Research Square

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Transcriptome analysis of scions grafted to potato rootstock for improving late blight resistance

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Abstract

Background: Late blight seriously threatens potato cultivation worldwide. The severe and widespread damage caused by the fungal pathogen can lead to drastic decreases in potato yield. Although grafting technology has been widely used to improve crop resistance, the effects of grafting on potato late blight resistance as well as the associated molecular mechanisms remain unclear. Therefore, we performed RNA transcriptome sequencing analysis and the late blight resistance testing of the scion when the potato late blight-resistant variety Qingshu 9 and the susceptible variety Favorita were used as the rootstock and scion, respectively, and *vice versa.* The objective of this study was to evaluate the influence of the rootstock on scion disease resistance and to clarify the related molecular mechanisms.

Results: A Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis revealed that the expression levels of genes related to plant–pathogen interactions, plant mitogen-activated protein kinase (MAPK) signaling pathways, and plant hormone signal transduction pathways were significantly up-regulated in the scion when Qingshu 9 was used as the rootstock. These genes included late blight response genes encoding calcium-dependent protein kinases (CDPKs), chitin elicitor receptor kinases (CERKs), LRR receptor serine/threonine protein kinases (LRR-LRKs), NPR family proteins in the salicylic acid synthesis pathway, and MAPKs. When Favorita was used as the rootstock, the expression levels of the late blight response genes were not up-regulated in the Qingshu 9 scion, but the expression levels of the genes related to proline metabolism, fatty acid chain elongation, and diterpenoid biosynthesis pathways were down-regulated. Resistance results showed that self-grafting of the susceptible variety and grafting with the resistant variety as the rootstock increased the resistance of the susceptible scion to late blight. However, the resistance was stronger after grafting with the resistant variety as the rootstock. Using the susceptible variety as the rootstock decreased the late blight resistance of the resistant scion.

Conclusions: Our results showed that changes to the expression of disease resistance genes in the scion after grafting are associated with late blight resistance. The results provide the basis for exploring the molecular mechanism underlying the effects of rootstocks on scion disease resistance.

Background

Potato (*Solanum tuberosum* L.), which is an annual solanaceous herb native to the Andes in South America, was introduced to China in the 17th century and has become the fourth most important food crop in the world. Globally, China is the primary potato-producing country. Potato tubers are a rich source of nutrients, including starch, proteins, minerals, crude fiber, and anti-oxidative and anti-aging compounds. In addition to serving as a commonly consumed vegetable, potato is widely used in the textile, pharmaceutical, food, dye, paper, and other industries because of its high starch content. Thus, potato has extremely diverse uses, and its production can substantially affect national economies. However, potato yields are severely affected by pests and adverse environmental conditions. The most harmful threat to sustainable potato production worldwide is late blight caused by *Phytophthora*

infestans (Mont.) De Bary [1]. Late blight has drastically decreased potato yields, including losses of up to 100% in some cases. Annual direct economic losses due to late blight infections worldwide are as high as US $6.7 billion, which corresponds to 15% of the total potato output [2]. Consequently, developing viable methods for preventing and controlling late blight during potato cultivation is critical. Favorita, which is a high-yielding potato variety that produces high-quality tubers, was introduced to China from the Netherlands in 1981 by the China-owned Assets Supervision and Administration Bureau of the Central Ministry of Agriculture. Favorita is one of the main early-maturing potato varieties cultivated in China. However, because it is susceptible to late blight, strict planting conditions are required during its cultivation. An outbreak of late blight will seriously impact the yield and quality of Favorita potatoes [3]. Therefore, there is a critical need for enhancing the late blight resistance of Favorita.

Grafting, which is an ancient agricultural technique dating back to 424 BC, is a vegetative hybrid cultivation method in which two cut plants are joined and allowed to heal to develop into a new plant. Buds or branches are usually used as the scion, whereas the root stem serves as the rootstock; the scion is attached to an incision in the rootstock, after which the fused plant materials heal to form a grafted plant [4]. In agriculture, grafting technology is mainly used to increase crop yield, improve the branching structure, and enhance crop resistance to biotic and abiotic stresses [5]. Crop grafting technology has been applied in China relatively extensively. Specifically, it has been widely used to breed stress-resistant tomato, eggplant, pepper, and melon varieties as well as to elucidate the mechanisms underlying the stress resistance of these crops [6]. In studies using drought-tolerant tobacco varieties as rootstocks, Huo (2016) and other researchers concluded that grafted tobacco plants can improve their drought resistance by regulating antioxidant enzyme activities and stress-responsive gene expression [7]. Wang et al. (2015) grafted tomato to purple potato rootstock and observed that grafting significantly increased the tomato yield and decreased the incidence of bacterial wilt, without affecting fruit quality [8]. Additionally, grafting cucumber to Yunnan black-seed pumpkin rootstock can increase cucumber resistance to blight [9]. Therefore, choosing an appropriate rootstock can increase plant stress resistance and yield. Grafting is also relevant for studying long-distance signaling in plants [10]. Numerous studies proved that RNA, proteins, hormones, and even chloroplast and nuclear genomes can be transported from the rootstock to the scion [11-13]. Changes to plant traits may be closely related to the exchange of material between the scion and rootstock. However, the molecular basis of plant trait modifications and the physiological or biochemical changes after grafting remain unknown. Transcriptome analyses of grafted plants can reveal the specific genes involved in regulating the physiological responses induced by grafting [14] as well as the differentially expressed genes (DEGs) in the transcriptional network and main metabolic pathways influencing plant growth, development, and responses to environmental stresses before and after grafting[15]. The plant hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are important signaling molecules involved in abiotic and biotic stress responses [16-18]. Genes related to disease responses and calcium-dependent signaling are also crucial for plant stress responses [16].

In this study, we used the early-maturing and susceptible potato variety Favorita (abbreviated as “F”) and the mid-late maturing and highly late blight-resistant potato variety Qingshu 9 (abbreviated as “Q”) as test materials. These varieties were grafted onto each other as scions and rootstocks. On the basis of the potato genome sequence, we analyzed the transcriptome data for the scion after grafting. Moreover, we conducted resistance tests to explore the effect of potato grafting on scion gene expression and late blight resistance.

Results

2.1 Transcriptome sequencing and assembly

The transcriptomes of the third leaves from the top of the analyzed plants were sequenced with the Illumina high-throughput sequencing platform (Fig. 1), resulting in 6.5×10^7, 5.7×10^7, 6.1×10^7, and 5.5×10^7 raw reads, which were filtered to obtain 6.3×10^7, 5.6×10^7, 6.1×10^7, and 5.4×10^7 clean reads for the F/Q, Q/F, F, and Q leaves, respectively. The data error rate was 0.03% for all leaves. Additionally, for the F/Q, Q/F, F, and Q leaves, the Q30 was 93.66%, 93.76%, 93.86%, and 92.82% and the GC content was 42.38%, 42.31%, 42.24%, and 42.18%, respectively. The clean reads were aligned to the reference genome sequence using the HISAT program (i.e., hierarchical indexing for spliced alignment of transcripts). The average mapping rate for the F/Q, Q/F, F, and Q leaves was 87.61%, 86.09%, 82.43%, and 85.78%, respectively. The similarity of the mapping rate among the samples indicated that the clean read data were comparable between samples. Therefore, the transcriptome sequencing results were reliable and appropriate for further analyses (Table 1).

2.2 Screening of DEGs

Using a gene expression level fold-change > 1 and a *p*-value < 0.05 as the criteria, we detected 8,022 DEGs (Fig. 2). The F/Q *vs* F comparison revealed 3,153 up-regulated genes and 4,389 down-regulated genes. In contrast, the Q/F *vs* Q comparison identified 329 up-regulated genes and 151 down-regulated genes.

2.3 Gene Ontology (GO) enrichment analysis of DEGs

The DEGs were functionally annotated based on GO classifications (Fig. 3), which divided the genes into the following three main categories: biological process (BP), cellular component (CC), and molecular function (MF). Regarding the DEGs identified in the F/Q *vs* F comparison, the genes grouped in the BP category were mainly related to photosynthesis. The DEGs in the CC category were primarily associated with thylakoids, photosynthetic membranes, and photosynthetic systems, whereas the DEGs in the MF category were mainly involved in cell regulation and peptidase catalytic activity. Of the DEGs detected in the Q/F *vs* Q comparison, those grouped in the BP category were mainly associated with responses to injury and stress. The DEGs in the CC category mainly affected the cell wall and external packaging structure. The DEGs belonging to the MF category were primarily related to cell regulation and peptidase catalytic activity.

2.4 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs

The DEGs identified in the two comparisons were also analyzed using the KEGG pathway database (Fig. 4). The top 20 enriched KEGG pathways were identified. More specifically, in the F/Q *vs* F comparison, the up-regulated DEGs were mainly associated with the mRNA monitoring pathway, autophagy - other, glutathione metabolism, plant–pathogen interactions, plant hormone signal transduction, nitrogen metabolism, and the mitogen-activated protein kinase (MAPK) signaling pathway, whereas the down-regulated DEGs were primarily associated with ribosomes and the photosynthesis antenna protein as well as with photosynthesis, phenylpropane biosynthesis, cyanoamino acid metabolism, steroid biosynthesis, and pentose and glucuronide conversions. Regarding the Q/F *vs* Q comparison, the up-regulated DEGs were mainly related to the protein processing pathway in the endoplasmic reticulum, whereas the down-regulated DEGs were involved in fatty acid elongation, arginine and proline metabolism, and diterpenoid biosynthetic pathways.

2.4.1 Effects of grafting on the expression of genes related to plant–pathogen interactions

The KEGG pathway analysis revealed that 36 plant–pathogen interaction-related genes were more highly expressed in the F/Q leaves than in the F leaves (Fig. 5). These genes encoded 10 calcium-dependent protein kinases (CDPKs), five cyclic nucleotide-gated ion channel (CNGC) proteins, three chitin elicitor receptor kinases (CERKs), two LRR receptor serine/threonine protein kinases (LRR-LRKs), and two WRKY transcription factors. On the basis of the results of the KEGG pathway analysis of the DEGs in the Q/F *vs* Q comparison, the expression levels of only three heat shock protein (HtpG) genes were up-regulated.

2.4.2 Effects of grafting on the expression of genes involved in MAPK signaling pathways

The KEGG pathway analysis indicated that 31 MAPK signaling pathway-related genes had higher expression levels in the F/Q leaves than in the F leaves (Fig. 6). These genes included six encoding MAPKs, five encoding ethylene-insensitive proteins, and three encoding the abscisic acid (ABA) receptor PYL. Additionally, the DEGs more highly expressed in Q/F leaves than in Q leaves included a serine/threonine protein kinase (SRK2) gene and a protein phosphatase (PP2C) gene.

2.4.3 Effects of grafting on the expression of plant hormone signaling-related genes

Plant hormone signal transduction is affected by grafting. In the F/Q *vs* F comparison, the up-regulated genes were mainly associated with the ET, SA, and JA pathways. Among the genes in the ET signal transduction pathway, the expression levels of one ETR gene, one EIN2 gene, four EIN3 genes, and one ERF1/2 gene were up-regulated. Six DEGs were related to the JA signal transduction pathway, of which four were up-regulated genes and two were down-regulated genes. The up-regulated genes encoded JA synthase, whereas the down-regulated genes were TIFY family genes. There were 11 up-regulated genes and one down-regulated gene involved in the SA pathway, including three NPR family genes, five TGA family genes, and two genes encoding disease-related proteins (Fig. 7). The up-regulated DEGs in the Q/F *vs* Q comparison encoded a SRK2 and a PP2C.

2.5 Resistance of potato to late blight after grafting

At 35 days after grafting, the F/F, F/Q, Q/Q, and Q/F scion leaves were collected for an *in vitro* inoculation assay, with leaves from ungrafted F and Q plants serving as controls. The resistance of the inoculated leaves was analyzed on the seventh day. The susceptibility to infection was evaluated based on the resistance index. The ungrafted F leaf was susceptible (S) to infection. Similarly, the self-grafted F/F leaf was also S, but its disease index (56.7) was lower than that of the F leaf (76.6). Compared with the F/F and F leaves, the F/Q leaf was more resistant to infection [i.e., moderately resistant (MR)]. The ungrafted Q leaf and the self-grafted Q/Q leaf were highly resistant (HR) to disease. Compared with the Q and Q/Q leaves, the Q/F leaf was less resistant to infection [i.e., resistant (R)] (Fig. 8).

Discussion

3.1 Analysis of the effect of grafting on potato late blight resistance based on transcriptome sequencing data

In order to explore the effect of rootstock on the gene expression of scion after grafting two different potato varieties, we used the potato late blight-resistant variety Qingshu 9 and the susceptible cultivar Favorita for grafting. Then the transcriptomes of the stable F/Q and Q/F scions were sequenced with the Illumina HiSeq 4000 sequencing platform, with ungrafted F and Q used as the controls. A total of 7,542 DEGs were identified in the F/Q *vs* F comparison, including 3,152 up-regulated genes and 4,389 down-regulated genes. The 480 DEGs revealed by the Q/F *vs* Q comparison consisted of 329 up-regulated genes and 151 down-regulated genes. The DEGs were functionally characterized based on the enriched GO terms. Most of the DEGs were related to wound responses, cell parts, responses to stimulation, biological regulation, and catalytic activities. The transcription of many genes related to cell rearrangements, cell division, the metabolic mode, and stress responses changed in the scion after grafting.

The enriched KEGG pathways indicated that compared with the corresponding expression in the ungrafted F, the expression levels of some stress-related genes were significantly up-regulated in the F scion. These genes were mainly related to plant–pathogen interactions, plant MAPK signaling, and plant hormone signaling. This was consistent with the results of earlier KEGG pathway enrichment analyses of the DEGs identified after the transcriptome sequencing of grafted tomato scions by Wang Hui et al. and the DEGs revealed by the transcriptome sequencing of grafted litchi sections by Chen Zhen et al. [17; 18]. Grafting increases the oxidative stress of plants, leading to the stimulation of the antioxidant defense system in the scion as well as the up-regulated expression of related genes. The expression levels of genes related to auxin, gibberellin, ABA, ET, SA, and JA also change. For example, the (auxin inlux carrier) Aux1 family, (small auxin-up RNA) SAUR family, and transcription factor family genes are all involved in the regulation of plant growth and development. In the current study, the expression levels of these genes were up-regulated. Some of these up-regulated genes encoded CDPKs, CERKs, LRR-LRKs, NPR proteins involved in the SA synthesis pathway, and MAPKs, which are all potato late blight response proteins [19].

Because of their effects on plant interactions with pathogens, the MAPK signaling pathway and plant hormone signal transduction influence the resistance reaction of many crops [20-22]. We speculated that the up-regulated expression of these genes may be increasing resistance of the F scion to late blight. The Q/F *vs* Q comparison revealed relatively few DEGs. However, the identified up-regulated genes were mainly involved in the synthesis of the endoplasmic reticulum, endocytosis, and cell-to-cell movement. None of the up-regulated genes were related to potato responses to late blight. This may have been because Q is a disease-resistant variety in which these genes are already highly expressed. The down-regulated genes mainly contributed to proline metabolism, fatty acid chain elongation, and diterpenoid biosynthesis pathways. Such genes are involved in the plant resistance response. The down-regulated expression of these genes may be related to the resistance of the Q scion to late blight.

3.2 Effects of grafting on potato late blight resistance

To explore the influence of the rootstock on late blight resistance for the gene expression changes of scion, we tested the late blight resistance of the separated leaves of the scion. Our analysis indicated that the resistance index of F increased from S to MR after it was grafted onto Q rootstock. Moreover, although self-grafted F had the same resistance index as the ungrafted F, it had a lower disease index than the ungrafted F. Self-grafting and grafting to the resistant rootstock improved the resistance of the susceptible scion to late blight. However, grafting to the disease-resistant variety resulted in stronger resistance. Additionally, the grafting of Q to F rootstock decreased the resistance of the Q scion from HR to R, indicating that using a susceptible variety as the rootstock will decrease the resistance of the infected scion to late blight.

Conclusions

The above-mentioned results indicate that the self-grafting of susceptible potato varieties and the grafting to disease-resistant varieties as the rootstock can increase the resistance of susceptible potato varieties to late blight, but the resistance is greater when resistant varieties are used as the rootstock. In contrast, using susceptible varieties as the rootstock decreases disease resistance. The late blight resistance is associated with the changes to the expression of resistance-related genes in the scion. The DEGs identified in this study are potential candidate genes for future functional analyses. Moreover, the study findings may provide the basis for future investigations of the molecular mechanism underlying the enhanced disease resistance of scions resulting from potato xenografting.

Methods

5.1 Materials

The potato late blight-resistant variety Qingshu 9 and the susceptible variety Favorita were provided by the Biotechnology Institute of the Guizhou Academy of Agricultural Sciences, Guiyang city, Guizhou

province, China. *Phytophthora infestans* W1 was provided by Guizhou University/Guizhou Provincial Biochemical Engineering Center, Guiyang city, Guizhou province, China.

5.2 Methods

5.2.1 Grafting

For both varieties, virus-free potato blocks with only one bud eye were sown in an 11-cm diameter pot filled with sterile nutrient soil and then placed in a glass greenhouse at the Institute of Agricultural Bioengineering of Guizhou University on June 26, 2019. Plants were grafted on July 26, 2019 using the “splice method. Specifically, virus-free potato segments were placed in sterile nutrient soil. Uniformly growing 4-week-old plants with non-hollow branches were selected for grafting. Healthy young shoots (4–5 cm) with 4–5 leaves were used as scions, whereas healthy young shoots were cut 2–3 cm above the soil level to produce the rootstocks. During grafting, a 3-cm deep vertical incision was made in the middle of the rootstock. The scion was cut into wedges, inserted into the incision, and immediately covered with plastic wrap. Finally, the graft union was secured with a grafting clip, after which the seedlings were covered with plastic cups (Fig. 9). The grafting procedure was completed in a glass greenhouse.

5.2.2 Transcriptome sequencing after grafting

On August 26, 2019, the third leaf from the top of uniformly growing and healthy plants (F/Q, Q/F, and ungrafted F and Q) was collected for the subsequent transcriptome sequencing analysis with the Illumina HiSeq 4000 high-throughput platform. The sequencing was completed with three biological replicates. The RNA extraction and transcriptome sequencing were performed by Beijing Nuohezhiyuan Technology Co., Ltd.

5.2.3 Evaluation of late blight resistance after grafting

Stably growing grafted plants were examined to assess their resistance to late blight, with the ungrafted F and Q plants sown on June 26, 2019 used as the controls. Late blight resistance was evaluated using the *in vitro* leaf inoculum method. Before inoculating the leaves, *P. infestans* cultured for 15 days was added to test tubes containing sterile water. The solution was then passed through 1–2 layers of filter paper, after which the filtrate was examined with a microscope to confirm the production of sporangia. When the concentration reached 20–25/μL, the sporangia were placed in a refrigerator at 4 °C for 1 h to promote the release of zoospores. The healthy third leaf (from the top of the plants) was collected for inoculations. Leaf samples were collected from 30 plants for the F and Q ungrafted controls and for the F/F, Q/Q, Q/F, and F/Q grafted samples. The leaves were placed in plastic Petri dishes with the abaxial side facing up, after which they were covered with wet filter paper and sprayed with 2 mL distilled water. Using a pipette, the leaves were inoculated with a 20-μL *P. infestans* suspension. The inoculation site was located next to the main vein. The Petri dishes were sealed with Parafilm and then incubated at 22 °C with a 16-h light/8-h dark cycle. The leaves were checked for disease symptoms daily, with a particular

focus on moisture retention. Symptoms were detectable at 5 days after the inoculation, and the size of the diseased area was measured on day 7. The longest and widest diseased spots were recorded [length (L) and width (W) being perpendicular], after which the lesion area was calculated using the following formula: $A = 1/4 \times \pi \times L \times W$. Late blight disease severity was assessed using the following levels [23]: level 1: no symptoms or the lesion area was less than 3%; level 2: the lesion area was between 3% and 10%, with no chlorosis and water immersion around the dead tissue; level 3: the lesion area was between 10% and 30%, and the surrounding area was soaked and contained white mycelia; level 4: the lesion area was between 30% and 60%, with obvious white mycelia; and level 5: the lesion area was greater than 60%, with obvious rotted tissue. The disease index (%) was calculated with the following formula: (Σ disease level × number of plants at that disease level)/ (highest disease level × number of surveyed plants) × 100.

Declarations

(1) Ethics approval and consent to participate

We does not contain any studies with human or animal subjects.

(2) Consent for publication

My manuscript doesn’t contain any individual person’s data in any form (including any individual details, images or videos).

(3) Availability of data and materials

The datasets used and/or analysed during the current study are available from the author on reasonable request.

(4) Competing interests

The authors declare that they have no competing interests.

(5) Funding

This study was funded by National Major Project of Cultivating New Varieties of Genetically Modified Organisms (NO.2016ZX08010003-009) and Guizhou Province High-level Innovative Talent Training Program Project ([2016]4003). During my study, these two funding bodies provided the financial guarantee for my reagent.

(6) Authors' contributions

The corresponding author DGZ provides ideas for the design of the thesis, guides during the experiment, and revises the article. The author of this article YXL designs the experiment according to the corresponding author's ideas, and conducts the experiment operation and writing the manuscript.

All authors have read and approved the manuscript.

(7) Acknowledgments

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Abbreviations

Full name	Abbreviations
Kyoto Encyclopedia of Genes and Genomes	KEGG
Plant mitogen-activated protein kinase	MAPK
Calcium-dependent protein kinases	CDPKs
Chitin elicitor receptor kinases	CERKs
LRR receptor serine/threonine protein kinases	LRR-LRKs
Nucleotide-gated ion channel	CNGC
Salicylic acid	SA
Jasmonic acid	JA
Ethylene	ET
Auxin inlux carrier	Aux1
Small auxin-up RNA	SAUR
Heat shock protein	HtpG
Serine/threonine protein kinase	SRK2
Protein phosphatase	PP2C
Abscisic acid	ABA
Gene Ontology	GO
Biological process	BP
Cellular component	CC
Molecular function	MF
Differentially expressed genes	DEGs

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Tables

	Raw reads	clean reads	error_rate /%	Q30 /%	GC_pct /%	Total map /%	Unique map /%
F	6.1×10^7	$6.1 \times 10_7$	0.03	93.86	42.24	82.43	79.68
Q	$5.5\times10_7$	$5.4 \times 10_7$	0.03	92.82	42.18	85.78	83.17
F/Q	$6.5\times10_7$	$6.3 \times 10_7$	0.03	93.66	42.38	87.61	85.25
Q/F	$5.7\times10_7$	$5.6 \times 10_7$	0.03	93.76	42.31	86.09	83.42

Table 1. Summary of sequence reads for four RNA samples including two control groups (F, Q) and two grafted groups (F/Q, Q/F).

Figures

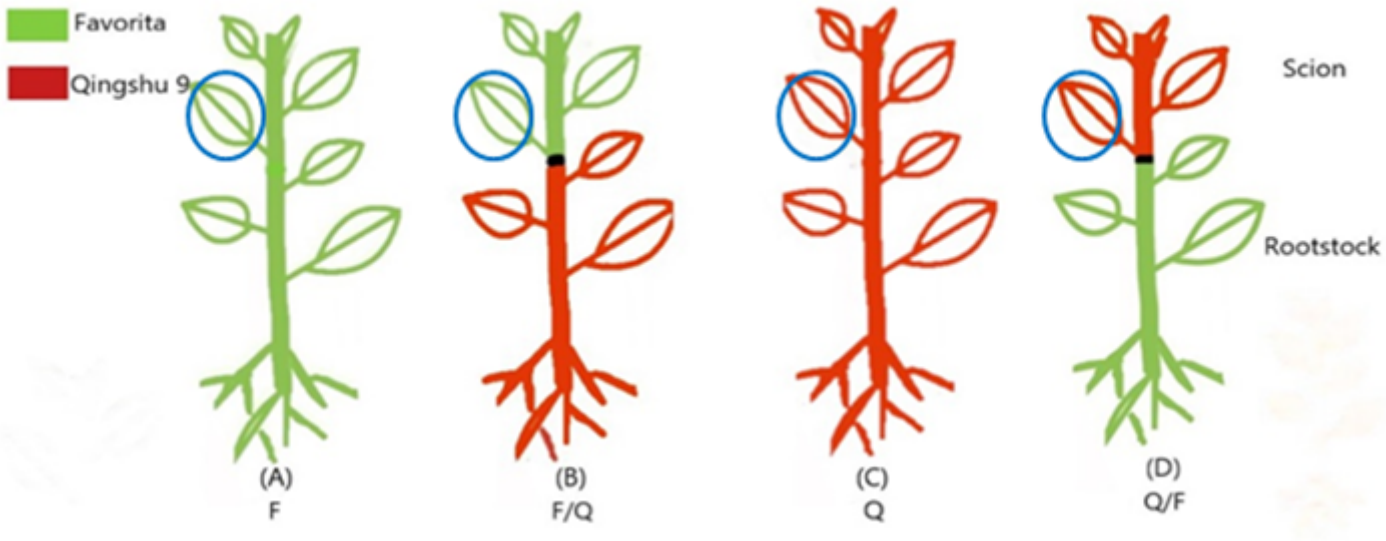

Figure 1

Illustration of F and Q grafting. The green plant is F and the red is Q. The position of the leaves collected for transcriptome sequencing is circled.

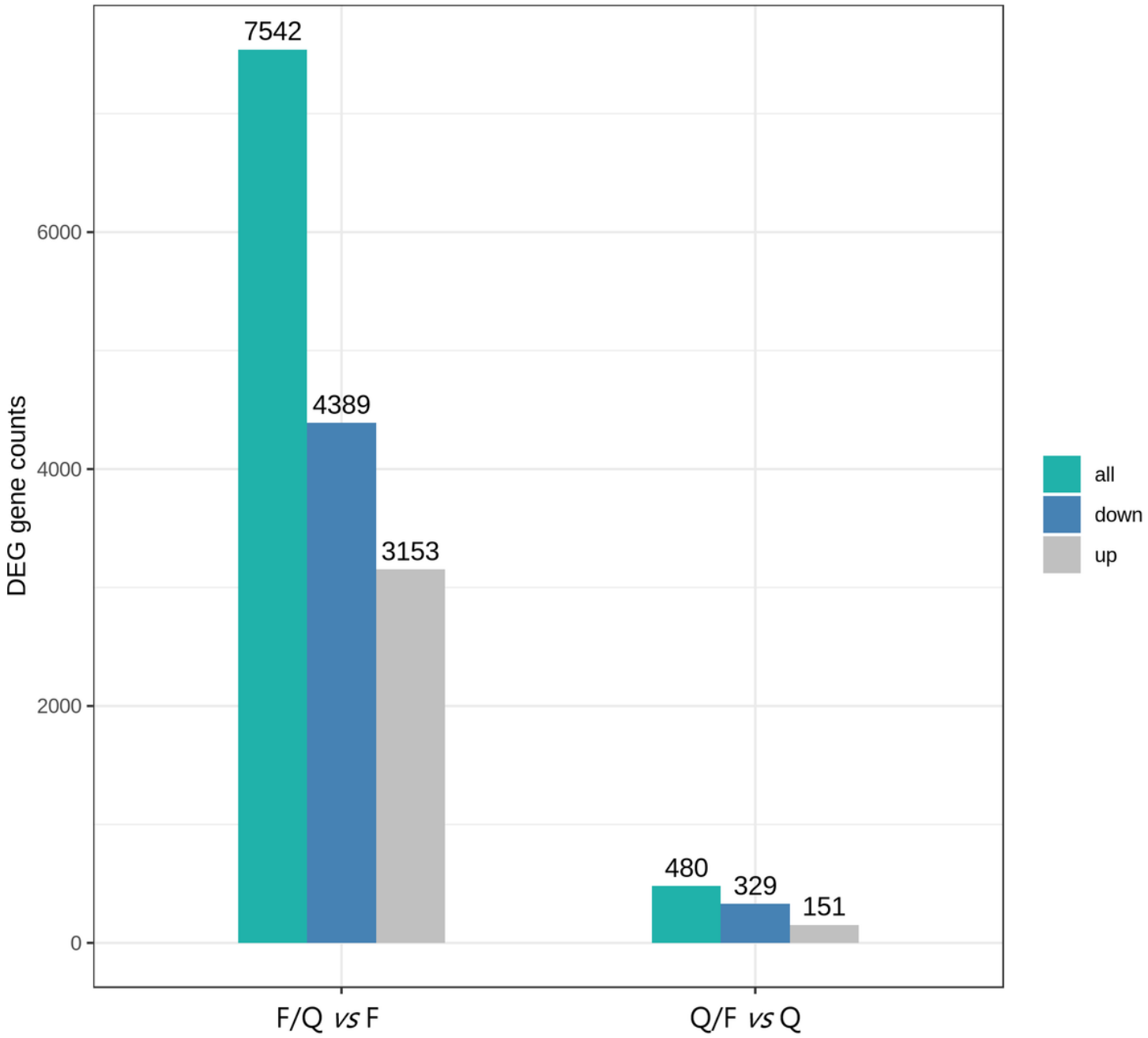

Figure 2

Number of DEGs detected between ungrafted and grafted potato seedlings (F vs F/Q and Q vs Q/F).

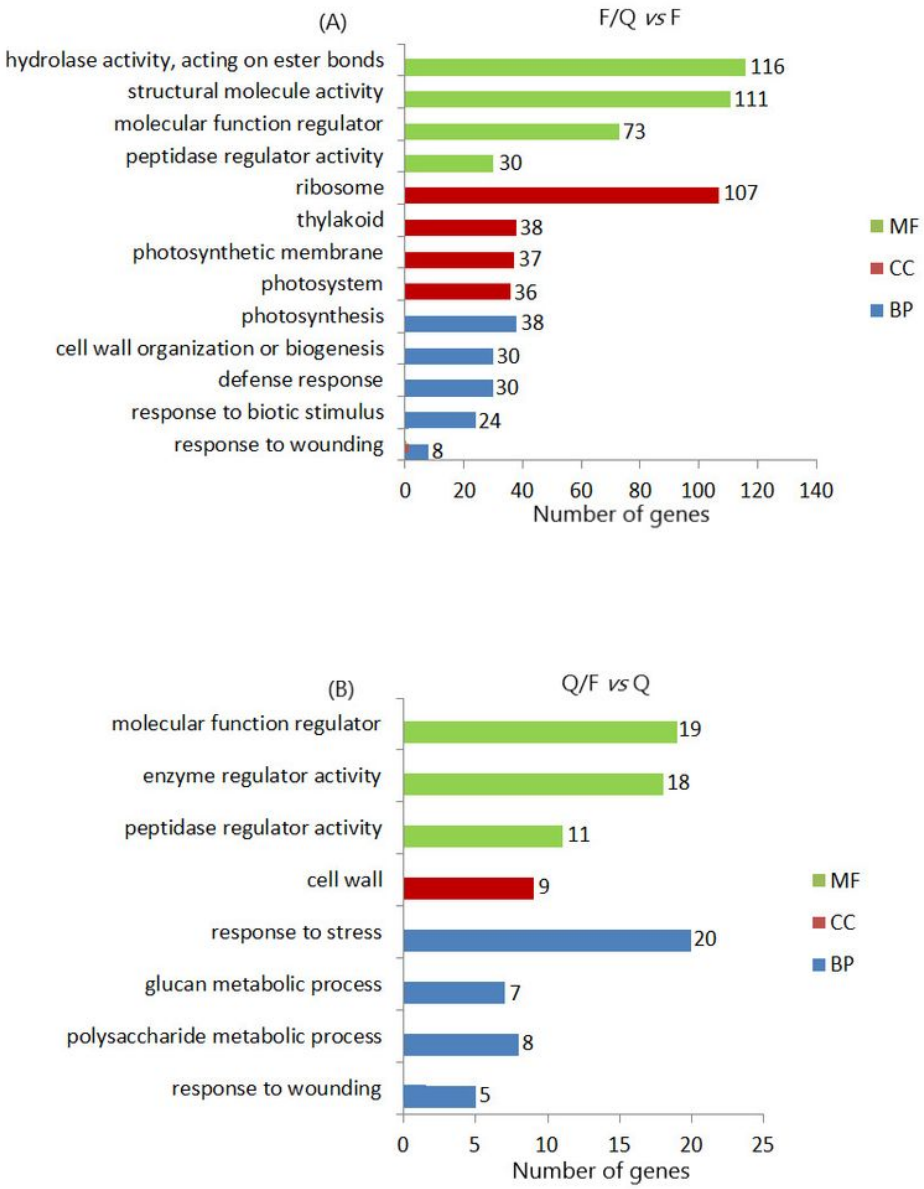

Figure 3

GO statistics and enrichment analysis of identified DEGs detected between ungrafted and grafted potato seedlings. (A) The DEGs between F and F/Q. (B) The DEGs between Q and Q/F.

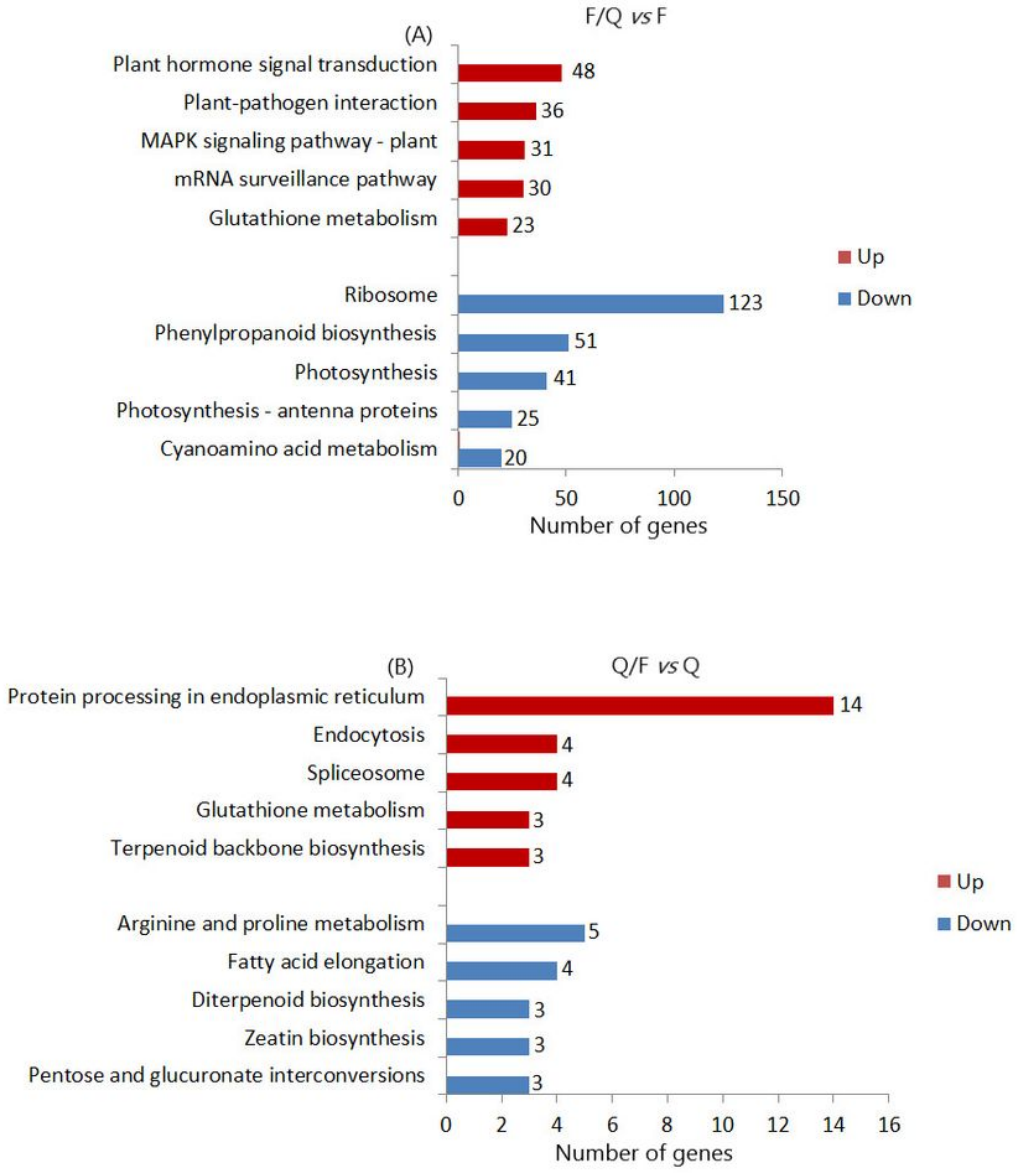

Figure 4

The five most significantly enriched KEGG pathways among DEGs detected between ungrafted and grafted potato seedlings. (A) The DEGs between F and F/Q. (B) The DEGs between Q and Q/F.

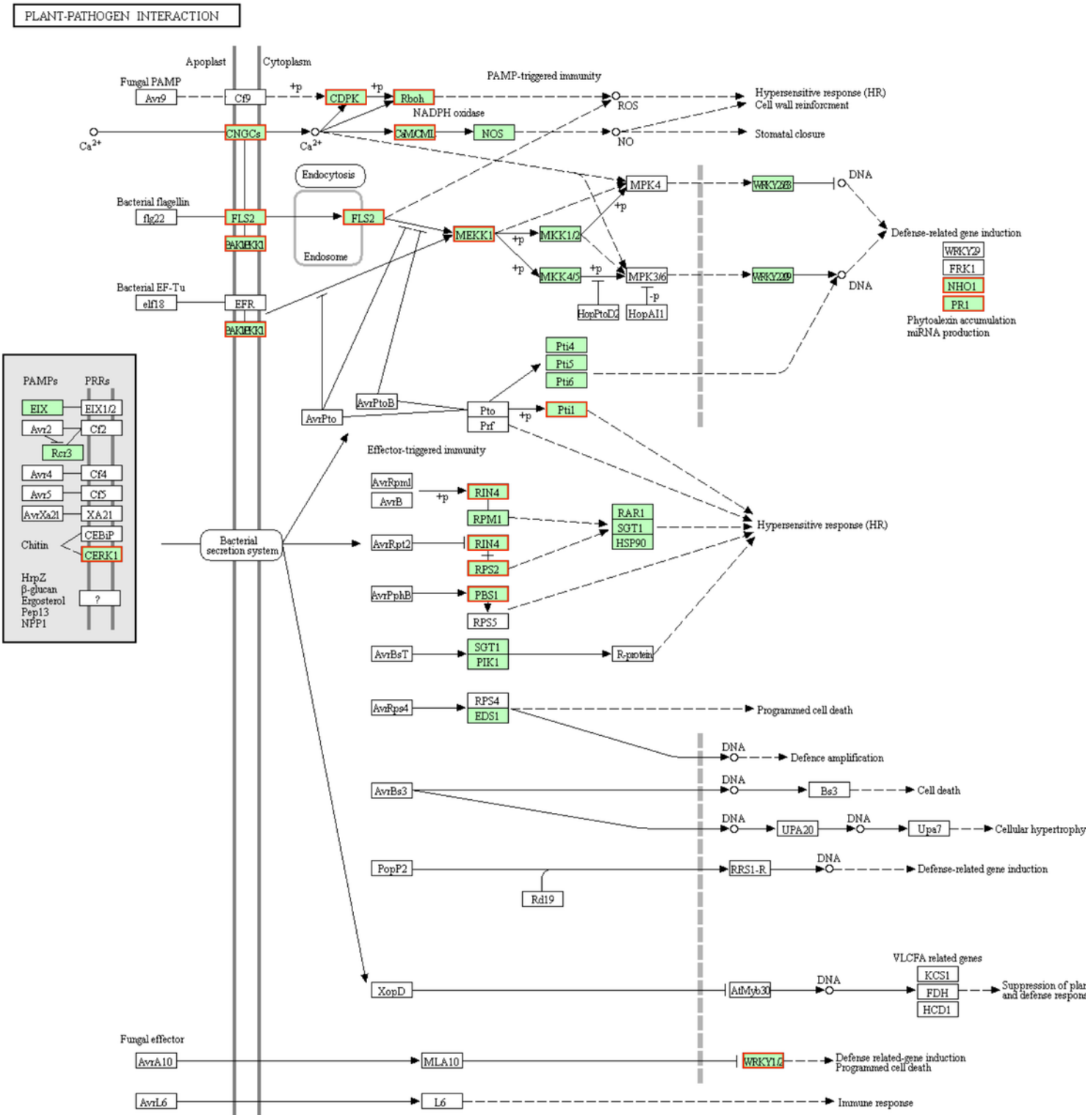

Figure 5

Up-regulated genes in the plant–pathogen interaction signaling pathway (F/Q vs F). The up-regulated genes are indicated by red boxes.

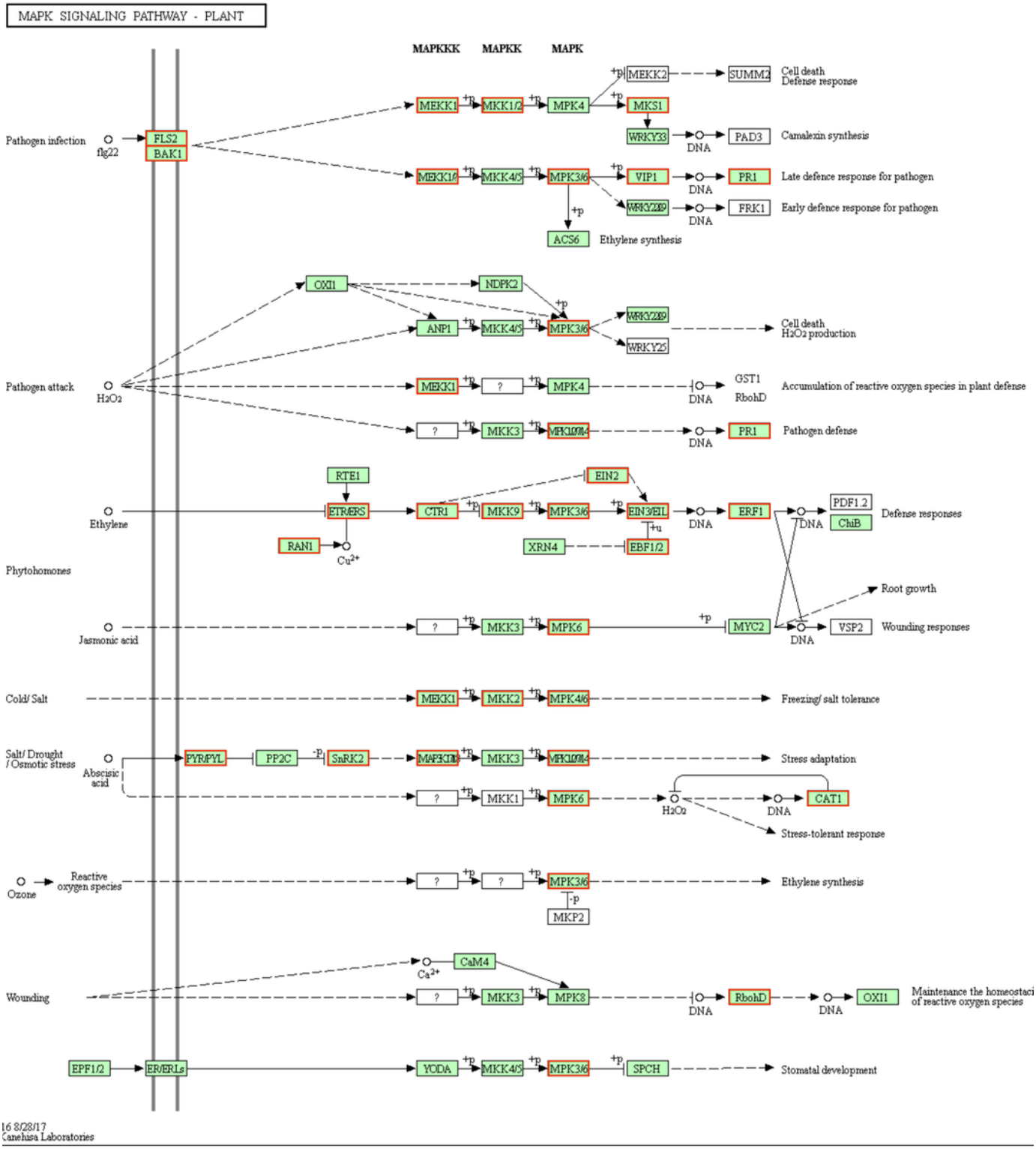

Figure 6

Up-regulated genes in the MAPK signaling pathway (F/Q vs F). The up-regulated genes are indicated by red boxes.

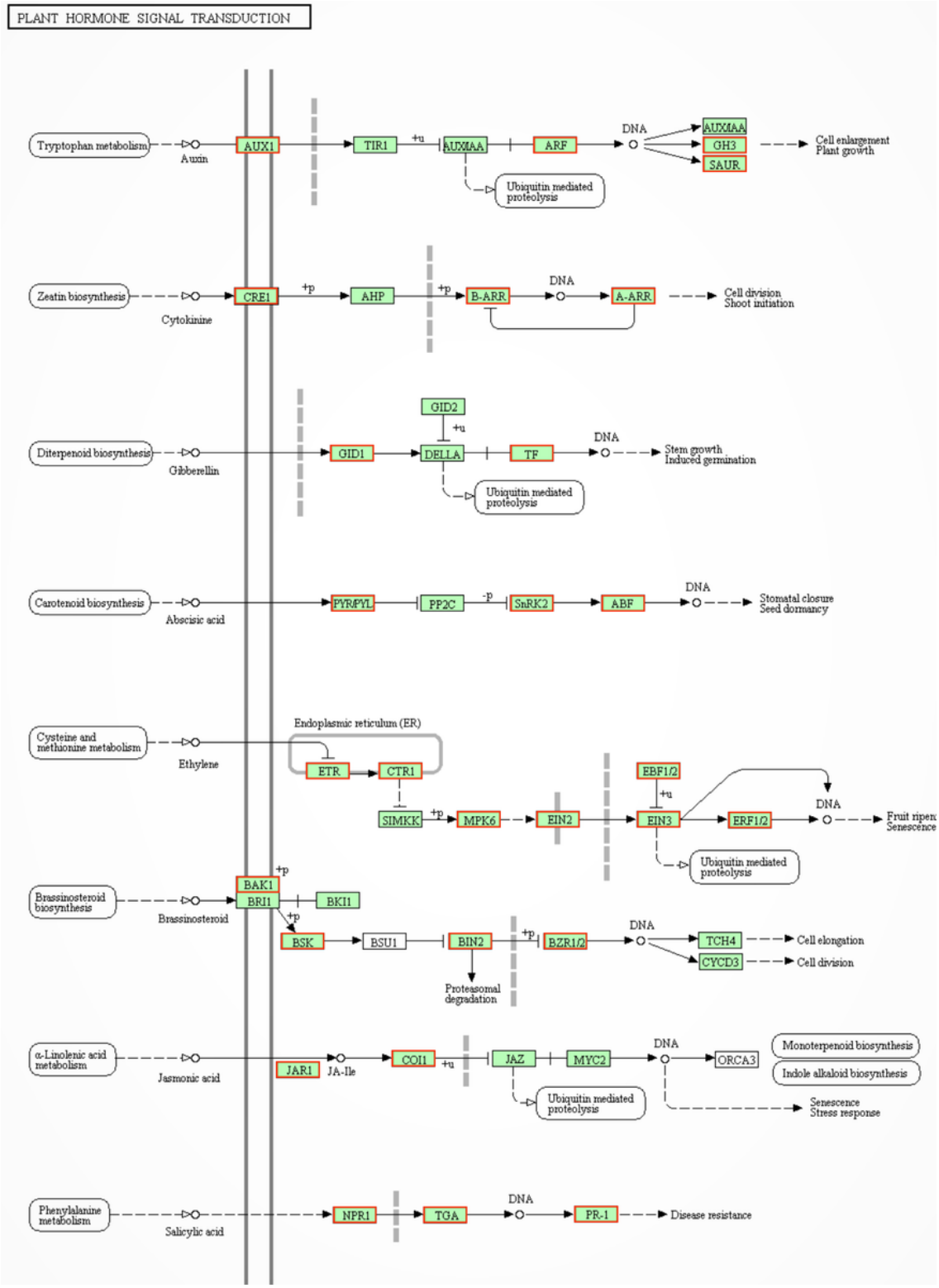

Figure 7

Up-regulated genes in the plant hormone signal transduction pathway (F/Q vs F). The up-regulated genes are indicated by red boxes.

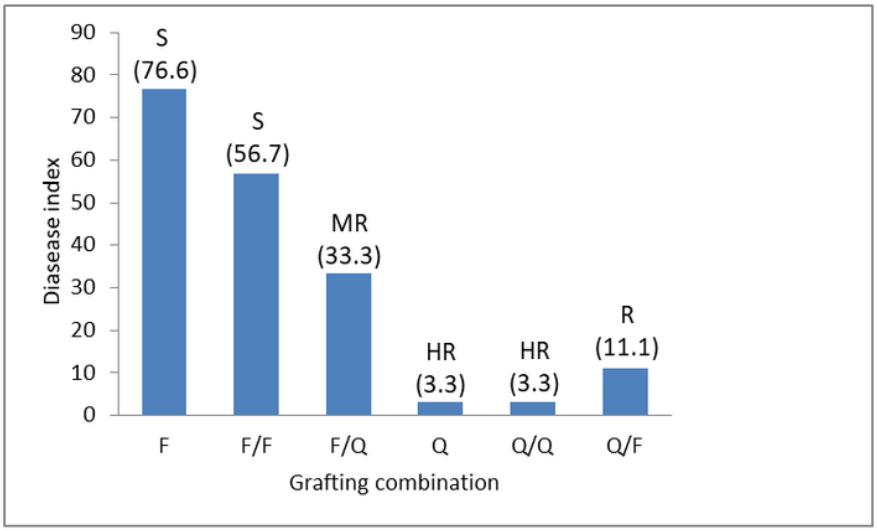

Figure 8

Late blight index results of the separated leaves after inoculating P. infestans on the 7th day. The disease index grade of ungrafted F leaf and self-grafted F/F leaf were both susceptible (S). The F/Q leaf was more resistant to infection [i.e., moderately resistant (MR)]. The ungrafted Q leaf and the self-grafted Q/Q leaf were highly resistant (HR) to disease. Compared with the Q and Q/Q leaves, the Q/F leaf was less resistant to infection [i.e., resistant (R)]. Index grade: (1) I: Immune, disease index (DI) = 0; (2) HR: Highly resistant, $0 \leq DI \leq 10$; (3) R: Resistant, $10 \leq DI \leq 30$; (4) MR: Moderately resistant, $30 \leq DI \leq 50$; (5) S: Susceptible, $DI > 50$

Figure 9

Image of a grafted plant. Healthy young shoots (4–5 cm) with 4–5 leaves were used as scions, healthy young shoots were cut 2–3 cm above the soil level to produce the rootstocks. A 3-cm deep vertical incision was made in the middle of the rootstock. The scion was cut into wedges, inserted into the incision.