.69% | 50.84% | 51.08% | 54.95% | 53.77% | 55.66% |

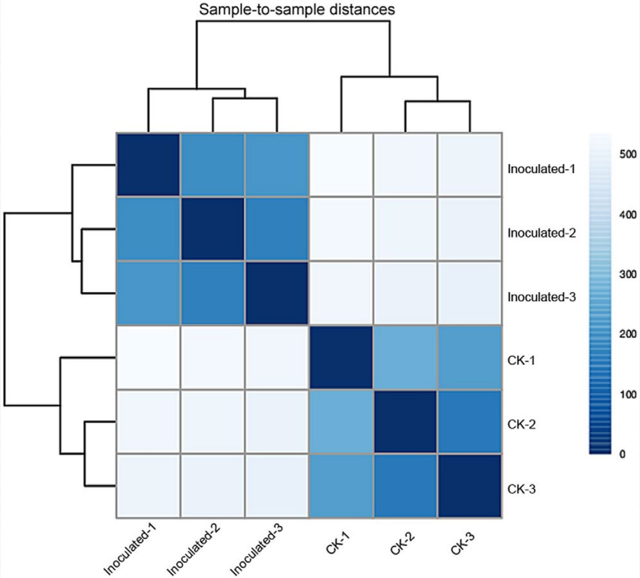
# DEGs were recognized based on FPKM value



For CK-1 DEGs, 9496 (FPKM > = 10), 33,690 (FPKM 1–10), 6827 (FPKM 0.5–1), and 57,532 (FPKM 0.–0.5) genes were differentially expressed. An approximately similar response was observed in CK-2 and CK-3 samples.

For Inoculated-1 DEGs, 5150 (FPKM > = 10), 27,077 (FPKM 1–10), 8799 (FPKM 0.5–1), and 66,499 (FPKM 0.-0.5) genes were differentially expressed. The differential expression of genes indicates the genetic difference between mock and infected plants.

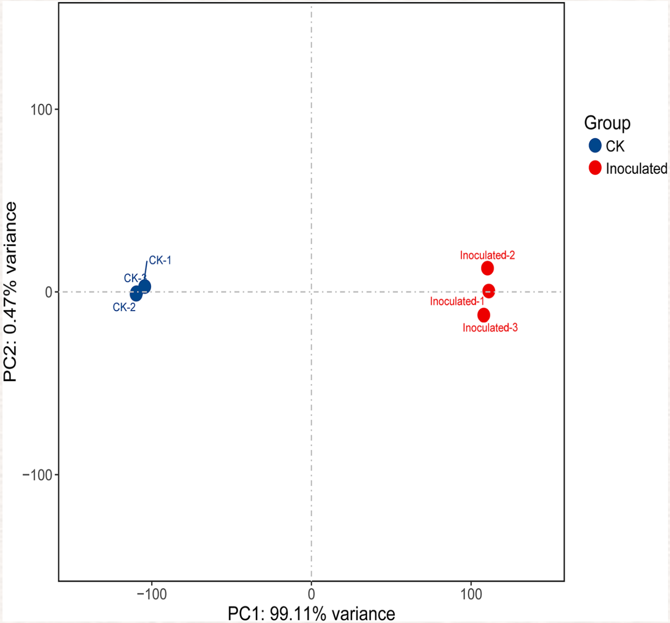
# Biological Replicates



Biological replicates of every sample were clustered together.

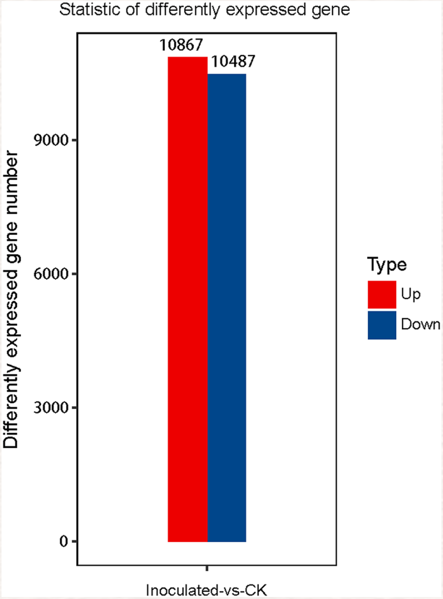
Sample-to-sample clustering analysis demonstrated that the gene expression level between replicates was reproducible and that batch effects were controlled.

# Principal Component Analysis (PCA)



Indicating that there is good reproducibility among the biological replicates of the same treatments but differences between the treatments.

# Identification of differentially expressed genes (DEGs)



The differentially expressed genes were recognized in *T. controversa*- and mock-infected libraries. In this comparison, 10,867 (up-regulated) and 10,487 (down-regulated) genes were expresse.

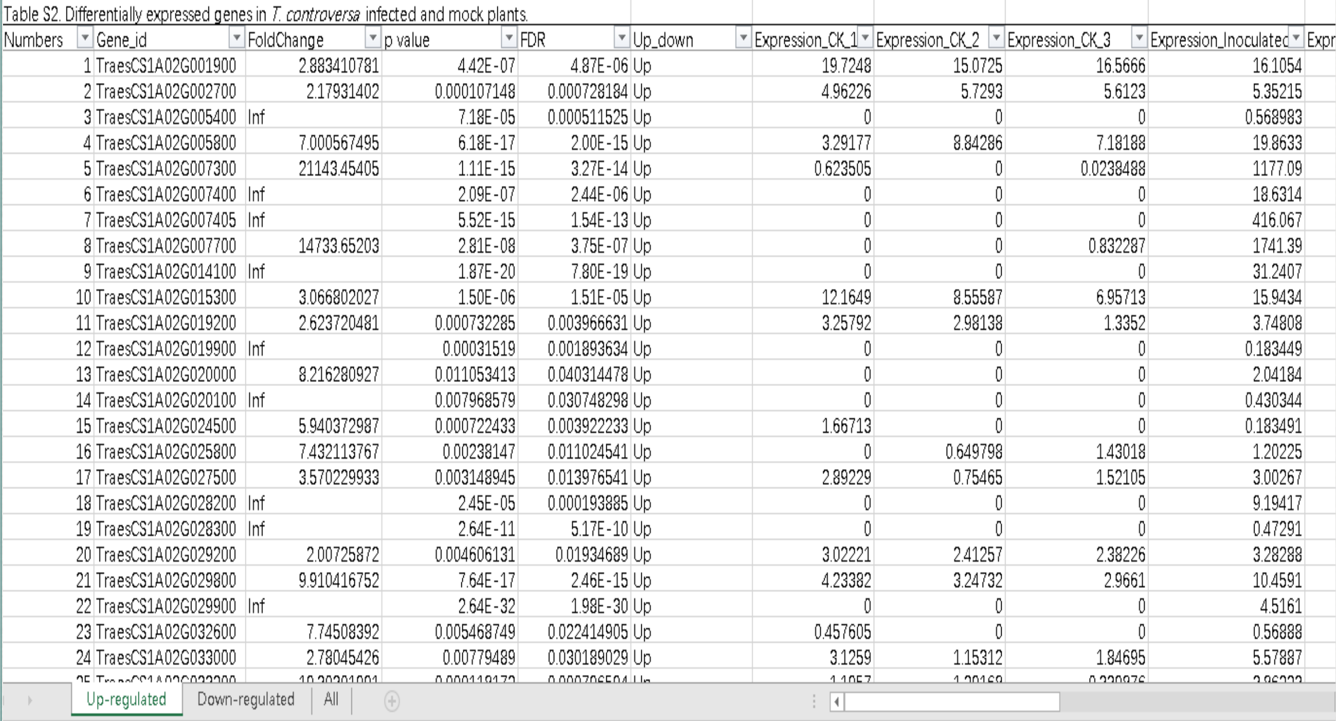
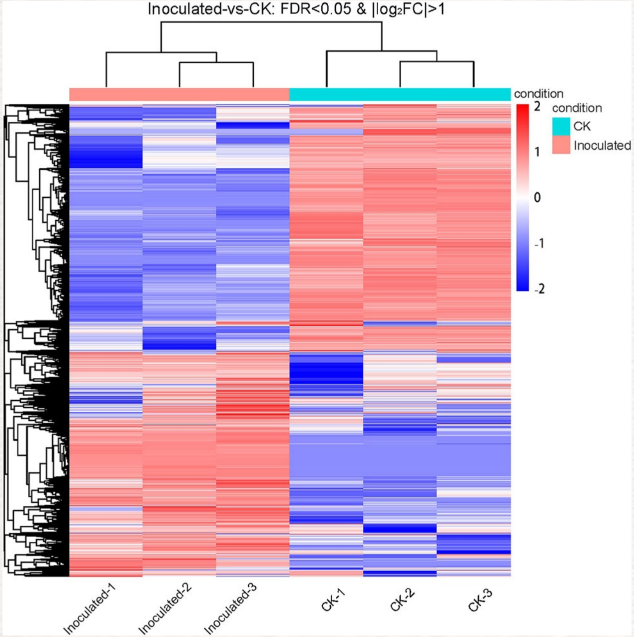


Table S2. Differentially expressed genes in *T. controversa* infected and mock plants.

| Numbers | Gene_id | FoldChange | p value | FDR | Up_down | Expression_CK_1 | Expression_CK_2 | Expression_CK_3 | Expression_Inoculated | Expr |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | TraesCS1A02G001900 | 2.883410781 | 4.42E-07 | 4.87E-06 | Up | 19.7248 | 15.0725 | 16.5666 | 16.1054 | |
| 2 | TraesCS1A02G002700 | 2.17931402 | 0.000107148 | 0.000728184 | Up | 4.96226 | 5.7293 | 5.6123 | 5.35215 | |
| 3 | TraesCS1A02G005400 | Inf | 7.18E-05 | 0.000511525 | Up | 0 | 0 | 0 | 0.568983 | |
| 4 | TraesCS1A02G005800 | 7.000567495 | 6.18E-17 | 2.00E-15 | Up | 3.29177 | 8.84286 | 7.18188 | 19.8633 | |
| 5 | TraesCS1A02G007300 | 21143.45405 | 1.11E-15 | 3.27E-14 | Up | 0.623505 | 0 | 0.0238488 | 1177.09 | |
| 6 | TraesCS1A02G007400 | Inf | 2.09E-07 | 2.44E-06 | Up | 0 | 0 | 0 | 18.6314 | |
| 7 | TraesCS1A02G007405 | Inf | 5.52E-15 | 1.54E-13 | Up | 0 | 0 | 0 | 416.067 | |
| 8 | TraesCS1A02G007700 | 14733.65203 | 2.81E-08 | 3.75E-07 | Up | 0 | 0 | 0.832287 | 1741.39 | |
| 9 | TraesCS1A02G014100 | Inf | 1.87E-20 | 7.80E-19 | Up | 0 | 0 | 0 | 31.2407 | |
| 10 | TraesCS1A02G015300 | 3.066802027 | 1.50E-06 | 1.51E-05 | Up | 12.1649 | 8.55587 | 6.95713 | 15.9434 | |
| 11 | TraesCS1A02G019200 | 2.623720481 | 0.000732285 | 0.003966631 | Up | 3.25792 | 2.98138 | 1.3352 | 3.74808 | |
| 12 | TraesCS1A02G019900 | Inf | 0.00031519 | 0.001893634 | Up | 0 | 0 | 0 | 0.183449 | |
| 13 | TraesCS1A02G020000 | 8.216280927 | 0.011053413 | 0.040314478 | Up | 0 | 0 | 0 | 2.04184 | |
| 14 | TraesCS1A02G020100 | Inf | 0.007968579 | 0.030748298 | Up | 0 | 0 | 0 | 0.430344 | |
| 15 | TraesCS1A02G024500 | 5.940372987 | 0.000722433 | 0.003922233 | Up | 1.66713 | 0 | 0 | 0.183491 | |
| 16 | TraesCS1A02G025800 | 7.432113767 | 0.00238147 | 0.011024541 | Up | 0 | 0.649798 | 1.43018 | 1.20225 | |
| 17 | TraesCS1A02G027500 | 3.570229933 | 0.003148945 | 0.013976541 | Up | 2.89229 | 0.75465 | 1.52105 | 3.00267 | |
| 18 | TraesCS1A02G028200 | Inf | 2.45E-05 | 0.000193885 | Up | 0 | 0 | 0 | 9.19417 | |
| 19 | TraesCS1A02G028300 | Inf | 2.64E-11 | 5.17E-10 | Up | 0 | 0 | 0 | 0.47291 | |
| 20 | TraesCS1A02G029200 | 2.00725872 | 0.004606131 | 0.01934689 | Up | 3.02221 | 2.41257 | 2.38226 | 3.28288 | |
| 21 | TraesCS1A02G029800 | 9.910416752 | 7.64E-17 | 2.46E-15 | Up | 4.23382 | 3.24732 | 2.9661 | 10.4591 | |
| 22 | TraesCS1A02G029900 | Inf | 2.64E-32 | 1.98E-30 | Up | 0 | 0 | 0 | 4.5161 | |
| 23 | TraesCS1A02G032600 | 7.74508392 | 0.005468749 | 0.022414905 | Up | 0.457605 | 0 | 0 | 0.56888 | |
| 24 | TraesCS1A02G033000 | 2.78045426 | 0.00779489 | 0.030189029 | Up | 3.1259 | 1.15312 | 1.84695 | 5.57887 | |

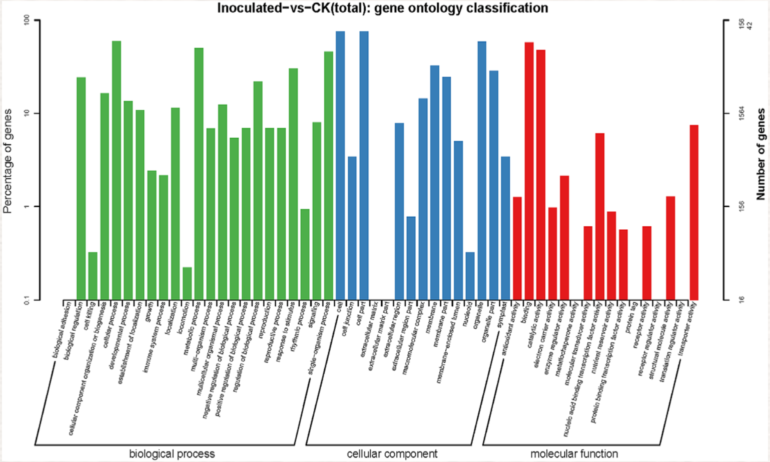
# Hierarchical clustering analysis



To elucidate the transcriptional changes occurring after *T. controversa* infection, we demonstrated the expression pattern by using hierarchical clustering analysis.

On behalf of the analysis, the expression levels of *T. controversa*-infected and mock-infected plants were different from each other but were similar in the replication of *T. controversa*-infected and mock-infected plants.

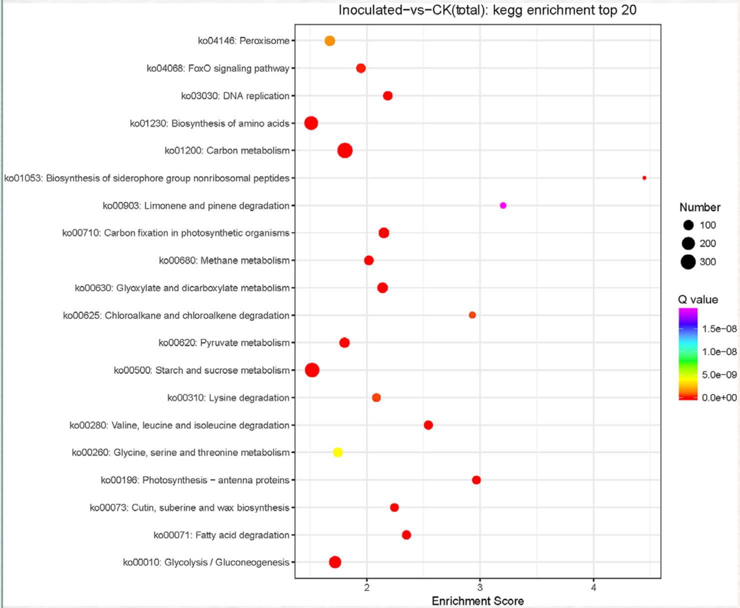
# GO enrichment analysis of DEGs



GO was mainly associated with cellular process, metabolic process and single-organism process.

Meanwhile, in the cellular component category, DEGs were primarily associated with cell, cell part, and organelle.

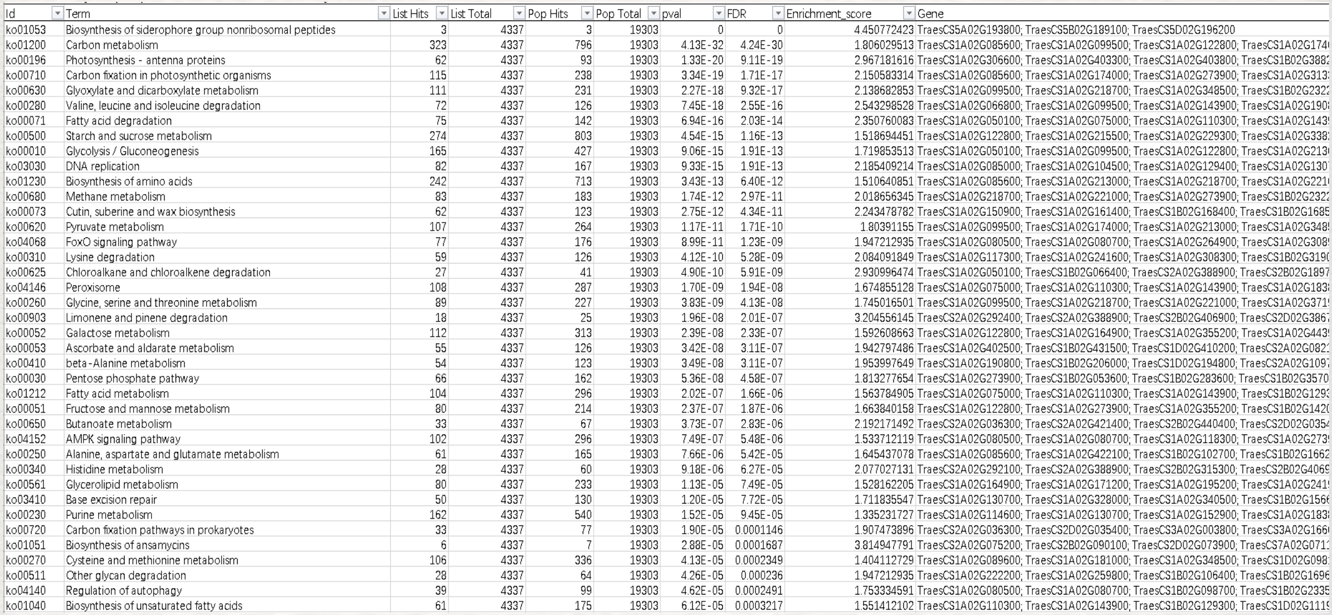
# KEGG pathway analysis



For DEGs, 205 different pathways were identified. However, the top 20 KEGG enrichment pathways were primarily activated.

The pathway of biosynthesis of siderophore group nonribosomal peptides was activated slightly during the interaction

# KEGG pathway analysis



| Id | Term | List Hits | List Total | Pop Hits | Pop Total | pval | FDR | Enrichment_score | Gene |
|---|---|---|---|---|---|---|---|---|---|
| ko01053 | Biosynthesis of siderophore group nonribosomal peptides | 3 | 4337 | 3 | 19303 | 0 | 0 | 4.450772423 | TraesCS5A02G193800; TraesCS5B02G189100; TraesCS5D02G196200 |
| ko01200 | Carbon metabolism | 323 | 4337 | 796 | 19303 | 4.13E-32 | 4.24E-30 | 1.806029513 | TraesCS1A02G085600; TraesCS1A02G099500; TraesCS1A02G122800; TraesCS1A02G174 |
| ko00196 | Photosynthesis - antenna proteins | 62 | 4337 | 93 | 19303 | 1.33E-20 | 9.11E-19 | 2.967181616 | TraesCS1A02G306600; TraesCS1A02G403300; TraesCS1A02G403800; TraesCS1B02G3882 |
| ko00710 | Carbon fixation in photosynthetic organisms | 115 | 4337 | 238 | 19303 | 3.34E-19 | 1.71E-17 | 2.150583314 | TraesCS1A02G085600; TraesCS1A02G174000; TraesCS1A02G273900; TraesCS1A02G313 |
| ko00630 | Glyoxylate and dicarboxylate metabolism | 111 | 4337 | 231 | 19303 | 2.27E-18 | 9.32E-17 | 2.138682853 | TraesCS1A02G099500; TraesCS1A02G218700; TraesCS1A02G348500; TraesCS1B02G2322 |
| ko00280 | Valine, leucine and isoleucine degradation | 72 | 4337 | 126 | 19303 | 7.45E-18 | 2.55E-16 | 2.543298528 | TraesCS1A02G066800; TraesCS1A02G099500; TraesCS1A02G143900; TraesCS1A02G190 |
| ko00071 | Fatty acid degradation | 75 | 4337 | 142 | 19303 | 6.94E-16 | 2.03E-14 | 2.350760083 | TraesCS1A02G050100; TraesCS1A02G075000; TraesCS1A02G110300; TraesCS1A02G143 |
| ko00500 | Starch and sucrose metabolism | 274 | 4337 | 803 | 19303 | 4.54E-15 | 1.16E-13 | 1.518694451 | TraesCS1A02G122800; TraesCS1A02G215500; TraesCS1A02G229300; TraesCS1A02G338 |
| ko00010 | Glycolysis / Gluconeogenesis | 165 | 4337 | 427 | 19303 | 9.06E-15 | 1.91E-13 | 1.719853513 | TraesCS1A02G050100; TraesCS1A02G099500; TraesCS1A02G122800; TraesCS1A02G213 |
| ko03030 | DNA replication | 82 | 4337 | 167 | 19303 | 9.33E-15 | 1.91E-13 | 2.185409214 | TraesCS1A02G085000; TraesCS1A02G104500; TraesCS1A02G129400; TraesCS1A02G130 |
| ko01230 | Biosynthesis of amino acids | 242 | 4337 | 713 | 19303 | 3.43E-13 | 6.40E-12 | 1.510640851 | TraesCS1A02G085600; TraesCS1A02G213000; TraesCS1A02G218700; TraesCS1A02G221 |
| ko00680 | Methane metabolism | 83 | 4337 | 183 | 19303 | 1.74E-12 | 2.97E-11 | 2.018656345 | TraesCS1A02G218700; TraesCS1A02G221000; TraesCS1A02G273900; TraesCS1B02G2322 |
| ko00073 | Cutin, suberine and wax biosynthesis | 62 | 4337 | 123 | 19303 | 2.75E-12 | 4.34E-11 | 2.243478782 | TraesCS1A02G150900; TraesCS1A02G161400; TraesCS1B02G168400; TraesCS1B02G1685 |
| ko00620 | Pyruvate metabolism | 107 | 4337 | 264 | 19303 | 1.17E-11 | 1.71E-10 | 1.80391155 | TraesCS1A02G099500; TraesCS1A02G174000; TraesCS1A02G213000; TraesCS1A02G348 |
| ko04068 | FoxO signaling pathway | 77 | 4337 | 176 | 19303 | 8.99E-11 | 1.23E-09 | 1.947212935 | TraesCS1A02G080500; TraesCS1A02G080700; TraesCS1A02G264900; TraesCS1A02G308 |
| ko00310 | Lysine degradation | 59 | 4337 | 126 | 19303 | 4.12E-10 | 5.28E-09 | 2.084091849 | TraesCS1A02G117300; TraesCS1A02G241600; TraesCS1A02G308300; TraesCS1B02G3190 |
| ko00625 | Chloroalkane and chloroalkene degradation | 27 | 4337 | 41 | 19303 | 4.90E-10 | 5.91E-09 | 2.930996474 | TraesCS1A02G050100; TraesCS1B02G066400; TraesCS2A02G388900; TraesCS2B02G1897 |
| ko04146 | Peroxisome | 108 | 4337 | 287 | 19303 | 1.70E-09 | 1.94E-08 | 1.674855128 | TraesCS1A02G075000; TraesCS1A02G110300; TraesCS1A02G143900; TraesCS1A02G183 |
| ko00260 | Glycine, serine and threonine metabolism | 89 | 4337 | 227 | 19303 | 3.83E-09 | 4.13E-08 | 1.745016501 | TraesCS1A02G099500; TraesCS1A02G218700; TraesCS1A02G221000; TraesCS1A02G371 |
| ko00903 | Limonene and pinene degradation | 18 | 4337 | 25 | 19303 | 1.96E-08 | 2.01E-07 | 3.204556145 | TraesCS2A02G292400; TraesCS2A02G388900; TraesCS2B02G406900; TraesCS2D02G3867 |
| ko00052 | Galactose metabolism | 112 | 4337 | 313 | 19303 | 2.39E-08 | 2.33E-07 | 1.592608663 | TraesCS1A02G122800; TraesCS1A02G164900; TraesCS1A02G355200; TraesCS1A02G443 |
| ko00053 | Ascorbate and aldarate metabolism | 55 | 4337 | 126 | 19303 | 3.42E-08 | 3.11E-07 | 1.942797486 | TraesCS1A02G402500; TraesCS1B02G431500; TraesCS1D02G410200; TraesCS2A02G0821 |
| ko00410 | beta-Alanine metabolism | 54 | 4337 | 123 | 19303 | 3.49E-08 | 3.11E-07 | 1.953997649 | TraesCS1A02G190800; TraesCS1B02G206000; TraesCS1D02G194800; TraesCS2A02G1097 |
| ko00030 | Pentose phosphate pathway | 66 | 4337 | 162 | 19303 | 5.36E-08 | 4.58E-07 | 1.813277654 | TraesCS1A02G273900; TraesCS1B02G053600; TraesCS1B02G283600; TraesCS1B02G3570 |
| ko01212 | Fatty acid metabolism | 104 | 4337 | 296 | 19303 | 2.02E-07 | 1.66E-06 | 1.563784905 | TraesCS1A02G075000; TraesCS1A02G110300; TraesCS1A02G143900; TraesCS1B02G1293 |
| ko00051 | Fructose and mannose metabolism | 80 | 4337 | 214 | 19303 | 2.37E-07 | 1.87E-06 | 1.663840158 | TraesCS1A02G122800; TraesCS1A02G273900; TraesCS1A02G355200; TraesCS1B02G1420 |
| ko00650 | Butanoate metabolism | 33 | 4337 | 67 | 19303 | 3.73E-07 | 2.83E-06 | 2.192171492 | TraesCS2A02G036300; TraesCS2A02G421400; TraesCS2B02G440400; TraesCS2D02G0354 |
| ko04152 | AMPK signaling pathway | 102 | 4337 | 296 | 19303 | 7.49E-07 | 5.48E-06 | 1.533712119 | TraesCS1A02G080500; TraesCS1A02G080700; TraesCS1A02G118300; TraesCS1A02G273 |
| ko00250 | Alanine, aspartate and glutamate metabolism | 61 | 4337 | 165 | 19303 | 7.66E-06 | 5.42E-05 | 1.645437078 | TraesCS1A02G085600; TraesCS1A02G422100; TraesCS1B02G102700; TraesCS1B02G1662 |
| ko00340 | Histidine metabolism | 28 | 4337 | 60 | 19303 | 9.18E-06 | 6.27E-05 | 2.077027131 | TraesCS2A02G292100; TraesCS2A02G388900; TraesCS2B02G315300; TraesCS2B02G4069 |
| ko00561 | Glycerolipid metabolism | 80 | 4337 | 233 | 19303 | 1.13E-05 | 7.49E-05 | 1.528162205 | TraesCS1A02G164900; TraesCS1A02G171200; TraesCS1A02G195200; TraesCS1A02G241 |
| ko03410 | Base excision repair | 50 | 4337 | 130 | 19303 | 1.20E-05 | 7.72E-05 | 1.711835547 | TraesCS1A02G130700; TraesCS1A02G328000; TraesCS1A02G340500; TraesCS1B02G1566 |
| ko00230 | Purine metabolism | 162 | 4337 | 540 | 19303 | 1.52E-05 | 9.45E-05 | 1.335231727 | TraesCS1A02G114600; TraesCS1A02G130700; TraesCS1A02G152900; TraesCS1A02G183 |
| ko00720 | Carbon fixation pathways in prokaryotes | 33 | 4337 | 77 | 19303 | 1.90E-05 | 0.0001146 | 1.907473896 | TraesCS2A02G036300; TraesCS2D02G035400; TraesCS3A02G003800; TraesCS3A02G166 |
| ko01051 | Biosynthesis of ansamycins | 6 | 4337 | 7 | 19303 | 2.88E-05 | 0.0001687 | 3.814947791 | TraesCS2A02G075200; TraesCS2B02G090100; TraesCS2D02G073900; TraesCS7A02G071 |
| ko00270 | Cysteine and methionine metabolism | 106 | 4337 | 336 | 19303 | 4.13E-05 | 0.0002349 | 1.404112729 | TraesCS1A02G089600; TraesCS1A02G181000; TraesCS1A02G348500; TraesCS1D02G098 |
| ko00511 | Other glycan degradation | 28 | 4337 | 64 | 19303 | 4.26E-05 | 0.000236 | 1.947212935 | TraesCS1A02G222200; TraesCS1A02G259800; TraesCS1B02G106400; TraesCS1B02G1696 |
| ko04140 | Regulation of autophagy | 39 | 4337 | 99 | 19303 | 4.62E-05 | 0.0002491 | 1.753334591 | TraesCS1A02G080500; TraesCS1A02G080700; TraesCS1B02G098700; TraesCS1B02G2335 |
| ko01040 | Biosynthesis of unsaturated fatty acids | 61 | 4337 | 175 | 19303 | 6.12E-05 | 0.0003217 | 1.551412102 | TraesCS1A02G110300; TraesCS1A02G143900; TraesCS1B02G129300; TraesCS1D02G111 |

# Identification of DEGs related to disease resistance

Differential expression of disease resistance genes, including pathogenesis-related (PR) genes, WRKY transcription factors and protein kinase genes (PRRs) were then identified.

215 PR genes were chaged (135 up-regulated, 80 down-regulated)

57 WRKY transcription factors were identified (44 up-regulated. 13 down regulated）

761protein kinase genes were identified (found 50 MAPK genes).

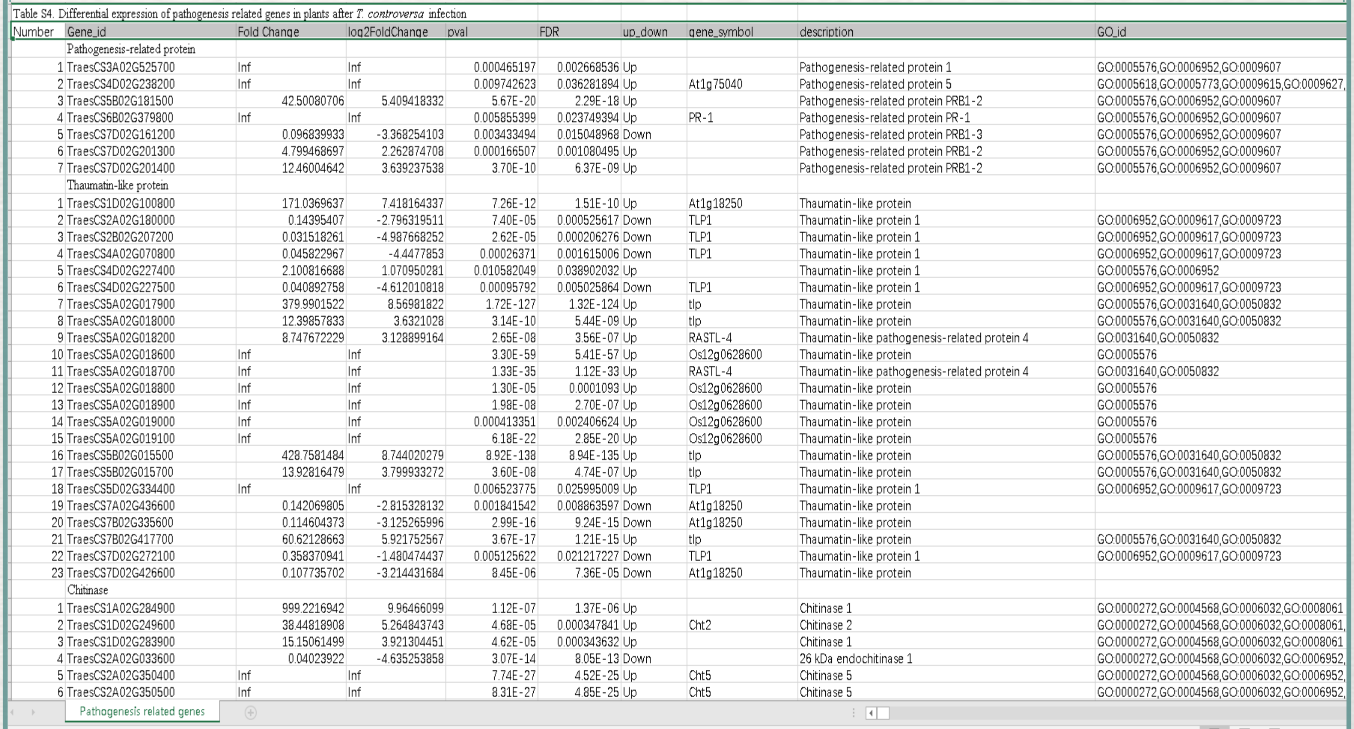


Table S4. Differential expression of pathogenesis related genes in plants after *T. controversa* infection

| Number | Gene_id | Fold Change | log2FoldChange | pval | FDR | up_down | gene_symbol | description | GO_id |
|---|---|---|---|---|---|---|---|---|---|
| | Pathogenesis-related protein | | | | | | | | |
| 1 | TraesCS3A02G525700 | Inf | Inf | 0.000465197 | 0.002668536 | Up | | Pathogenesis-related protein 1 | GO:0005576,GO:0006952,GO:0009607 |
| 2 | TraesCS4D02G238200 | Inf | Inf | 0.009742623 | 0.036281894 | Up | At1g75040 | Pathogenesis-related protein 5 | GO:0005618,GO:0005773,GO:0009615,GO:0009627, |
| 3 | TraesCS5B02G181500 | 42.50080706 | 5.409418332 | 5.67E-20 | 2.29E-18 | Up | | Pathogenesis-related protein PRB1-2 | GO:0005576,GO:0006952,GO:0009607 |
| 4 | TraesCS6B02G379800 | Inf | Inf | 0.005855399 | 0.023749394 | Up | PR-1 | Pathogenesis-related protein PR-1 | GO:0005576,GO:0006952,GO:0009607 |
| 5 | TraesCS7D02G161200 | 0.096839933 | -3.368254103 | 0.003433494 | 0.015048968 | Down | | Pathogenesis-related protein PRB1-3 | GO:0005576,GO:0006952,GO:0009607 |
| 6 | TraesCS7D02G201300 | 4.799468697 | 2.262874708 | 0.000166507 | 0.001080495 | Up | | Pathogenesis-related protein PRB1-2 | GO:0005576,GO:0006952,GO:0009607 |
| 7 | TraesCS7D02G201400 | 12.46004642 | 3.639237538 | 3.70E-10 | 6.37E-09 | Up | | Pathogenesis-related protein PRB1-2 | GO:0005576,GO:0006952,GO:0009607 |
| | Thaumatin-like protein | | | | | | | | |
| 1 | TraesCS1D02G100800 | 171.0369637 | 7.418164337 | 7.26E-12 | 1.51E-10 | Up | At1g18250 | Thaumatin-like protein | |
| 2 | TraesCS2A02G180000 | 0.14395407 | -2.796319511 | 7.40E-05 | 0.000525617 | Down | TLP1 | Thaumatin-like protein 1 | GO:0006952,GO:0009617,GO:0009723 |
| 3 | TraesCS2B02G207200 | 0.031518261 | -4.987668252 | 2.62E-05 | 0.000206276 | Down | TLP1 | Thaumatin-like protein 1 | GO:0006952,GO:0009617,GO:0009723 |
| 4 | TraesCS4A02G070800 | 0.045822967 | -4.4477853 | 0.00026371 | 0.001615006 | Down | TLP1 | Thaumatin-like protein 1 | GO:0006952,GO:0009617,GO:0009723 |
| 5 | TraesCS4D02G227400 | 2.100816688 | 1.070950281 | 0.010582049 | 0.038902032 | Up | | Thaumatin-like protein 1 | GO:0005576,GO:0006952 |
| 6 | TraesCS4D02G227500 | 0.040892758 | -4.612010818 | 0.00095792 | 0.005025864 | Down | TLP1 | Thaumatin-like protein 1 | GO:0006952,GO:0009617,GO:0009723 |
| 7 | TraesCS5A02G017900 | 379.9901522 | 8.56981822 | 1.72E-127 | 1.32E-124 | Up | tlp | Thaumatin-like protein | GO:0005576,GO:0031640,GO:0050832 |
| 8 | TraesCS5A02G018000 | 12.39857833 | 3.6321028 | 3.14E-10 | 5.44E-09 | Up | tlp | Thaumatin-like protein | GO:0005576,GO:0031640,GO:0050832 |
| 9 | TraesCS5A02G018200 | 8.747672229 | 3.128899164 | 2.65E-08 | 3.56E-07 | Up | RASTL-4 | Thaumatin-like pathogenesis-related protein 4 | GO:0031640,GO:0050832 |
| 10 | TraesCS5A02G018600 | Inf | Inf | 3.30E-59 | 5.41E-57 | Up | Os12g0628600 | Thaumatin-like protein | GO:0005576 |
| 11 | TraesCS5A02G018700 | Inf | Inf | 1.33E-35 | 1.12E-33 | Up | RASTL-4 | Thaumatin-like pathogenesis-related protein 4 | GO:0031640,GO:0050832 |
| 12 | TraesCS5A02G018800 | Inf | Inf | 1.30E-05 | 0.0001093 | Up | Os12g0628600 | Thaumatin-like protein | GO:0005576 |
| 13 | TraesCS5A02G018900 | Inf | Inf | 1.98E-08 | 2.70E-07 | Up | Os12g0628600 | Thaumatin-like protein | GO:0005576 |
| 14 | TraesCS5A02G019000 | Inf | Inf | 0.000413351 | 0.002406624 | Up | Os12g0628600 | Thaumatin-like protein | GO:0005576 |
| 15 | TraesCS5A02G019100 | Inf | Inf | 6.18E-22 | 2.85E-20 | Up | Os12g0628600 | Thaumatin-like protein | GO:0005576 |
| 16 | TraesCS5B02G015500 | 428.7581484 | 8.744020279 | 8.92E-138 | 8.94E-135 | Up | tlp | Thaumatin-like protein | GO:0005576,GO:0031640,GO:0050832 |
| 17 | TraesCS5B02G015700 | 13.92816479 | 3.799933272 | 3.60E-08 | 4.74E-07 | Up | tlp | Thaumatin-like protein | GO:0005576,GO:0031640,GO:0050832 |
| 18 | TraesCS5D02G334400 | Inf | Inf | 0.006523775 | 0.025995009 | Up | TLP1 | Thaumatin-like protein 1 | GO:0006952,GO:0009617,GO:0009723 |
| 19 | TraesCS7A02G436600 | 0.142069805 | -2.815328132 | 0.001841542 | 0.008863597 | Down | At1g18250 | Thaumatin-like protein | |
| 20 | TraesCS7B02G335600 | 0.114604373 | -3.125265996 | 2.99E-16 | 9.24E-15 | Down | At1g18250 | Thaumatin-like protein | |
| 21 | TraesCS7B02G417700 | 60.62128663 | 5.921752567 | 3.67E-17 | 1.21E-15 | Up | tlp | Thaumatin-like protein | GO:0005576,GO:0031640,GO:0050832 |
| 22 | TraesCS7D02G272100 | 0.358370941 | -1.480474437 | 0.005125622 | 0.021217227 | Down | TLP1 | Thaumatin-like protein 1 | GO:0006952,GO:0009617,GO:0009723 |
| 23 | TraesCS7D02G426600 | 0.107735702 | -3.214431684 | 8.45E-06 | 7.36E-05 | Down | At1g18250 | Thaumatin-like protein | |
| | Chitinase | | | | | | | | |
| 1 | TraesCS1A02G284900 | 999.2216942 | 9.96466099 | 1.12E-07 | 1.37E-06 | Up | | Chitinase 1 | GO:0000272,GO:0004568,GO:0006032,GO:0008061 |
| 2 | TraesCS1D02G249600 | 38.44818908 | 5.264843743 | 4.68E-05 | 0.000347841 | Up | Cht2 | Chitinase 2 | GO:0000272,GO:0004568,GO:0006032,GO:0008061, |
| 3 | TraesCS1D02G283900 | 15.15061499 | 3.921304451 | 4.62E-05 | 0.000343632 | Up | | Chitinase 1 | GO:0000272,GO:0004568,GO:0006032,GO:0008061 |
| 4 | TraesCS2A02G033600 | 0.04023922 | -4.635253858 | 3.07E-14 | 8.05E-13 | Down | | 26 kDa endochitinase 1 | GO:0000272,GO:0004568,GO:0006032,GO:0006952, |
| 5 | TraesCS2A02G350400 | Inf | Inf | 7.74E-27 | 4.52E-25 | Up | Cht5 | Chitinase 5 | GO:0000272,GO:0004568,GO:0006032,GO:0006952, |
| 6 | TraesCS2A02G350500 | Inf | Inf | 8.31E-27 | 4.85E-25 | Up | Cht5 | Chitinase 5 | GO:0000272,GO:0004568,GO:0006032,GO:0006952, |

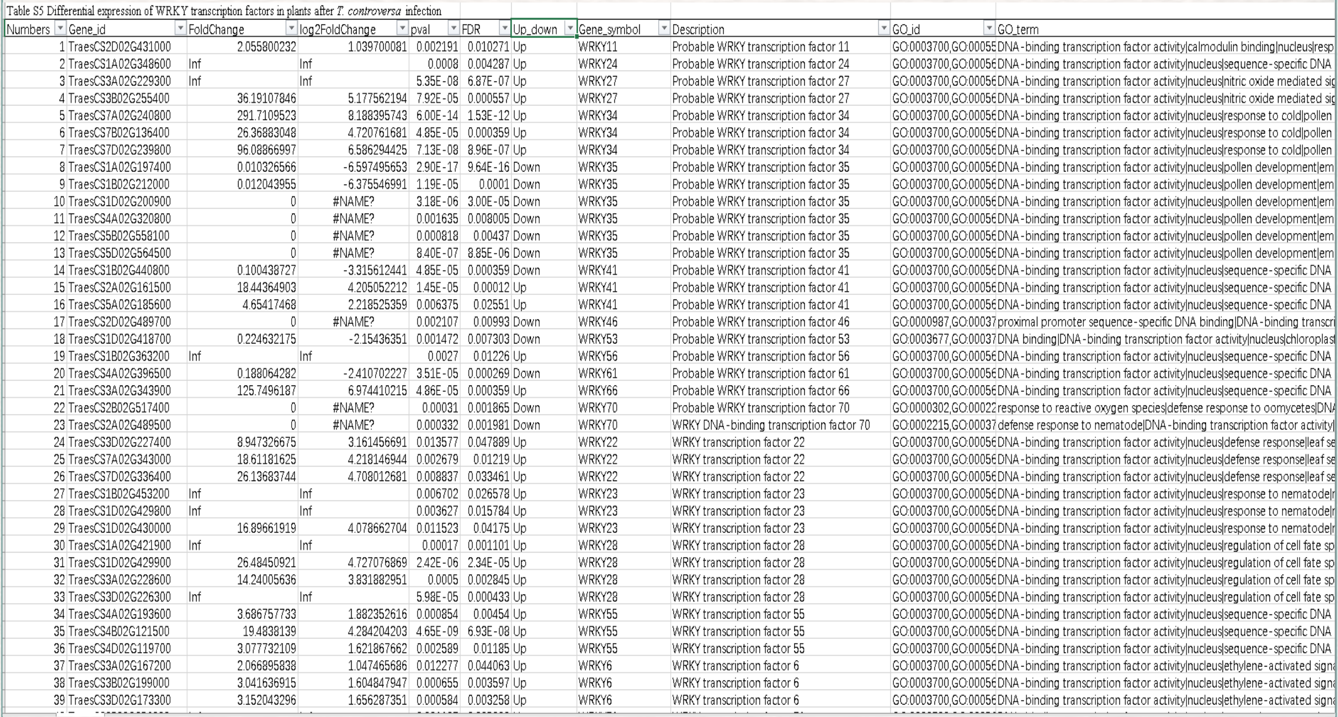


Table S5 Differential expression of WRKY transcription factors in plants after *T. controversa* infection

| Numbers | Gene_id | FoldChange | log2FoldChange | pval | FDR | Up_down | Gene_symbol | Description | GO_id | GO_term |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | TraesCS2D02G431000 | 2.055800232 | 1.039700081 | 0.002191 | 0.010271 | Up | WRKY11 | Probable WRKY transcription factor 11 | GO:0003700,GO:00055 | DNA-binding transcription factor activity\|calmodulin binding\|nucleus\|resp |
| 2 | TraesCS1A02G348600 | Inf | Inf | 0.0008 | 0.004287 | Up | WRKY24 | Probable WRKY transcription factor 24 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|sequence-specific DNA |
| 3 | TraesCS3A02G229300 | Inf | Inf | 5.35E-08 | 6.87E-07 | Up | WRKY27 | Probable WRKY transcription factor 27 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|nitric oxide mediated sig |
| 4 | TraesCS3B02G255400 | 36.19107846 | 5.177562194 | 7.92E-05 | 0.000557 | Up | WRKY27 | Probable WRKY transcription factor 27 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|nitric oxide mediated sig |
| 5 | TraesCS7A02G240800 | 291.7109523 | 8.188395743 | 6.00E-14 | 1.53E-12 | Up | WRKY34 | Probable WRKY transcription factor 34 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|response to cold\|pollen |
| 6 | TraesCS7B02G136400 | 26.36883048 | 4.720761681 | 4.85E-05 | 0.000359 | Up | WRKY34 | Probable WRKY transcription factor 34 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|response to cold\|pollen |
| 7 | TraesCS7D02G239800 | 96.08866997 | 6.586294425 | 7.13E-08 | 8.96E-07 | Up | WRKY34 | Probable WRKY transcription factor 34 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|response to cold\|pollen |
| 8 | TraesCS1A02G197400 | 0.010326566 | -6.597495653 | 2.90E-17 | 9.64E-16 | Down | WRKY35 | Probable WRKY transcription factor 35 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|pollen development\|em |
| 9 | TraesCS1B02G212000 | 0.012043955 | -6.375546991 | 1.19E-05 | 0.0001 | Down | WRKY35 | Probable WRKY transcription factor 35 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|pollen development\|em |
| 10 | TraesCS1D02G200900 | 0 | #NAME? | 3.18E-06 | 3.00E-05 | Down | WRKY35 | Probable WRKY transcription factor 35 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|pollen development\|em |
| 11 | TraesCS4A02G320800 | 0 | #NAME? | 0.001635 | 0.008005 | Down | WRKY35 | Probable WRKY transcription factor 35 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|pollen development\|em |
| 12 | TraesCS5B02G558100 | 0 | #NAME? | 0.000818 | 0.00437 | Down | WRKY35 | Probable WRKY transcription factor 35 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|pollen development\|em |
| 13 | TraesCS5D02G564500 | 0 | #NAME? | 8.40E-07 | 8.85E-06 | Down | WRKY35 | Probable WRKY transcription factor 35 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|pollen development\|em |
| 14 | TraesCS1B02G440800 | 0.100438727 | -3.315612441 | 4.85E-05 | 0.000359 | Down | WRKY41 | Probable WRKY transcription factor 41 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|sequence-specific DNA |
| 15 | TraesCS2A02G161500 | 18.44364903 | 4.205052212 | 1.45E-05 | 0.00012 | Up | WRKY41 | Probable WRKY transcription factor 41 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|sequence-specific DNA |
| 16 | TraesCS5A02G185600 | 4.65417468 | 2.218525359 | 0.006375 | 0.02551 | Up | WRKY41 | Probable WRKY transcription factor 41 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|sequence-specific DNA |
| 17 | TraesCS2D02G489700 | 0 | #NAME? | 0.002107 | 0.00993 | Down | WRKY46 | Probable WRKY transcription factor 46 | GO:0000987,GO:00037 | proximal promoter sequence-specific DNA binding\|DNA-binding transcri |
| 18 | TraesCS1D02G418700 | 0.224632175 | -2.15436351 | 0.001472 | 0.007303 | Down | WRKY53 | Probable WRKY transcription factor 53 | GO:0003677,GO:00037 | DNA binding\|DNA-binding transcription factor activity\|nucleus\|chloroplast |
| 19 | TraesCS1B02G363200 | Inf | Inf | 0.0027 | 0.01226 | Up | WRKY56 | Probable WRKY transcription factor 56 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|sequence-specific DNA |
| 20 | TraesCS4A02G396500 | 0.188064282 | -2.410702227 | 3.51E-05 | 0.000269 | Down | WRKY61 | Probable WRKY transcription factor 61 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|sequence-specific DNA |
| 21 | TraesCS3A02G343900 | 125.7496187 | 6.974410215 | 4.86E-05 | 0.000359 | Up | WRKY66 | Probable WRKY transcription factor 66 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|sequence-specific DNA |
| 22 | TraesCS2B02G517400 | 0 | #NAME? | 0.00031 | 0.001865 | Down | WRKY70 | Probable WRKY transcription factor 70 | GO:0000302,GO:00022 | response to reactive oxygen species\|defense response to oomycetes\|DNA |
| 23 | TraesCS2A02G489500 | 0 | #NAME? | 0.000332 | 0.001981 | Down | WRKY70 | WRKY DNA-binding transcription factor 70 | GO:0002215,GO:00037 | defense response to nematode\|DNA-binding transcription factor activity\| |
| 24 | TraesCS3D02G227400 | 8.947326675 | 3.161456691 | 0.013577 | 0.047889 | Up | WRKY22 | WRKY transcription factor 22 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|defense response\|leaf se |
| 25 | TraesCS7A02G343000 | 18.61181625 | 4.218146944 | 0.002679 | 0.01219 | Up | WRKY22 | WRKY transcription factor 22 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|defense response\|leaf se |
| 26 | TraesCS7D02G336400 | 26.13683744 | 4.708012681 | 0.008837 | 0.033461 | Up | WRKY22 | WRKY transcription factor 22 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|defense response\|leaf se |
| 27 | TraesCS1B02G453200 | Inf | Inf | 0.006702 | 0.026578 | Up | WRKY23 | WRKY transcription factor 23 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|response to nematode\|r |
| 28 | TraesCS1D02G429800 | Inf | Inf | 0.003627 | 0.015784 | Up | WRKY23 | WRKY transcription factor 23 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|response to nematode\|r |
| 29 | TraesCS1D02G430000 | 16.89661919 | 4.078662704 | 0.011523 | 0.04175 | Up | WRKY23 | WRKY transcription factor 23 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|response to nematode\|r |
| 30 | TraesCS1A02G421900 | Inf | Inf | 0.00017 | 0.001101 | Up | WRKY28 | WRKY transcription factor 28 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|regulation of cell fate sp |
| 31 | TraesCS1D02G429900 | 26.48450921 | 4.727076869 | 2.42E-06 | 2.34E-05 | Up | WRKY28 | WRKY transcription factor 28 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|regulation of cell fate sp |
| 32 | TraesCS3A02G228600 | 14.24005636 | 3.831882951 | 0.0005 | 0.002845 | Up | WRKY28 | WRKY transcription factor 28 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|regulation of cell fate sp |
| 33 | TraesCS3D02G226300 | Inf | Inf | 5.98E-05 | 0.000433 | Up | WRKY28 | WRKY transcription factor 28 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|regulation of cell fate sp |
| 34 | TraesCS4A02G193600 | 3.686757733 | 1.882352616 | 0.000854 | 0.00454 | Up | WRKY55 | WRKY transcription factor 55 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|sequence-specific DNA |
| 35 | TraesCS4B02G121500 | 19.4838139 | 4.284204203 | 4.65E-09 | 6.93E-08 | Up | WRKY55 | WRKY transcription factor 55 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|sequence-specific DNA |
| 36 | TraesCS4D02G119700 | 3.077732109 | 1.621867662 | 0.002589 | 0.01185 | Up | WRKY55 | WRKY transcription factor 55 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|sequence-specific DNA |
| 37 | TraesCS3A02G167200 | 2.066895838 | 1.047465686 | 0.012277 | 0.044063 | Up | WRKY6 | WRKY transcription factor 6 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|ethylene-activated signa |
| 38 | TraesCS3B02G199000 | 3.041636915 | 1.604847947 | 0.000655 | 0.003597 | Up | WRKY6 | WRKY transcription factor 6 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|ethylene-activated signa |
| 39 | TraesCS3D02G173300 | 3.152043296 | 1.656287351 | 0.000584 | 0.003258 | Up | WRKY6 | WRKY transcription factor 6 | GO:0003700,GO:00056 | DNA-binding transcription factor activity\|nucleus\|ethylene-activated signa |

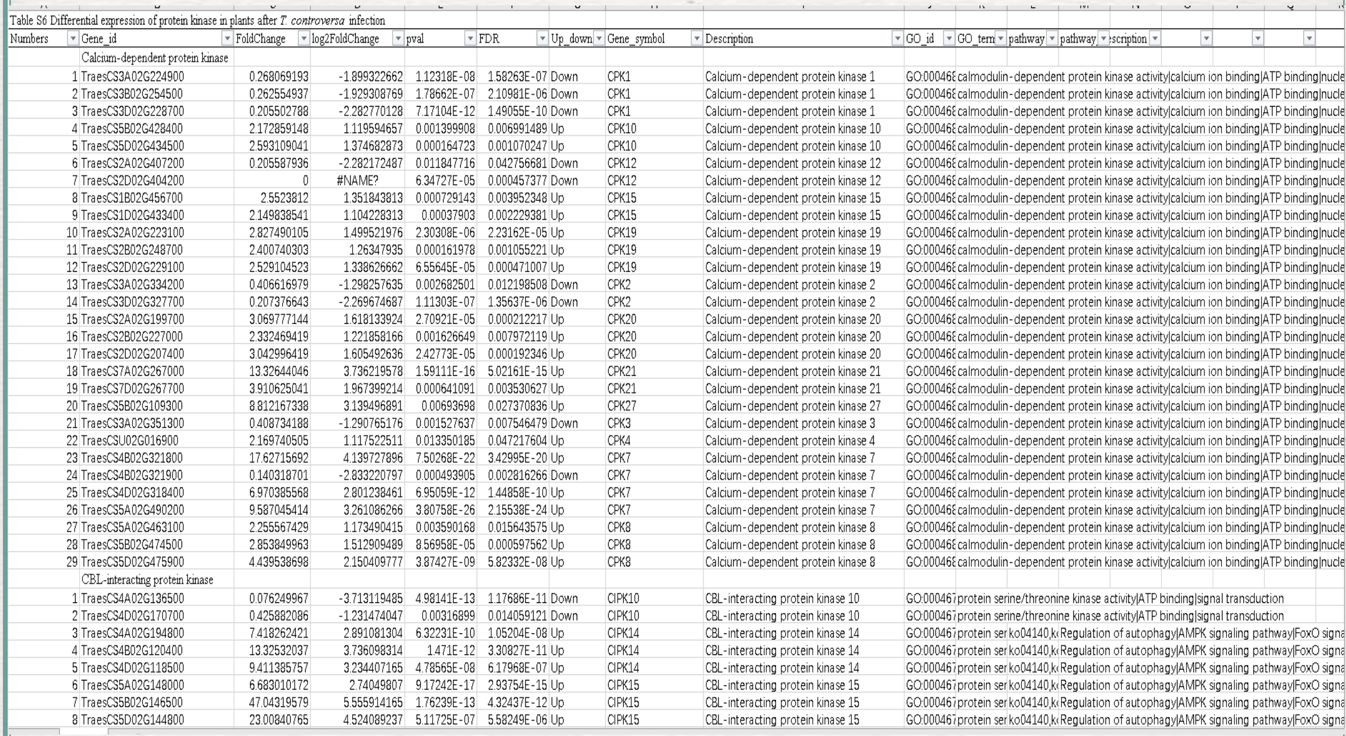


Table S6 Differential expression of protein kinase in plants after *T. controversa* infection

| Numbers | Gene_id | FoldChange | log2FoldChange | pval | FDR | Up_down | Gene_symbol | Description | GO_id | GO_term | pathway | pathway_escription |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Calcium-dependent protein kinase | | | | | | | | | | | |
| 1 | TraesCS3A02G224900 | 0.268069193 | -1.899322662 | 1.12318E-08 | 1.58263E-07 | Down | CPK1 | Calcium-dependent protein kinase 1 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 2 | TraesCS3B02G254500 | 0.262554937 | -1.929308769 | 1.78662E-07 | 2.10981E-06 | Down | CPK1 | Calcium-dependent protein kinase 1 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 3 | TraesCS3D02G228700 | 0.205502788 | -2.282770128 | 7.17104E-12 | 1.49055E-10 | Down | CPK1 | Calcium-dependent protein kinase 1 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 4 | TraesCS5B02G428400 | 2.172859148 | 1.119594657 | 0.001399908 | 0.006991489 | Up | CPK10 | Calcium-dependent protein kinase 10 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 5 | TraesCS5D02G434500 | 2.593109041 | 1.374682873 | 0.000164723 | 0.001070247 | Up | CPK10 | Calcium-dependent protein kinase 10 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 6 | TraesCS2A02G407200 | 0.205587936 | -2.282172487 | 0.011847716 | 0.042756681 | Down | CPK12 | Calcium-dependent protein kinase 12 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 7 | TraesCS2D02G404200 | 0 | #NAME? | 6.34727E-05 | 0.000457377 | Down | CPK12 | Calcium-dependent protein kinase 12 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 8 | TraesCS1B02G456700 | 2.5523812 | 1.351843813 | 0.000729143 | 0.003952348 | Up | CPK15 | Calcium-dependent protein kinase 15 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 9 | TraesCS1D02G433400 | 2.149838541 | 1.104228313 | 0.00037903 | 0.002229381 | Up | CPK15 | Calcium-dependent protein kinase 15 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 10 | TraesCS2A02G223100 | 2.827490105 | 1.499521976 | 2.30308E-06 | 2.23162E-05 | Up | CPK19 | Calcium-dependent protein kinase 19 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 11 | TraesCS2B02G248700 | 2.400740303 | 1.26347935 | 0.000161978 | 0.001055221 | Up | CPK19 | Calcium-dependent protein kinase 19 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 12 | TraesCS2D02G229100 | 2.529104523 | 1.338626662 | 6.55645E-05 | 0.000471007 | Up | CPK19 | Calcium-dependent protein kinase 19 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 13 | TraesCS3A02G334200 | 0.406616979 | -1.298257635 | 0.002682501 | 0.012198508 | Down | CPK2 | Calcium-dependent protein kinase 2 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 14 | TraesCS3D02G327700 | 0.207376643 | -2.269674687 | 1.11303E-07 | 1.35637E-06 | Down | CPK2 | Calcium-dependent protein kinase 2 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 15 | TraesCS2A02G199700 | 3.069777144 | 1.618133924 | 2.70921E-05 | 0.000212217 | Up | CPK20 | Calcium-dependent protein kinase 20 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 16 | TraesCS2B02G227000 | 2.332469419 | 1.221858166 | 0.001626649 | 0.007972119 | Up | CPK20 | Calcium-dependent protein kinase 20 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 17 | TraesCS2D02G207400 | 3.042996419 | 1.605492636 | 2.42773E-05 | 0.000192346 | Up | CPK20 | Calcium-dependent protein kinase 20 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 18 | TraesCS7A02G267000 | 13.32644046 | 3.736219578 | 1.59111E-16 | 5.02161E-15 | Up | CPK21 | Calcium-dependent protein kinase 21 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 19 | TraesCS7D02G267700 | 3.910625041 | 1.967399214 | 0.000641091 | 0.003530627 | Up | CPK21 | Calcium-dependent protein kinase 21 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 20 | TraesCS5B02G109300 | 8.812167338 | 3.139496891 | 0.00693698 | 0.027370836 | Up | CPK27 | Calcium-dependent protein kinase 27 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 21 | TraesCS3A02G351300 | 0.408734188 | -1.290765176 | 0.001527637 | 0.007546479 | Down | CPK3 | Calcium-dependent protein kinase 3 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 22 | TraesCSU02G016900 | 2.169740505 | 1.117522511 | 0.013350185 | 0.047217604 | Up | CPK4 | Calcium-dependent protein kinase 4 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 23 | TraesCS4B02G321800 | 17.62715692 | 4.139727896 | 7.50268E-22 | 3.42995E-20 | Up | CPK7 | Calcium-dependent protein kinase 7 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 24 | TraesCS4B02G321900 | 0.140318701 | -2.833220797 | 0.000493905 | 0.002816266 | Down | CPK7 | Calcium-dependent protein kinase 7 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 25 | TraesCS4D02G318400 | 6.970385568 | 2.801238461 | 6.95059E-12 | 1.44858E-10 | Up | CPK7 | Calcium-dependent protein kinase 7 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 26 | TraesCS5A02G490200 | 9.587045414 | 3.261086266 | 3.80758E-26 | 2.15538E-24 | Up | CPK7 | Calcium-dependent protein kinase 7 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 27 | TraesCS5A02G463100 | 2.255567429 | 1.173490415 | 0.003590168 | 0.015643575 | Up | CPK8 | Calcium-dependent protein kinase 8 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 28 | TraesCS5B02G474500 | 2.853849963 | 1.512909489 | 8.56958E-05 | 0.000597562 | Up | CPK8 | Calcium-dependent protein kinase 8 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| 29 | TraesCS5D02G475900 | 4.439538698 | 2.150409777 | 3.87427E-09 | 5.82332E-08 | Up | CPK8 | Calcium-dependent protein kinase 8 | GO:000468 | calmodulin-dependent protein kinase activity\|calcium ion binding\|ATP binding\|nucle | | |
| | CBL-interacting protein kinase | | | | | | | | | | | |
| 1 | TraesCS4A02G136500 | 0.076249967 | -3.713119485 | 4.98141E-13 | 1.17686E-11 | Down | CIPK10 | CBL-interacting protein kinase 10 | GO:000467 | protein serine/threonine kinase activity\|ATP binding\|signal transduction | | |
| 2 | TraesCS4D02G170700 | 0.425882086 | -1.231474047 | 0.00316899 | 0.014059121 | Down | CIPK10 | CBL-interacting protein kinase 10 | GO:000467 | protein serine/threonine kinase activity\|ATP binding\|signal transduction | | |
| 3 | TraesCS4A02G194800 | 7.418262421 | 2.891081304 | 6.32231E-10 | 1.05204E-08 | Up | CIPK14 | CBL-interacting protein kinase 14 | GO:000467 | protein ser | ko04140,k | Regulation of autophagy\|AMPK signaling pathway\|FoxO signa |
| 4 | TraesCS4B02G120400 | 13.32532037 | 3.736098314 | 1.471E-12 | 3.30827E-11 | Up | CIPK14 | CBL-interacting protein kinase 14 | GO:000467 | protein ser | ko04140,k | Regulation of autophagy\|AMPK signaling pathway\|FoxO signa |
| 5 | TraesCS4D02G118500 | 9.411385757 | 3.234407165 | 4.78565E-08 | 6.17968E-07 | Up | CIPK14 | CBL-interacting protein kinase 14 | GO:000467 | protein ser | ko04140,k | Regulation of autophagy\|AMPK signaling pathway\|FoxO signa |
| 6 | TraesCS5A02G148000 | 6.683010172 | 2.74049807 | 9.17242E-17 | 2.93754E-15 | Up | CIPK15 | CBL-interacting protein kinase 15 | GO:000467 | protein ser | ko04140,k | Regulation of autophagy\|AMPK signaling pathway\|FoxO signa |
| 7 | TraesCS5B02G146500 | 47.04319579 | 5.555914165 | 1.76239E-13 | 4.32437E-12 | Up | CIPK15 | CBL-interacting protein kinase 15 | GO:000467 | protein ser | ko04140,k | Regulation of autophagy\|AMPK signaling pathway\|FoxO signa |
| 8 | TraesCS5D02G144800 | 23.00840765 | 4.524089237 | 5.11725E-07 | 5.58249E-06 | Up | CIPK15 | CBL-interacting protein kinase 15 | GO:000467 | protein ser | ko04140,k | Regulation of autophagy\|AMPK signaling pathway\|FoxO signa |

# Quantitative real-time PCR

To verify the DEGs obtained by RNA-seq, the expression levels of eight genes were examined by quantitative real-time PCR (qPCR). The expression pattern of validated genes was similar to the results obtained from RNA-Seq. The qPCR results showed that seven genes were up-regulated and Lipase was determined to be down-regulated by both RNA-Seq and qRT-PCR analyses. Hence, the qRT-PCR results confirmed the RNA-Seq data.

| Gene ID | Genes annotation | FDR | FPKM | qRT-PCR | Validated |
|---|---|---|---|---|---|
| TraesCS3A02G525700 | Pathogenesis-related protein-1 | 2.67E-03 | Inf (Verr low) | 1.26 ± 0.21 up | Yes |
| TraesCS7D02G351300 | Chitinase 1 | 4.35E-09 | 5.93 up | 4.78 ± 0.32 up | Yes |
| TraesCS1D02G249600 | Chitinase 2 | 3.48E-04 | 5.26 up | 3.21 ± 0.11 up | Yes |
| TraesCS2B02G369000 | Chitinase 4 | 2.62E-07 | 5.26 up | 2.34 ± 0.10 up | Yes |
| TraesCS3D02G227400 | WRKY22 | 4.09E-02 | 5.26 up | 2.33 ± 0.60 up | Yes |
| TraesCS1A02G348600 | WRKY24 | 4.29E-03 | Inf (Verr low) | 0.96 ± 0.07 up | Yes |
| TraesCS1A02G094700 | Lipase | 3.10E-02 | -3.08 down | −2.02 ± 0.02 down | Yes |
| TraesCS1A02G249600 | Endo-1,4-beta-glucanase | 5.37E-03 | 1.09 up | 0.94 ± 0.14 up | Yes |

# Discussion

- In this experiment, we employed RNA-Seq to perform a transcriptomic study of wheat following *T. controversa* infection and identified the DGEs.

- The identification of DGEs of PR genes, WRKY transcription factors and PRRs will help the breeding of disease-resistant varieties.

- Overall, our findings provide a genome-wide gene expression profile for wheat plants infected with *T. controversa* and may help to elucidate the regulatory mechanisms governing the response of wheat to this pathogen.

# Thanks you for your attention!