

Transcriptome analysis revealed that grafting improves the resistance of pepper to *Phytophthora capsici* by fine-tuning growth-defense tradeoff

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Abstract

Grafting has been widely used to defense soil borne diseases and nematodes in vegetables production. However, the underlying mechanism of grafting-improved disease resistance is largely unknown. In this study, grafting cv. 'Ledu' scion to cv. 'Jingxin No. 5' rootstocks improved the resistance of pepper to *Phytophthora capsici*. To gain insights into the regulatory networks related to grafting, we performed transcriptome analysis of grafting and control pepper plants with or without *P. capsici* inoculation. RNA-seq analysis revealed that *P. capsici* infection largely re-programmed the pepper transcriptome and differentially expressed genes (DEGs) functionally annotated to metabolism processes including photosynthesis, response to stimulus, enzyme activity, and transcription were significantly enriched. Furthermore, the expression levels of most DEGs induced by *P. capsici* infection, such as genes functionally related to plant hormone signal transduction, plant-pathogen interaction, photosynthesis, reactive oxygen species, tend to recover to the control levels in grafting pepper plants, which help pepper maintain moderate plant defense response and considerable accumulation level of assimilation product, therefore fine-tuning the dynamic balance between pepper growth-defense tradeoffs. Taken together, our results suggest the dynamic transcriptional programming in grafting pepper that underpin *P. capsici* disease and providing insight that the fine-tuning balance between growth and defense of grafting pepper.

Keywords: *Capsicum annuum*; late blight disease; rootstock; RNA-seq

Introduction

Pepper (*Capsicum* spp., mainly *C. annuum* L.), a member of the Solanaceae family, is an economically and socially important crops worldwide (Penella *et al.*, 2017). In China, the cultivation area of pepper is more

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than 2.1 million ha and the annual output value is over 40 billion US dollars (Zou *et al.*, 2020). As excellent sources of many essential nutrients for humans, the global demand of pepper fruits is growing and how to increase crop production, fruit quality and the plant resistance to environmental stresses has drawn more and more attention (Yin *et al.*, 2021).

Late blight disease, caused by soil borne pathogen *Phytophthora capsici*, is one of the most devastating diseases that seriously threaten the sustainable production of pepper (Shi *et al.*, 2021). Worldwide annually losses due to this disease is more than 100 million dollars (Barchenger *et al.*, 2017). Numerous attempts have been made to improve the disease resistance of pepper crops. Cultural practices such as crop rotation and irrigation management are recommended, but pesticide is generally applied to control the diseases. However, fungicide resistance development and phytotoxicity are problems (Zhang *et al.*, 2019b). Another way to improve pepper resistance to soilborne diseases is resistant breeding, but that is very difficult to achieve and requires much time and effort. Many researchers are focusing on alternative control methods.

Grafting is one of the most popular and valuable technique used to against soil borne diseases and nematodes in vegetables production (Tsaballa *et al.*, 2013). Comparing with time-consuming breeding, grafting is simple, convenience and environmental-friendly. Grafting of pepper has been proved to alleviate negative effect of biotic (e.g., *Phytophthora* blight and bacterial wilt) and abiotic stresses (e.g., salt and low temperature) (Jang *et al.*, 2012; Gilardi *et al.*, 2013). However, until now, pepper grafting has been less exploited in improving pepper resistance since: (1) stresses tolerance pepper genotypes that can be used as stock are still scarcity (Penella *et al.*, 2017), (2) underlying mechanisms about grafting alleviates stress, which is crucial for carrying out more phenotypical screenings of various rootstock-scion combinations (Naegele *et al.*, 2014).

In this study, we found that using cv. 'Jingxin No. 5' as rootstocks can significantly improve the resistance level of cv 'Ledu' scion to *Phytophthora capsici*, the cause agent of pepper late blight. However, physiological and molecular mechanisms involved in scion and pepper rootstock interaction under *Phytophthora* blight stress are still unknown. Thus, in this study, we performed a comparative transcriptome analysis of the grafting and control pepper plants with or without *P. capsici* inoculation, which will to provide guidelines for a more efficient and sustainable use of rootstocks 'Jingxin No. 5' in improving resistance of pepper to *Phytophthora* blight disease.

Materials and Methods

Plant material and pathogen inoculation

Healthy and uniform pepper (*Capsicum annuum*. L) seeds (susceptible cv. 'Ledu' scion and resistant cv. 'Jingxin No. 5' rootstocks, supplied by Qinghai Academy of Agriculture and Forestry Sciences, China) were pre-germinated in an incubator, and then were planted in a solar greenhouse. When seedlings were grown into 4 to 6 leaves stage, seedlings with stem diameter larger than 2 mm were chosen to perform the grafting. The split-grafting was applied to construct hetero-grafted pepper seedlings. Briefly, the upper parts of rootstocks were cut off, with the first pair of true leaves being left, and separate the stem from the middle with a 1 cm notch. The lower part of scion was cut off and three to four true leaves were kept. Then the stem of scion was cut into 1 cm wedge and inserted into the notch of rootstock.

Grafting and nature growing pepper plants were cultured in green house for 20 days and then were used for inoculation and sampling. For pathogen inoculation, *Phytophthora capsici* isolate PcXN1314 was cultured on RSA medium for two weeks, then was washed with cold sterile distilled water to harvest sporangia. Then sporangia suspension was adjusted to 15,000 spores per mL and kept at 4 °C for 2 h to release motile zoospores. The zoospores suspension was sprayed on pepper leaves. Three days after inoculation, three kinds of leaf samples including *P. capsici* inoculation 'Ledu' scion with 'Jingxin No. 5' rootstocks, *P. capsici* inoculation

'Ledu', and control of un-inoculation 'Ledu' were collected for RNA-seq sequencing. Each sample contains three biological replications.

Paired-end strand-specific RNA sequencing

Total RNA was extracted from pepper samples using RNeasy Mini Kit (QIAGEN, Beijing, China) and treated with RNase-free DNase I (Takara, Dalian, China) to degrade residual genomic DNA. RNA quality and quantity were further validated by a Nanodrop 2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Total RNA was treated by the Ribo-Zero rRNA Removal Kit (Epicentre, Madison, WI, USA) to remove rRNA, and then 1 µg of rRNA-depleted total RNA was used to prepare the sequencing library. Sequencing of each library was conducted on the Illumina HiSeq 2500 platform to produce strand specific 150 bp pair-end reads. Reads were deposited in NCBI SRA database under project accession PRJNA728756.

Read mapping and transcriptome assembling

Sequencing raw reads were pre-processed to remove barcode and adaptor sequences, then to remove the low-quality and shorter reads. Bowtie was used to filter out reads belonging to rRNAs. TopHat2 was used to map clean reads to pepper reference genome *Capsicum.annuum*. L_Zunla-1_Release_2.0. Cufflinks was used to assemble transcripts and calculate expression levels represented by FPKM values (fragments per kilobase of exon per million fragments mapped) (Qin *et al.*, 2014). R package gmodels was used to perform principal component analysis (PCA) and R package edgeR was used to identify significantly different expression genes (filtering parameters $FDR < 0.05$ and $|\log_2FC| > 1$) (<http://www.rproject.org/>).

Functional enrichment test of different expression genes (DEGs)

To infer the potential biological functions of DEGs, Blast2GO was firstly used to functional annotate pepper genes. And several commonly used databases including Nr, Pfam, KOG/COG, and Swiss-Prot were also used to perform gene function annotation (Yin *et al.*, 2018b). GO terms and KEGG pathways of the DEGs were manually extracted and submitted to an online tool argriGO to obtain the GO functional clues of these DEGs (Zhu *et al.*, 2019).

Validation of RNA-Seq Data by Quantitative Real-Time PCR (qRT-PCR)

RNA preparation with three biological replicates for each sample was conducted as described above. The first-strand cDNA synthesis was performed using a Prime-Script™ II First Strand cDNA synthesis kit (Takara Bio, Dalian, China) according to the manufacturer's instructions. The primer sets for each gene were designed by Primer Premier 5.0, and their sequences are listed in Supplementary Files S1. qRT-PCR was carried out on an ABI 7500 Real-Time PCR System (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with SYBR Premix Ex Taq™ II kit (Takara). Expression was calculated as $2^{-\Delta\Delta CT}$ and normalized to that of the reference gene *Actin* (Jiang *et al.*, 2021).

Results and Discussion

*Grafting 'Ledu' scion to 'Jingxin No. 5' rootstocks improved the resistance performance of pepper plants to *Phytophthora capsici**

Selection of *P. capsici* resistance cultivars and used them as rootstocks is a promising approach to decrease the negative effects of pathogen on pepper (Gilardi *et al.*, 2013). In this study, we found 'Jingxin No. 5' was a potential rootstock that can be used to improve *P. capsici* resistance. As can be seen in Figure 1, disease symptom appeared in leaves and stems of pepper cultivar 'Ledu' after five days of *P. capsici* spores being inoculated,

suggesting that ‘Ledu’ is a susceptibility cultivar (LDS, ‘Ledu’ susceptibility). In contrast, grafting cv. ‘Ledu’ scion to cv. ‘Jingxin No. 5’ rootstocks can largely improve the resistance to *P. capsici* (GR, grafting resistance).



Figure 1. Grafting enhances the resistance performance of pepper plants
CK, control plant; LDS, cv. ‘Ledu’ susceptibility to *P. capsici*; GR, grafting resistance plant (grafting cv. ‘Ledu’ scion to cv. ‘Jingxin No. 5’ rootstocks).

Summary of RNA-seq data

Although grafting has been reported to improve tolerance to environmental stresses, insights into the molecular regulation mechanisms of grafting have remained future objectives. Furthermore, genes and gene networks underlying resistant improvement mechanisms of grafted pepper to *P. capsici* are still unknown. In this study, through RNA-seq analysis, 887 million 150-nt pair-end raw reads were obtained (Table 1). After low quality reads filtering, ~886 million clean reads (99.90%) were produced and an average of ~98 million reads was obtained for each sample. Using the TopHat2 software with default settings, a total of 703,453,864 reads (79.37%) were successfully mapped to the pepper genome, most of which were uniquely-mapped reads (75.49%). Collectively, 22,072 genes showed expression signal in at least one sample, with the number of expressed genes ranging from 14,481 (LDS-2) to 16,495 (CK-1) (Table 1, Supplementary Files S2). Besides, a total of 30,800 new transcripts were assembled, with the number ranging from 25,850 in CK-2 to 27,106 in CK-3.

Table 1. Overview of RNA-seq data and mapping results

Sample	RNA-seq reads			Expressed genes	
	Raw	Clean (% of raw)	Unique mapped (% of clean)	Known genes ^a	New transcripts ^b
CK-1	105961146	105873258 (99.92%)	79371481 (74.97%)	16495 (46.68%)	26674
CK-2	75729778	75648984 (99.89%)	57697575 (76.27%)	14537 (41.14%)	25850
CK-3	106361480	106246668 (99.89%)	81842756 (77.04%)	15555 (44.02%)	27106
LDS-1	92577412	92501070 (99.92%)	70528216 (76.25%)	15989 (45.25%)	26651
LDS-2	107816886	107688840 (99.88%)	79007886 (73.37%)	14481 (40.98%)	25928
LDS-3	104151762	104038326 (99.89%)	78663110 (75.62%)	15890 (44.97%)	26364
GR-1	71297300	71211298 (99.88%)	53746784 (75.49%)	15302 (43.30%)	26184
GR-2	121011522	120906480 (99.91%)	90362253 (74.74%)	15292 (43.28%)	26706
GR-3	102311614	102197462 (99.89%)	78513865 (76.83%)	15827 (44.79%)	26871
Total	887218900	886312386 (99.90%)	669733926 (75.49%)	22072 (62.46%)	30800

^a Assembled genes belonging to known 35,335 genes reported by (Qin *et al.*, 2014). ^b new transcripts assembled in this study.

Gene expression profiles and qRT-PCR validation

To validate the repeatability between biological replications and difference between treatments, principal component analysis (PCA) was performed (Figure 2A) (Yin *et al.*, 2018b). The result showed that the first and second principal components (PC1 and PC2) accounted for 70.9 and 16.3% variability in the RNA-Seq dataset. Samples belong to three treatments were separately distributed and three biological replications belong to same treatment were clustered together, indicating a stable and distinct response of pepper plants to corresponding treatment. Then, qRT-PCR was conducted on a randomly selected seven genes to confirm the expression profiling of RNA-seq data. Results showed that the gene expression patterns determined by qRT-PCR were fairly consistent with that of RNA-seq analysis ($R^2 = 0.96$, Figure 2B, 2C), which confirmed the reliability of RNA-seq.

To systematically explore the transcriptomic dynamics, we conducted pair-wised comparisons between different treatments of interest (Figure 2D) and differentially expressed genes (DEGs) were identified. Totally, 8,861 DEGs genes were identified in different pairs of treatments, of which 2,990, 6,381 and 3,798 were found between LDS and GR, CK and LDS, and CK and GR, respectively (Figure 2D). We then analyzed the putative functions of these DEGs based on their associated annotations.

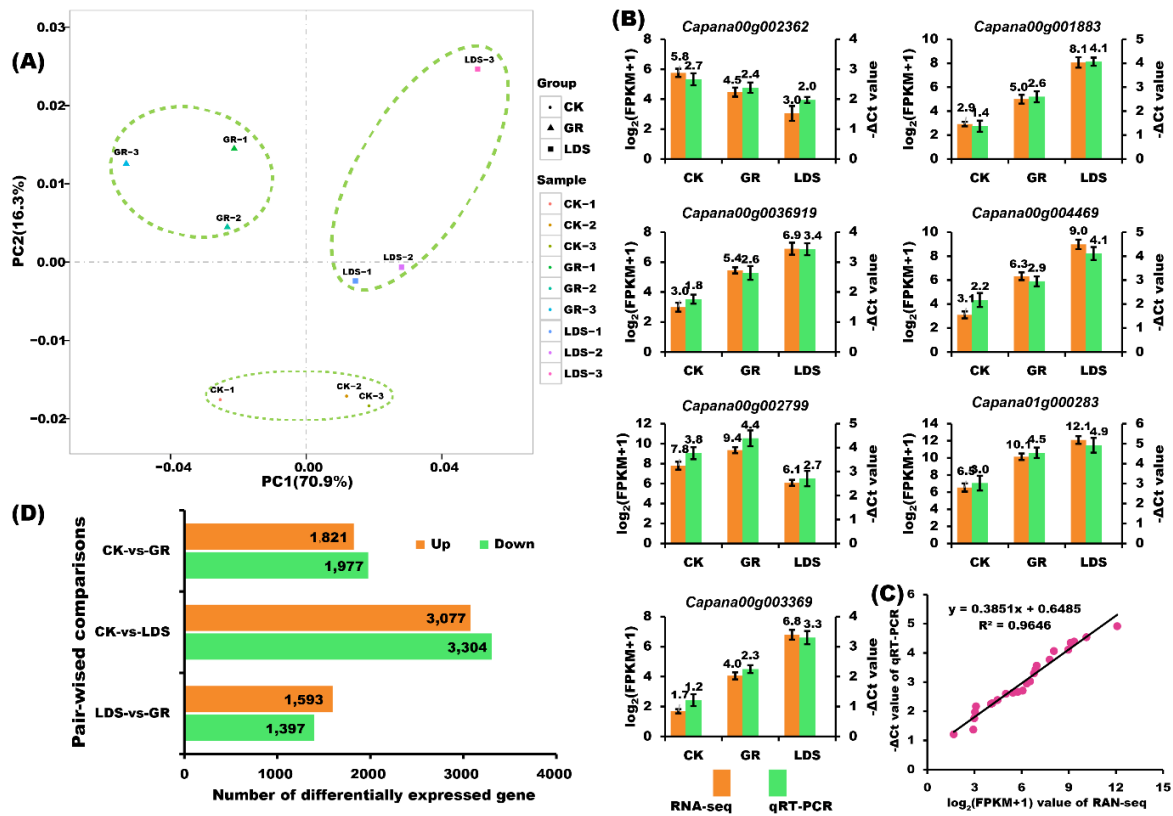


Figure 2. RNA-seq data and genes expression analysis (A) Principal component analysis. (B) Gene expression patterns determined by RNA-seq and qRT-PCR. *Capana00g002362*, transporter precursor chloroplast phosphate; *Capana00g001883*, tetraspanin-8-like; *Capana00g003691*, NHL domain-containing protein; *Capana00g004469*, PAR1 protein; *Capana00g002799*, chlorophyll a-b binding protein 1B; *Capana01g000283*, PR5-like protein; *Capana00g003369*, diacylglycerol kinase. (C) Correlation analysis between log₂(FPKM+1) values of RNA-seq and -ΔCt value of qRT-PCR. (D) Number of differentially expressed genes.

Transcriptomic response to P. capsici infection

Disease affects the photosynthesis of plant and induces the expression of genes that are functionally associated with stress response. The effects of both biotic and abiotic stresses on pepper plants include a wide range of physiological, metabolic and genomic changes that provoke alterations in photosynthesis, reactive oxygen species (ROS) accumulation, and an imbalance uptake of nutrients (Penella *et al.*, 2017). In this study, comparing to CK, *P. capsici* infection (LDS) caused thousands of genes up-/down-regulated (3,304/3,077) in the leaves (Figure 1D). Most different expression genes were annotated to metabolic process, catalytic activity, binding, cellular process, single-organism process, and cell part GO terms (Figure 3A). Interestingly, most significant enrichment GO terms belonging to cellular component were associated with photosynthesis, including plastid thylakoid (GO:0031976), plastid envelope (GO:0009526), chloroplast part (GO:0044434), and chloroplast thylakoid (GO:0044434) (Figure 3B). Consistent with the pathogen infection, genes belonging to response to stimulus (GO:0050896) was significant enriched. And genes associated with biosynthetic (GO:0006779 porphyrin-containing compound biosynthetic process), energy (GO:0006739 NADP metabolic process), and plastid membrane organization (GO:0009668) were also significant enriched, reflecting the main metabolism changes of pepper after infection by pathogen. GO and KEGG analysis of these DEG genes suggested that molecular functions such as transcription (GO:0001071 nucleic acid binding transcription factor activity), lyase (GO:0016830 carbon-carbon lyase activity), hydrolase (GO:0004553 hydrolase activity, hydrolyzing O-glycosyl compounds), oxidoreductase activity (GO:0016491), and antioxidant activity (GO:0016209) were also significant enriched, reflecting the main changes of metabolism processes after infection by pathogen (Figure 3C).

Grafting largely changes transcriptomic response of pepper to P. capsici infection

To reveal the effect of grafting on the pepper transcriptome under *P. capsici* infection, samples were analyzed and 1,397 and 1,593 up- and down-regulated DEGs were identified (Figure 2D). Most DEGs were annotated to metabolic process, catalytic activity, binding, cellular process, single-organism process, and cell part GO terms (Figure 4A). Under the cellular component category, different expression genes were mainly significantly enriched to GO terms related to photosystem (GO:0009521), chloroplast part (GO:0044434), and plastid thylakoid (GO:0031976), implying the considerable change of photosystem between LDS and GR pepper plants (Figure 4B). As to biological process category, GO terms related to metabolism and energy were significantly enriched, including generation of precursor metabolites and energy (GO:0006091), NADP metabolic process (GO:0006739), sulfur amino acid biosynthetic process (GO:0000097), and porphyrin-containing compound biosynthetic process (GO:0006779). Under molecular function category, several catalytic activities were enriched, such as hydrolase (GO:0016798, hydrolase activity, acting on glycosyl bonds), lyase (GO:0016835, carbon-oxygen lyase activity), kinase (GO:0004672, protein kinase activity), transferase (GO:0046912, transferase activity, transferring acyl groups, acyl groups converted into alkyl on transfer), oxidoreductase (GO:0016627, oxidoreductase activity, acting on the CH-CH group of donors). Besides, nucleic acid binding transcription factor activity (GO:0001071) and tetrapyrrole binding (GO:0046906) were also significantly enriched. GO and KEGG analysis suggested that carbon metabolism, photosynthesis, plant hormone signal transduction associated processes and pathways represent the main transcriptome changes between the resistance GR and susceptibility LDS pepper (Figure 4B, 4C).

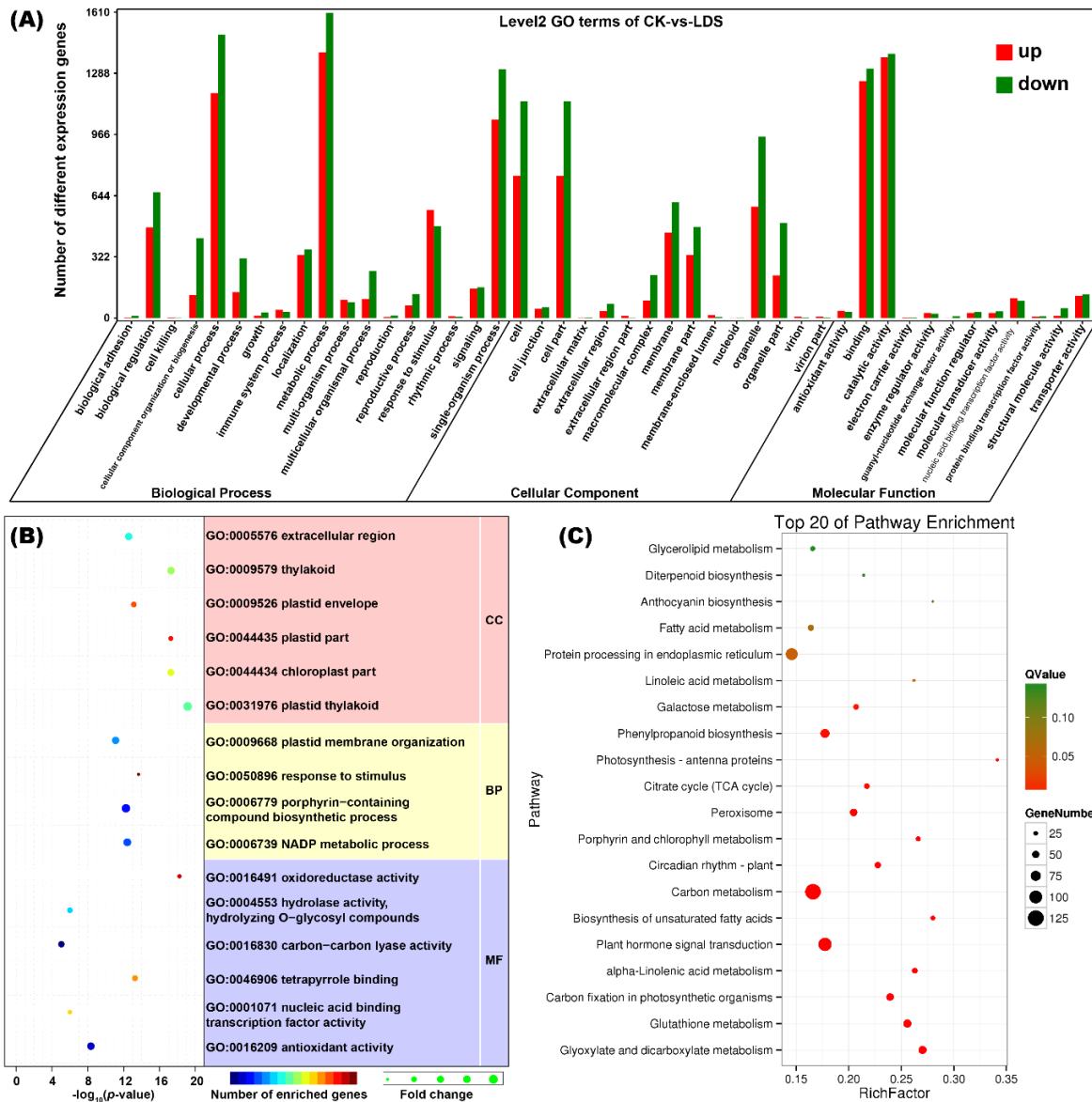


Figure 3. Enrichment of different expression genes between CK and LDS (A) GO terms statistics of different expression genes. (B) Enrichment of specific GO terms. (C) Enrichment of specific KEGG pathways.

Resistance-improvement mechanisms of grafting

To better unravel the regulating effect of grafting under pathogen stress, we extracted DEGs between CK and LDS samples, and explored their expression patterns in GR samples. Interestingly, the average magnitude of the increased expression levels for the up-regulated DEGs (CK-vs-LDS) was decreased 51.46% in comparison with the expression data in GR samples. Consistently, the averaged amplitude of change for the down-regulated DEGs (CK-vs-LDS) was decreased 58.16% in grafting plants under pathogen inoculation (Figure 5A). This result indicated that, after grafting cv. ‘Ledu’ scion to cv. ‘Jingxin No. 5’ rootstocks, the expression levels of most of the DEGs induced by *P. capsici* infection showed the trend to recover to the CK expression levels in GR.

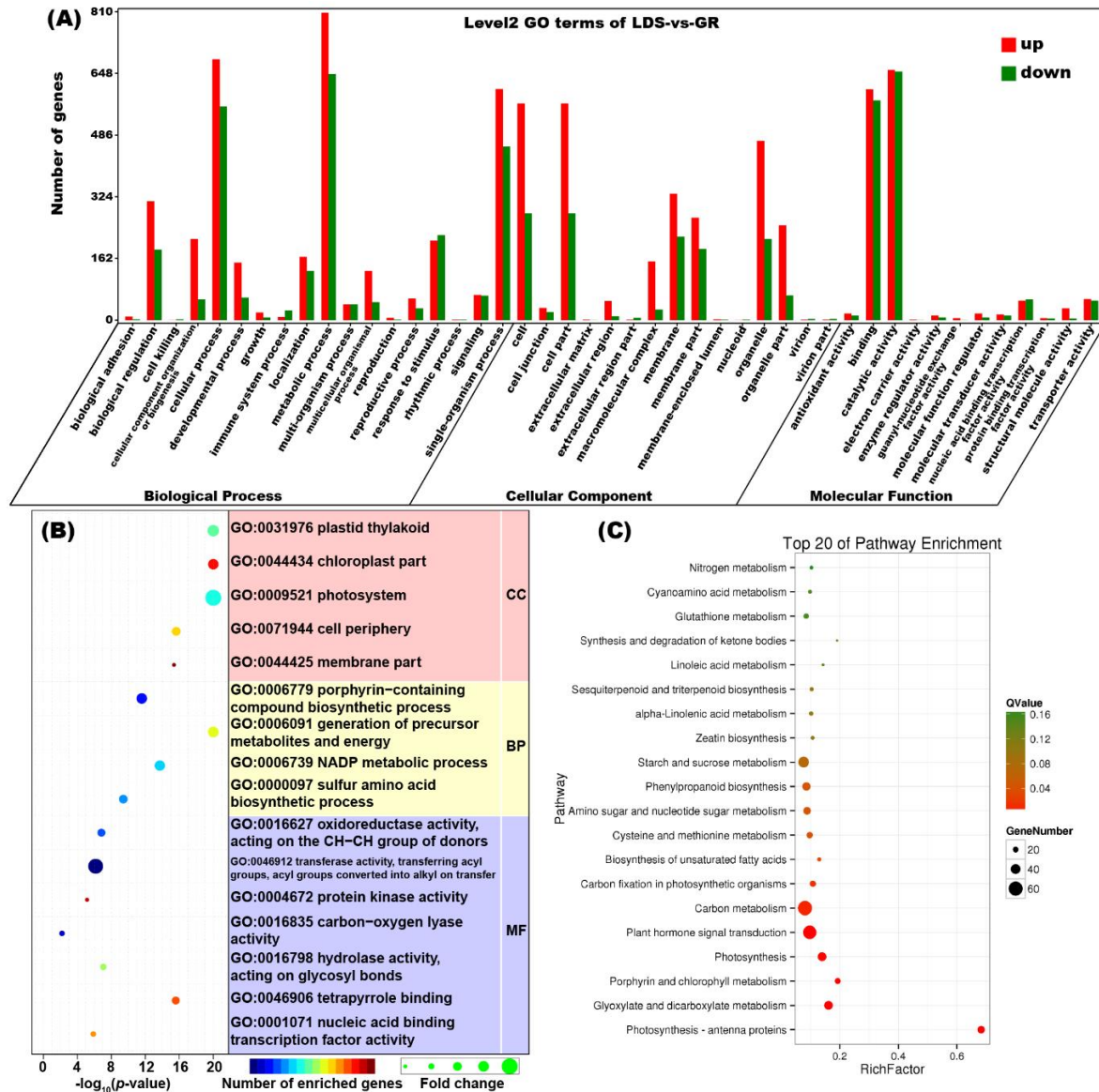


Figure 4. Enrichment of different expression genes between LDS and GR (A) GO terms statistics of different expression genes. (B) Enrichment of specific GO terms. (C) Enrichment of specific KEGG pathways.

To clearly demonstrate the dynamic change, DEGs belonging to KO pathways including Plant-pathogen interaction (ko04626), Plant hormone signal transduction (ko04075), Oxidative phosphorylation (ko00190), Photosynthesis (ko00195) and DEGs functionally related to Reactive Oxygen Species scavenging were specifically extracted and analyzed. In general, the expression levels of most DEGs, no matter up- or down-regulated genes in LDS, tend to back to CK level in GR (Figure 5B-5F) (For convenience, these up- or down-regulated CK-vs-LDS DEGs showing tendency to back to CK expression level in GR were defined as UpToNormal or DownToNormal patterns).

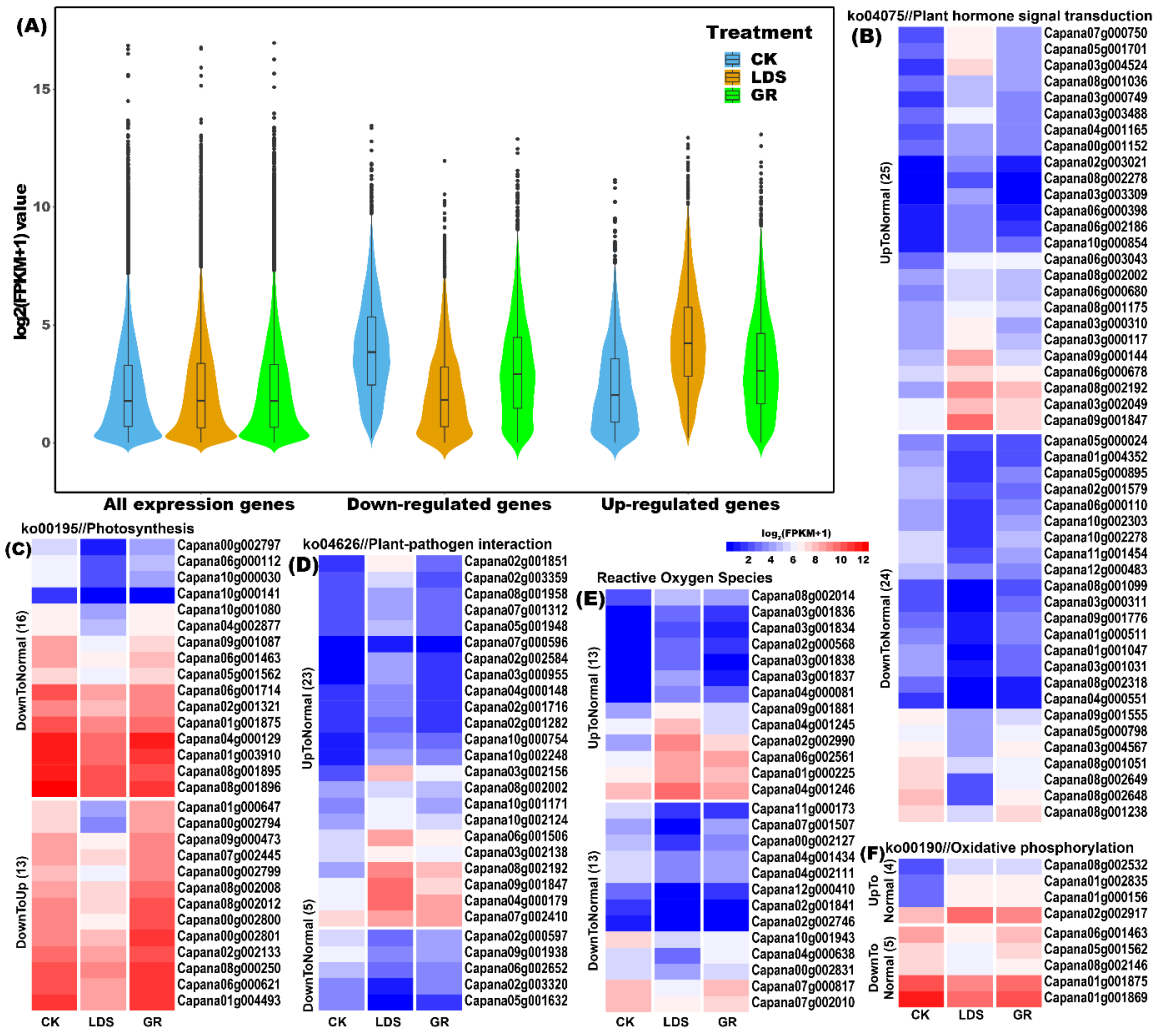


Figure 5. Effect of grafting on the expression dynamics of genes responded to *P. capsici* infection

(A) Overview dynamic change of all expression genes and DEGs. (B, C, D, F) Dynamic change of DEGs belonging to corresponding KO pathway. (E) Peroxidase, catalase, ascorbate peroxidase, cationic peroxidase, and peroxiredoxin DEGs involving in Reactive Oxygen Species scavenging, DownToNormal, down-regulated gene in LDS and expression level tend to back to CK level in GR. Similar as UpToNormal. DownToUp, down-regulated gene in LDS and expression level higher than CK in GR.

Plant-pathogen interaction (ko04626) pathway comprise genes involved in plant immunity and pathogen infection, which is the basis for effective regulation of plant defense systems and successful resistance to pathogens (Zhang *et al.*, 2019a). WRKYs are transcription factors involved in plant stress resistance under both biotic and abiotic stresses (Zhu *et al.*, 2019). In this study, WRKY transcription factors *Capana06g001506* and *Capana10g000754* showed “UpToNormal” pattern, which were up-regulated in both LDS and GR, whereas the expression level in GR tend to back to the CK level (Figure 5D). *Capana10g000754* is homologue to Arabidopsis *WRKY22*, which was rapidly and strongly induced upon submergence and insertion mutants showed lower resistance to *Pseudomonas syringae* (Hsu *et al.*, 2013). In this study, *Capana10g000754* was 8-fold up-regulated in LDS and 5-fold up-regulated in GR, whereas the up-regulated expression level was decreased 40% in GR comparing to LDS. Besides, calcium-binding protein CMLs are a group of Ca²⁺-dependent proteins that plays essential role in stress responses in the pathway of plant-pathogen interaction (ko04626) (Mo *et al.*, 2018). Many lines of genes involved in calcium signaling were significantly up-regulated under stress conditions. For example, CML45 was up-regulated by exogenous resistance inducer

in rice (An *et al.*, 2019). CML49 was overexpressed in chili pepper after infected by *Polyphagotarsonemus latus* (Patavardhan *et al.*, 2020). Here, four calcium-binding protein, *Capana02g003359* (homologue to CML45), *Capana10g002248* (homologue to CML49), *Capana03g000955*, and *Capana10g001171* showed “UpToNormal” patterns. Arabidopsis CML18 was reported to be involved in salt stress signaling (Yamaguchi *et al.*, 2005). *Capana02g000597*, homologue to CML18, was down-regulated in LDS and showed back to CK levels in GR (DownToNormal pattern).

It is well-known that hormones, such as ethylene (ET), jasmonic acid (JA), and salicylic acid (SA), are essential for plant immunity. These hormones play important roles in plant growth and development, as well as plant disease resistance (Zhou *et al.*, 2018; Zhu *et al.*, 2019). Here, 49 DEGs were functionally annotated to Plant hormone signal transduction (ko04075), including 25 up-regulated and 24 down-regulated genes that showed the regulation patterns of back to CK in GR. Ethylene-responsive transcription factors (ERF) play key roles in crop resistance to *Phytophthora* pathogens. For example, *GmERF113* increased resistance of soybean to *P. sojae* (Zhao *et al.*, 2017), while *StERF3* negatively regulated resistance of potato to *P. infestans* (Tian *et al.*, 2015), *NbERF173* increasing resistance of tobacco to *P. parasitica* (Yu *et al.*, 2020), *CaAP2/ERF064* inducing cell death and increasing resistance of pepper to *P. capsici* (Jin *et al.*, 2019). Consistently, in this study, we found three ERFs, *Capana03g003309*, *Capana03g004524*, and *Capana05g001701*, were significantly up-regulated in LDS and showed “UpToNormal” regulation pattern in GR (Figure 5B).

Oxidative phosphorylation plays a central role in the energy metabolism of plants (Farhat *et al.*, 2019). It provides most of the ATP for plants to support life, and provide energy for plant stress responses (Wilson, 2017). Here we found four up-regulated and five down-regulated genes were functionally annotated to Oxidative phosphorylation (ko00190) pathway (Figure 5F). Their expression levels in GR sample also showed the tendency to back to the expression level in CK, especially for these genes functionally associated with ATP synthase like *Capana05g001562*, *Capana06g001463*, and *Capana01g001875*, implying the recovery of energy metabolism in grafting pepper.

In higher plants, the production of reactive oxygen species (ROS) is a general defense event during plant-pathogen interactions (Camejo *et al.*, 2016). Following pathogen infection, genes encoding peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), cationic peroxidase, and peroxiredoxin (Prx) comprise an efficient defense system to achieve successful disease resistance (Mo *et al.*, 2018). Here, we found 26 DEGs, 13 up- and 13 down-regulated genes, showed the “UpToNormal” and “DownToNormal” expression patterns in GR pepper plants, which was presumably related to fine-tuning the ROS event (Figure 5E).

Robust defense response is efficient against biotrophic pathogens, whereas it does not protect plants against necrotrophic pathogens infection (Govrin and Levine, 2000). Take the hypersensitive cell death as an example, it was widely employed by plant in responding to pathogen infection (Yin *et al.*, 2018a). However, those necrotrophic fungi can exploit this host defense mechanism for their pathogenicity, which facilitate the colonization of pathogen in host plants (Govrin and Levine, 2000). *P. capsici* is a hemi-biotrophic pathogen, and the biotrophy to necrotrophy transition will happen after 24 to 48 hours of infection (Jupe *et al.*, 2013). In this study, large ratio of LDS-vs-CK DEGs were functionally annotated to Plant-pathogen interaction (ko04626), Plant hormone signal transduction (ko04075), Oxidative phosphorylation (ko00190), and Reactive Oxygen Species. Consistently, Jupe *et al.* (2013) reported that large number of genes related to defense response were differentially regulated during the transition, indicating the distinct transcriptional responses of the host that accompanies the initiation of necrotrophy by *P. capsici*, implying a pathogen-derived cue that causes host cell death. However, in this study, it should be noticed that these LDS-vs-CK DEGs generally showed “UpToNormal” (UpBackNormal) and “DownToNormal” expression patterns in grafting resistance pepper plants, which may due to the reason that grafting helps to maintain the balance between moderate plant defense response and host cell death, thus prevent the transition from biotrophy to necrotrophy and finally fine-tuning the resistance performance of grafting pepper.

P. capsici infection commonly cause foliar chlorosis and necrosis, therefore seriously affect leaf photosynthesis (Zhao *et al.*, 2011). Consistently, in this study, 29 DEGs functionally annotated to Photosynthesis (ko00195) were identified and all of them were down-regulated in LDS, implying the photosynthesis was decreased after infection. In contrast, among the 29 DEGs, 16 genes showed the tendency back to the CK expression level in GR. In particular, 13 DEGs showed higher expression levels in GR than CK (Figure 5C) (Defined as DownToUp expression patterns), suggesting that photosynthesis was largely recovered in grafting pepper under *P. capsici* infection, thus maintained a considerable accumulation level of assimilation product in pepper to support growth and defense response under *P. capsici* infection.

Growth-defense tradeoffs are thought to occur in plants due to resource restrictions, which demand prioritization towards either growth or defense, depending on external and internal factors (Huot *et al.*, 2014).

Plants have evolved a precise mechanism for fine-tuning the balance between growth and defense, which contributes to survival under adverse conditions (Li *et al.*, 2019). Interestingly, in this study, we found grafting help pepper maintain moderate plant defense response and maintain considerable accumulation level of assimilation product, thus fine-tuning the dynamic balance between growth and defense tradeoffs.

Conclusions

This study showed that grafting cv. ‘Ledu’ scion to cv. ‘Jingxin No. 5’ rootstocks could largely improve the resistance performance of pepper to *P. capsici*, indicating that ‘Jingxin No. 5’ is a appreciate rootstocks for control late blight disease by grafting. RNA-seq analysis suggested the dynamic transcriptional re-programming of grafting pepper underpin *P. capsici* disease. Further analysis revealed that grafting improves the resistance performance of pepper to *P. capsici* by fine-tuning the growth-defense tradeoffs to maintain the balance between moderate plant defense response and considerable accumulation level of assimilation product.

Authors’ Contributions

W.R. X., Q.Y. G., J.L. Y, L. H., and L.P. W. conceived the study, designed the experiments, and analyzed data. L. H., J.L. Y., L.P. W., and J.H. Y. conducted the experiments, prepared the samples, and analyzed data. J.L. Y conducted the RNA-seq data analyses. J.L. Y wrote the first draft, J.L. Y and L. H. finalized the manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- An M, Zhou T, Guo Y, Zhao X, Wu Y (2019). Molecular regulation of host defense responses mediated by biological Anti-TMV agent Ningnanmycin. *Viruses* 11:9. <https://doi.org/10.3390/v11090815>
- Barchenger DW, Lamour KH, Sheu ZM, Shrestha S, Kumar S, Lin SW, ... Bosland PW (2017). Intra- and intergenomic variation of ploidy and clonality characterize *Phytophthora capsici* on *Capsicum* sp. in Taiwan. *Mycological Progress* 16:955-963. <https://doi.org/10.1007/s11557-017-1330-0>
- Camejo D, Guzman Cedeno A, Moreno A (2016). Reactive oxygen species, essential molecules, during plant-pathogen interactions. *Plant Physiology and Biochemistry* 103:10-23. <https://doi.org/10.1016/j.plaphy.2016.02.035>
- Farhat N, Hichri S, Hildebrandt TM, Debez A, Braun HP (2019). Composition and stability of the oxidative phosphorylation system in the halophile plant *Cakile maritima*. *Frontiers in Plant Science* 10. <https://doi.org/10.3389/fpls.2019.01010>
- Gilardi G, Baudino M, Moizio M, Pugliese M, Garibaldi A, Gullino ML (2013). Integrated management of *Phytophthora capsici* on bell pepper by combining grafting and compost treatment. *Crop Protection* 53:13-19. <https://doi.org/10.1016/j.cropro.2013.06.008>
- Govrin EM, Levine A (2000). The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. *Current Biology* 10:751-757. [https://doi.org/10.1016/S0960-9822\(00\)00560-1](https://doi.org/10.1016/S0960-9822(00)00560-1)
- Hsu FC, Chou MY, Chou SJ, Li YR, Peng HP, Shih MC (2013). Submergence confers immunity mediated by the WRKY22 transcription factor in *Arabidopsis*. *Plant Cell* 25:2699-2713. <https://doi.org/10.1105/tpc.113.114447>
- Huot B, Yao J, Montgomery BL, He SY (2014). Growth-defense tradeoffs in plants: a balancing act to optimize fitness. *Molecular Plant* 7:1267-1287. <https://doi.org/10.1093/mp/ssu049>
- Jang Y, Yang E, Cho M, Um Y, Ko K, Chun C (2012). Effect of grafting on growth and incidence of Phytophthora blight and bacterial wilt of pepper (*Capsicum annuum* L.). *Horticulture Environment and Biotechnology* 53:9-19. <https://doi.org/10.1007/s13580-012-0074-7>
- Jiang X, Yin J, Wang L, Xi K, Zhu X, Li G, ... Liu Y (2021). Identification and evolutionary analysis of the metal-tolerance protein family in eight Cucurbitaceae species. *The Plant Genome* e20167. <https://doi.org/10.1002/tpg2.20167>
- Jin JH, Zhang HX, Ali M, Wei AM, Luo DX, Gong ZH (2019). The CaAP2/ERF064 regulates dual functions in pepper: plant cell death and resistance to *Phytophthora capsici*. *Genes* 10:541. <https://doi.org/10.3390/genes10070541>
- Jupe J, Stam R, Howden A, Morris J, Zhang R, Hedley P, ... Huitema E (2013). *Phytophthora capsici*-tomato interaction features dramatic shifts in gene expression associated with a hemi-biotrophic lifestyle. *Genome Biology* 14:R63. <https://doi.org/10.1186/gb-2013-14-6-r63>
- Li Y, Yang Y, Hu Y, Liu H, He M, Yang Z, ... Hou X (2019). DELLA and EDS1 form a feedback regulatory module to fine-tune plant growth-defense tradeoff in *Arabidopsis*. *Molecular Plant* 12:1485-1498. <https://doi.org/10.1016/j.molp.2019.07.006>
- Mo Z, Feng G, Su W, Liu Z, Peng F (2018). Transcriptomic analysis provides insights into grafting union development in pecan (*Carya illinoensis*). *Genes* 9. <https://doi.org/10.3390/genes9020071>
- Naegle RP, Boyle S, Quesada Ocampo LM, Hausbeck MK (2014). Genetic diversity, population structure, and resistance to *Phytophthora capsici* of a worldwide collection of eggplant germplasm. *Plos One* 9:e95930. <https://doi.org/10.1371/journal.pone.0095930>
- Patavardhan SS, Subba P, Najar A, Awasthi K, D'Souza L, Prasad TSK, ... Nivas SK (2020). Plant-pathogen interactions: broad mite (*Polyphagotarsonemus latus*)-induced proteomic changes in chili pepper plant (*Capsicum frutescens*). *Omics* 24:714-725. <https://doi.org/10.1089/omi.2020.0080>
- Penella C, Nebauer SG, López Galarza S, Quiñones A, San Bautista A, Calatayud Á (2017). Grafting pepper onto tolerant rootstocks: An environmental-friendly technique overcome water and salt stress. *Scientia Horticulturae* 226:33-41. <https://doi.org/10.1016/j.scienta.2017.08.020>

- Qin C, Yu C, Shen Y, Fang X, Chen L, Min J, ... Zhang Z (2014). Whole-genome sequencing of cultivated and wild peppers provides insights into *Capsicum* domestication and specialization. *Proceedings of the National Academy of Sciences* 111:5135-5140. <https://doi.org/10.1073/pnas.1400975111>
- Shi J, Ye W, Ma D, Yin J, Zhang Z, Wang Y, ... Qiao Y (2021). Improved whole genome sequence of *Phytophthora capsici* generated by long-read sequencing. *Molecular Plant-Microbe Interactions* 34:866-869. <https://doi.org/10.1094/MPMI-12-20-0356-A>
- Tian Z, He Q, Wang H, Liu Y, Zhang Y, Shao F, ... Xie C (2015). The potato ERF transcription factor StERF3 negatively regulates resistance to *Phytophthora infestans* and salt tolerance in potato. *Plant and Cell Physiology* 56:992-1005. <https://doi.org/10.1093/pcp/pcv025>
- Tsaballa A, Athanasiadis C, Pasentsis K, Ganopoulos I, Nianiou Obeidat I, Tsaftaris A (2013). Molecular studies of inheritable grafting induced changes in pepper (*Capsicum annuum*) fruit shape. *Scientia Horticulturae* 149:2-8. <https://doi.org/10.1016/j.scienta.2012.06.018>
- Wilson DF (2017). Oxidative phosphorylation: regulation and role in cellular and tissue metabolism. *Journal of Physiology* 595:7023-7038. <https://doi.org/10.1113/JP273839>
- Yamaguchi T, Aharon GS, Sottosanto JB, Blumwald E (2005). Vacuolar Na⁺/H⁺ antiporter cation selectivity is regulated by calmodulin from within the vacuole in a Ca²⁺-and pH-dependent manner. *Proceedings of the National Academy of Sciences* 102:16107-16112. <https://doi.org/10.1073/pnas.0504437102>
- Yin J, Gu B, Huang G, Tian Y, Quan J, Lindqvist-Kreuzer H, ... Shan W (2018a). Conserved RXLR effector genes of *Phytophthora infestans* expressed at the early stage of potato infection are suppressive to host defense. *Frontiers in Plant Science* 8:2155. <https://doi.org/10.3389/fpls.2017.02155>
- Yin J, Yan J, Hou L, Jiang L, Xian W, Guo Q (2021). Identification and functional deciphering suggested the regulatory roles of long intergenic ncRNAs (lincRNAs) in increasing grafting pepper resistance to *Phytophthora capsici*. *BMC Genomics* 22:868. <https://doi.org/10.1186/s12864-021-08183-z>
- Yin JL, Liu MY, Ma DF, Wu JW, Li SL, Zhu YX, ... (2018b). Identification of circular RNAs and their targets during tomato fruit ripening. *Postharvest Biology and Technology* 136:90-98. <https://doi.org/10.1016/j.postharvbio.2017.10.013>
- Yu J, Chai C, Ai G, Jia Y, Liu W, Zhang X, ... Dou D (2020). A *Nicotiana benthamiana* AP2/ERF transcription factor confers resistance to *Phytophthora parasitica*. *Phytopathology Research* 2:4. <https://doi.org/10.1186/s42483-020-0045-3>
- Zhang L, Shang J, Wang W, Du J, Li K, Wu X, ... Yang W (2019a). Comparison of transcriptome differences in soybean response to soybean mosaic virus under normal light and in the shade. *Viruses* 11:793. <https://doi.org/10.3390/v11090793>
- Zhang P, Zhu Y, Ma D, Xu W, Zhou J, Yan H, ... Yin J (2019b). Screening, identification, and optimization of fermentation conditions of an antagonistic endophyte to wheat head blight. *Agronomy* 9:476. <https://doi.org/10.3390/agronomy9090476>
- Zhao D, Glynn NC, Glaz B, Comstock JC, Sood S (2011). Orange rust effects on leaf photosynthesis and related characters of sugarcane. *Plant Disease* 95:640-647. <https://doi.org/10.1094/pdis-10-10-0762>
- Zhao Y, Chang X, Qi D, Dong L, Wang G, Fan S, ... Zhang S (2017). A novel soybean ERF transcription factor, GmERF113, increases resistance to *Phytophthora sojae* infection in soybean. *Frontiers in Plant Science* 8:299. <https://doi.org/10.3389/fpls.2017.00299>
- Zhou R, Zhu YX, Zhao J, Fang ZW, Wang SP, Yin JL, ... Ma D (2018). Transcriptome-wide identification and characterization of potato circular RNAs in response to *Pectobacterium carotovorum* subspecies *brasiliense* infection. *International Journal of Molecular Sciences* 19:71. <https://doi.org/10.3390/ijms19010071>
- Zhu Y, Yin J, Liang Y, Liu J, Jia J, Huo H, ... Gong H (2019). Transcriptomic dynamics provide an insight into the mechanism for silicon-mediated alleviation of salt stress in cucumber plants. *Ecotoxicology and Environmental Safety* 174:245-254. <https://doi.org/10.1016/j.ecoenv.2019.02.075>
- Zou X, Ma Y, Dai X, Li X, Li S (2020). Spread and industry development of pepper in China. *Acta Horticulturae Sinica* 47:1715-1726. <https://doi.org/10.16420/j.issn.0513-353x.2020-0103>



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