Transcriptome Analysis of Grafted Potato Rootstock for Improvement of Scion Late-Blight Resistance

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Research article

Keywords: Potato late blight, Graft, Scion, Rootstock, Transcriptome sequencing

Posted Date: September 3rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-58639/v1

Abstract

Late blight seriously threatens potato cultivation worldwide. Damage caused by the fungus, which is severe and widespread, can lead to drastic reductions in potato yield and even total loss. Although grafting technology has been widely used to improve crop resistance, the effects and associated molecular mechanisms of grafting on resistance to potato late blight are unclear. In this study, we therefore performed RNA transcriptome sequencing of the scion when potato late blight-resistant variety Qingshu 9 and susceptible variety Favorita were used as the rootstock and scion, respectively, and vice versa. Using the sequencing results, we analyzed the influence of the rootstock on scion resistance and related molecular mechanisms. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis revealed that plant–pathogen interactions, the plant MAPK signaling pathway, and genes on the SA pathway were significantly up-regulated in the scion when Qingshu 9 was used as the rootstock. When Favorita was used as the rootstock, β-ketoacyl-CoA synthase-related genes in the scion, Qingshu 9, were significantly down-regulated. Resistance to late blight on scion leaves were also tested *in vitro*, which results consistent with those obtained by sequencing. All the generated evidence indicates that the use of resistant and susceptible varieties as rootstocks can respectively increase and reduce resistance to late blight. Our sequencing results further elucidate the molecular mechanism underlying the post-grafting effects of rootstocks on late blight resistance and provide a theoretical basis for the transfer of resistance genes between scions and rootstocks after grafting.

Introduction

Potato (*Solanum tuberosum L.*), an annual solanaceous herb native to the Andes in South America, was introduced into China in the 17th century and has become the fourth largest food crop. China now has become the first potato producing country in the world. Potato tubers have high nutritional value, which is being rich in starch, proteins, minerals, crude fiber, and anti-oxidative and anti-aging compounds. In addition to serving as a vegetable, potato is widely used in textile, pharmaceutical, food, dye, paper, and other industries because of its high starch content. Potato thus occupies an important position in daily life and the national economy. However, the yields are menaced by pests and adverse environmental conditions seriously. The most harmful threat is late blight caused by *Phytophthora infestans* (Mont.) De Bary, which threatens the survival of the global potato [1]. Late blight has drastically reduced potato yields and even led to complete production failures. At present, annual direct economic losses caused by late blight worldwide are as high as US $ 6.7 billion, which corresponds to 15% of total potato output [2]. So the prevention and control of late blight during potato planting and the selection of disease-resistant varieties are very important.

Grafting, an ancient agricultural technique dating back to 424 BC, is a vegetative hybrid cultivation method in which two cut plants are joined together and allowed to heal into a new plant. Buds or branches are usually used as the scion, and the root stem serves as the rootstock; the scion is attached to the incision of the rootstock to heal and grow into grafted plants [3]. In agriculture, grafting technology is mainly used to increase crop yield, improve branching structure, and enhance the biotic and abiotic stress

resistance of crops [4]. The application of crop grafting technology is relatively extensive in China. Grafting technology has been widely used in tomato, eggplant, pepper, and melon production to enhance their resistance—not only for breeding resistant varieties, but also to explore their resistance mechanisms [5]. In studies using drought-tolerant tobacco varieties as rootstocks, Huo (2016) and other researchers have found that grafted tobacco plants can improve their drought resistance by regulating antioxidant enzyme activity and stress-responsive gene expressions [6]. Wang et al. (2015) used purple potato as a rootstock for tomato grafting cultivation; according to their results, grafting significantly increased tomato yield and reduced the incidence of bacterial wilt without affecting fruit quality [7]. Grafting of Yunnan black-seed pumpkin rootstock to cucumber can increase cucumber resistance to blight [8]. Choosing the appropriate rootstock can thus improve the stress resistance and yield of plants. Grafting is also an effective tool for studying long-distance signaling in plants [9]. Numerous studies have shown that RNA, proteins, hormones, and even chloroplast and nuclear genomes can be transported from rootstock to scion [10-12]. It is speculated that the changes in plant traits are inferred to be closely related to the exchange of material between scion and rootstock. However, the underlying molecular mechanisms of alterations in plant traits and the physiological or biochemical changes after grafting are still unknown.

Among second-generation sequencing technologies, RNA sequencing is by far the most powerful tool available for comparative transcriptome analysis. This technique is highly repeatable with minimal systematic differences [13]. Transcriptome analysis of grafted plants can reveal specific genes involved in regulation of physiological responses induced by grafting [14]. Bioinformatics analysis of transcriptome data can be used to identify differentially expressed genes in the transcriptional network and major metabolic pathways of plant growth, development, and environmental stress responses before and after grafting [15]. The plant hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are important signaling molecules involved in both abiotic and biotic stress [16-18]. Genes related to disease-related (PR) responses and calcium-dependent signaling also play crucial roles in plant stress response [16]. So based on the potato genome data, transcriptome data are analyzed to determine the genes of plant resistance response in the scions when different varieties of potato are used as rootstock. Such an approach can clarify the effect of different resistant rootstocks on late blight resistance of scions after grafting.

In this study, the potato late blight-resistant variety Qingshu 9 and the susceptible variety Favorita were grafted onto each other as scions and rootstocks. The effects of the two rootstocks on late blight resistance were investigated, and the molecular mechanisms responsible for changes were analyzed at the transcriptome level. Our study of the transfer of resistance genes between rootstocks and scions after grafting has laid a foundation for further understanding of the molecular basis of rootstock-enhanced late blight resistance.

1 Results And Analysis

1.1 Effect of heterogeneous grafting on survival rate

The potato was grafted using the "split grafting method". Seven days after grafting, the survival rate of self-root grafting was 100%, and the survival rates of heterogeneous graft Q/F and F/Q were 93.9% and 90.9%, respectively (Table 1).

Table 1. Statistics of potato graft survival rate

	Graft combination	Number of plants	Deaths/strain	Survival rate/%
1	Q/F	33	2	93.9
2	F/Q	33	3	90.9
3	F/F	33	0	100
4	Q/Q	33	0	100

1.2 Resistance identification results of potato late blight after grafting

Thirty-five days after grafting, *in vitro* leaf grafting was inoculated on F/F, F/Q, Q/Q, and Q/F scion leaves. The control group had ungrafted F and Q seeded on the same day, and the resistance statistics were performed on the seventh day. The ungrafted F resistance index was susceptible (S), and the self-root grafted F/F resistance index was also S. However, the disease index was slightly lower than F: 76.6, which was 56.7. Compared with F/F and F, the resistance index of F/Q had been improved, which was due to medium resistance (MR). Hence, the ungrafted Q resistance index had high resistance (HR), and the self-root grafted Q/Q resistance index was also of HR. Compared to Q and Q/Q, the Q/F resistance index decreased to disease resistance (R). (Table 2)

Table 2. Statistics on the effects of grafting on potato late blight resistance

Grafting combination	Total grafting/strain	Number of outbreaks/strain	Incidence /%	Illness index	Resistance index
F	30	5	16.7	76.6	S
F/F	30	4	13.3	56.7	S
F/Q	30	4	13.3	33.3	MR
Q	30	1	3.3	3.3	HR
Q/Q	30	1	3.3	3.3	HR
Q/F	30	2	6.6	11.1	R

Note: (1) I: Immune, DI = 0; (2) HR: High resistance, 0 ≤ DI ≤ 10; (3) R: Disease resistance, 10 ≤ DI ≤ 30; (4) MR: Medium resistance, 30 ≤ DI ≤ 50 (5) S: Suffering from illness, DI ≥ 50

1.3 Transcriptome sequencing and assembly

Based on the transcriptome sequencing and data assembly analysis of the high-throughput sequencing platform (IlluminaHiseq 4000), the third leaves from the top of the grafted combination F/Q, Q/F, and ungrafted F, Q, were sequenced separately. Raw reads of 6.5×10^7, 5.7×10^7, 6.1×10^7, 5.5×10^7 were obtained, and the original sequences were filtered to obtain 6.3×10^7, 5.6×10^7, 6.1×10^7, and 5.4×10^7 clean reads. The data error rates were 0.03%; Q20 was 97.90%, 97.96%, 98.02%, and 97.54%, and GC content was 42.38%, 42.31%, 42.24%, and 42.18%. After obtaining clean reads, the clean reads were aligned to the reference genome sequence by Hierarchical Indexing for Spliced Alignment of Transcripts (HISAT). The average ratio of each sample was 87.61%, 86.09%, 82.43% and 85.78%. The uniform ratio between samples indicated that the clean reads data between samples were comparable. To conclude, the transcriptome sequencing results were credible.

1.4 Screening of differentially expressed genes (DEGs)

DEGs with a gene expression fold > 1 and a *p*-value of < 0.05 after multiple checks were selected for screening in both groups. A total of 8022 DEGs were screened (Fig. 1). By comparing F/Q with F and Q/F with Q, it was found that F/Q versus F had 3152 genes up-regulated and 4389 genes down-regulated; Q/F versus Q had 329 genes up-regulated and 151 genes down-regulated.

1.5 Differential *Gene Ontology* analysis

According to the *Gene Ontology* (GO) classification display (Fig. 2), GO functional analysis mainly included three categories, which were used to describe the biological process (BP), cellular component (CC), and molecular function (Molecular function, MF). In F/Q versus F, BP mainly annotated genes of photosynthesis. Differential genes in CC were higher in thylakoids, photosynthetic membranes, and photosynthetic systems. Molecular function was mainly concentrated in enzyme inhibitor activity, hydrolase activity, hydrolyzed O-glycosyl compounds, endopeptidase modulator activity, endopeptidase inhibitor activity, peptidase modulator activity, and in the peptidase inhibitor activity gene. Among them, the up-regulated genes were mainly enriched in cell recognition, pollination, pollen pistil interaction, multicellular biological processes, pollen recognition, etc. in BP, as well as ubiquitin protein transferase activity, ubiquitin-like protein transferase activity, peptidase activity, pattern binding, and polysaccharide binding genes in MF. Down-regulated genes were enriched in photosynthesis, translation, peptide biosynthesis processes, amide biosynthesis processes in BP, ribosomes, thylakoids, thylakoid parts, photosynthetic membranes, photosystems, and ribosomal structural components, structural molecule activity, enzyme inhibitor activity, hydrolase activity, genes acting on glycosyl bonds. In Q/F versus Q, BP mainly concentrated on response to injury and stress. The differential genes of CC were concentrated in the cell wall and external packaging structure. MF was mainly annotated to enzyme inhibitor activity, enzyme regulation activity, endopeptidase inhibitor activity, peptidase inhibitor activity, regulating molecular function and other genes. Among them, the up-regulated genes were mainly enriched in the two types of genes corresponding to stress and protein folding of BP, apoplasts, extracellular regions, cell

walls, external packaging structures in CC, folding protein binding, transferase activity, and transfer in MF Alkyl or aryl (except methyl) genes.

1.6 Differential gene KEGG analysis

The KEGG pathway analysis was performed on the differential genes in the two grafted combinations F/Q, Q/F scions and the non-grafted F and Q leaves of the control group (Fig. 3). The results showed that among the top 20 pathways with the highest enrichment, when F/Q was compared with F, the KEGG pathway, enriched by differentially up-regulated genes, mainly included the mRNA monitoring pathway, autophagy-other, glutathione metabolism, plant-pathogen interaction, plant hormone signal transduction, nitrogen metabolism, and the MAPK signaling pathway. Down-regulated differential genes were mainly enriched in ribosomes and photosynthesis-antenna protein, and involved in photosynthesis, phenylpropane biosynthesis, cyanoamino acid metabolism, steroid biosynthesis, Pentose, and glucuronide conversion. Compared with Q and Q/F, the up-regulated differential genes were mainly concentrated in the protein processing pathway in the endoplasmic reticulum. Down-regulated differential genes were involved in fatty acid elongation, arginine and proline metabolism, and diterpenoid biosynthetic pathways.

1.6.1 Effect of grafting on gene expression related to plant-pathogen interactions

The KEGG pathway analysis showed that 36 plant-pathogen interaction-related genes in the grafting combination F/Q scion were up-regulated compared to leaves in the same part of F. Among them were ten calcium-dependent protein kinases (CDPKs), five cyclic nucleotide-gated ion channel proteins (CNGC), three chitin trigger receptor receptor kinases (CERKs), two LRR receptor serine/Threonine protein kinases (LRR-LRKs), and two WRKY transcription factors. However, no such gene was found in the results of the KEGG analysis of the differential genes between the graft combination Q/F and its control Q.

1.6.2 Effects of grafting on the gene expression of MAPK signaling pathways in plants

The KEGG pathway analysis showed that 31 MAPK signal pathway-related genes in the grafting combination of the F/Q scion were up-regulated compared to leaves in the same part of F. Among these were six mitogen-activated protein kinases (MAPKs), five ethylene-insensitive proteins, three abscisic acid receptors PYL. There were no significant differences between the grafted combination of Q/F and the non-grafted Q, in the genes of this pathway

1.6.3 Effects of grafting on the expression of plant hormone signaling related genes

The jasmonate (JA) pathway and the salicylic acid (SA) pathway were the two main pathways involved in plant-induced defense. In F/Q versus F, there were 19 differential genes enriched to the JA pathway, of which four were up-regulated genes and 15 were down-regulated genes. In F/Q versus F, there were 19 differential genes enriched to the JA pathway, of which four were up-regulated genes and 15 were down-regulated genes. There were four JA synthetases in the up-regulated genes and 12 TIFY family genes in the down-regulated genes. There were 37 differential genes enriched in the SA pathway, of which 36 were

up-regulated genes; one was a down-regulated gene, and six were up-regulated genes, NPR family genes, 13 were TGA family genes, and 17 were disease-associated proteins.

1.6.4 Effect of grafting on the expression of fatty acid chain elongation related genes

Very long chain fatty acids (VLCFAs) refer to fatty acids with a hydrocarbon chain length of more than 18 carbon atoms. VLCFAs are not only the main components of the structure of biological membranes, but also participate in life activities in the body, as signal molecules. They play a significant role in the growth, development, and resistance to the external environment. During the synthesis of VLCFAs, the first condensation reaction is a rate-limiting step. The reaction is catalyzed by β-ketoacyl CoA synthetase, which is encoded by the 3-ketoacyl-CoA synthase (KCS) gene, which determines the carbon chain length [20]. The down-regulated differential genes in the grafted combination Q/F and ungrafted Q leaves are mainly concentrated in the fatty acid elongation pathway, and all 29 are KCS family genes. However, no significant differential expression of this pathway gene has been found in F/Q versus F.

2 Discussion

2.1 Analysis of graft survival rate

When grafting involves two different species or genera, a lack of affinity may occur, that is, incompatibility between the two plants after grafting. In agricultural production, a lack of compatibility between the scion and the rootstock usually results in incompatibility, resulting in low survival rates of grafted plants. The survival of grafted plants must be the first step in subsequent researches. Therefore, the choice of rootstock and grafting methods is very important. In this study, "split grafting" has been used to graft two potato varieties Qingshu 9 and Favorita, and the results show that the survival rate of self-root grafting can reach 100%, and the survival rate of heterogeneous grafting can reach more than 90%. In this experiment it can be seen that the "splicing method" is suitable for grafting two potato varieties.

2.2 Effects of grafting on potato late blight resistance

In order to explore the influence of rootstock on late scab resistance, this study used potato late blight resistant variety Qingshu 9 and the susceptible cultivar Favorita for grafting. The results showed that the resistance index of Favorita could be increased from susceptibility (S) to moderate resistance (MR) after grafting Favorita with Qingshu 9 as the rootstock. It was worth noting that although the self-grafted Favorita had the same resistance index as the non-grafted Favorita, the self-grafted Favorita had a lower disease index than the non-grafted Favorita. It was seen that both the self-grafted and resistant rootstocks could improve the resistance of the susceptible scions to late blight. After grafting Favorita as rootstock with Qingshu 9, the resistance of Qingshu 9 was reduced from high resistance (HR) to disease resistance (R), indicating that the susceptible variety as rootstock would reduce the resistance of the infected scion to late blight.

2.3 Analysis of the effect of grafting on potato late blight resistance based on transcriptome analysis

In order to further explore the reasons for the change in resistance after grafting, this study used the Illumina HiSeqTM4000 sequencing platform to perform transcriptome sequencing and sequencing on the third leaf from the top of the grafted combination F/Q, Q/F, and non-grafted F, Q, respectively. Through analysis, a total of 8022 differentially expressed genes were screened, including 7542 differentially expressed genes, 3152 up-regulated genes, and 4389 down-regulated genes in F/Q versus F. There were 480 differentially expressed genes in Q/F versus Q, 329 genes were up-regulated, and 151 genes were down-regulated, indicating that a disease-resistant rootstock had a greater effect on a disease-resistant scion than a susceptible rootstock.

2.3.1 Transcriptomics analysis of disease-resistant rootstocks to increase scion resistance

The KEGG enrichment showed that F/Q significantly up-regulated the plant-pathogen interactions and plant MAPK signaling pathway-related genes in the scion leaves compared to the non-grafted F. Among the 36 up-regulated genes in the plant pathogen interaction pathway, 10 were calcium-dependent protein kinases (CDPKs), and among the 31 up-regulated MAPK signaling pathways, six were mitogen-activated protein kinases (MAPKs). When plants were infected by pathogenic bacteria, they underwent a hypersensitivity reaction and produced necrotic cells to prevent further expansion of the pathogen. The CDPKs were the primary reactors that triggered active necrosis of cells. They could also be regulated by Ca^{2+} and phosphorylation and participate in defense signal transduction [5]. The MAPKs could activate the expression of defense genes, thereby improving plant stress resistance [21]. In addition, cyclic nucleotide gated ion-channel proteins (CNGCs), chitin trigger receptor kinases (CERKs), LRR receptor serine/threonine protein kinases, and the WRKY transcription factors in up-regulated genes were also involved in plant resistance. The CNGCs were the most important Ca^{2+} conduction channels involved in plant immune response [22]. Chitin elicitor-binding protein (CEBiP) in rice had to combine with ceramide kinases (CERKs) to transmit chitin signals from intracellular to extracellular, even as CERKs in *Arabidopsis* could independently sense and transmit chitin signals. Therefore, it helped plants to resist fungal attacks, indicating that CERKs played an important role in chitin signaling [23]. The Leucine Rich Repeat-Lectin receptor-like kinases (LRR-LRKs) played a significant role in plant growth and disease resistance. Recent studies showed that many of the externally induced resistance responses and R-gene-mediated immune responses were related to the *LRR-LRK* gene, such as the *ArabidopsisFLS2* gene, which senses flagellin, the *Xa21* gene that resists bacterial wilt in rice, and so on [24]. Among the signal transduction pathways in plants, the SA pathway had the most up-regulated genes. A total of 36 genes were up-regulated, which were enriched in the SA receptor NPR, transcription factor TGA family genes, and disease-related proteins, all of which were important genes in the SA pathway. Among the 19 differential genes in the JA pathway, only four genes were up-regulated and 15 genes were down-regulated. The down-regulated genes were mainly concentrated in the TIFY family. Besides, the JAZ protein and MYC were also down-regulated. The TIFY family was plant-specific and played a key role in, plant growth and development, hormone signal transduction, and stress response. The JAZ protein of

the TIFY family interacted with MYC2 to mediate jasmonic acid signal transduction in response to various abiotic stresses [25]. Both SA and JA were involved in the systematic response of plants to resist stress. However, SA was often considered as an antagonist of JA [26]. The differential expression of genes in the two pathways of this study also confirmed this result. The above results of F/Q versus F KEGG enrichment of differential genes, further elucidated the reasons for the increased resistance to late blight of Favorita after grafting with Qingshu 9.

2.3.2 Transcriptomics analysis of susceptible rootstocks to reduce scion resistance

The results of *in vitro* leaf resistance detection showed that the resistance of scion Qingshu 9 decreased after grafting with Favorita. Transcriptome analysis of Q/F versus Q showed that the down-regulated genes were mainly concentrated in the *KCS* genes in the fatty acid elongation pathway. KCS is a key enzyme that prolongs ultra-long-chain fatty acids. It is responsible for the synthesis of precursors of waxy components. Wax components on the surface of the plant body wax are important parts of the plant defense system. It plays an important role in preventing infection by pathogens, herbivorous insects, and resisting environmental stresses such as drought, ultraviolet damage, and frost [27]. The plant late blight resistance is reduced or related to this.

3 Conclusions

Grafting technology has a long history and is widely used in agriculture. Besides, it is known that after two plants are grafted, the growth and characteristics of the grafted plants will change. Furthermore, this is caused by the material exchange between the scion and the rootstock. Many more studies have shown that not only plant hormones, small molecule metabolites, sugars, inorganic salts, and the like, can be transported between anvils, and large molecular materials such as DNA, RNA, and proteins can also be transmitted between anvils [28]. However, the relation between transport processes and plant phenotypic changes has little to know.

Our results show that using potato resistant varieties as rootstocks can increase resistance to late blight, and using susceptible varieties as rootstocks can reduce resistance to late blight. From the results of transcriptome sequencing, it can be seen that when disease-resistant varieties are used as rootstocks, plant pathogen interactions, plant MAPK signaling pathways, and related genes on SA synthesis pathways in susceptible scions, are significantly up-regulated. When using susceptible varieties as rootstocks, β-ketoacyl-CoA synthase-related genes are significantly down-regulated in disease-resistant scions. This suggests that resistance-related genes may be transferred to the scion part through the rootstocks after grafting susceptible and disease-resistant potatoes with each other. A further study will focus on the transfer mode of the related genes of potato late blight resistance between scion and rootstock, after grafting, and further explore the reasons for the change in potato late blight after grafting.

4 Materials And Methods

4.1 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.

4.2 Methods

4.2.1 Grafting method

Grafting was performed using the “splice method.” In particular, virus-free potato pieces were planted in sterile nutrient soil. Four-week-old plants with consistent growth and non-hollow branches were selected for grafting. Healthy, young, 4-5-cm long shoots with four to five leaves were used as scions. Rootstocks were 2-3- cm above the soil level with two to three leaves. 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, and the seedlings were covered with plastic cups (Fig. 4). The entire growth process took place in a glasshouse.

4.2.2 Graft survival-rate statistics

On June 26, 2019, in the glass greenhouse of the Institute of Agricultural Bioengineering of the Guizhou University, two varieties of virus-free potato blocks, with only one bud eye, were sown in an 11-cm diameter flower pot filled with sterile nutrient soil. Grafting was performed on July 26, 2019 (Fig. 5), 33 plants were grafted for each combination, and the survival rate of the grafted seedlings was measured after seven days.

4.2.3 Identification of late blight resistance after grafting

After the growth of the grafted plants was stable, the resistance to late blight was determined with the ungrafted Favorita (F) and Qingshu 9 (Q) sowed on June 26, 2019. Late blight resistance was identified using the *in vitro* leaf inoculum method. Before inoculation, *Phytophthora infestans* that was cultured for 15 days was eluted into test tubes with sterile water, filtered with 1-2 layers of filter paper, and microscopy was used to make sporangia. When the concentration reached 20-25 cells/ μL, it was placed in a refrigerator at 4°C for 1 h to promote the release of zoospores. Then a consistent and healthy third leaf from top to bottom was taken for inoculation. 30 plants were taken for the ungrafted F and Q and for the grafted F/F, Q/Q, Q/F and F/Q. The isolated leaves were placed backside up in a plastic petri dish, covered with wet filter paper of the same size and sprayed with 2 mL of distilled water, and a pipette was used to inoculate a 20 μL suspension of *Phytophthora infestans* to the side of the main vein on the back of the leaf. After inoculation, the Petri dish was sealed with a Parafilm sealing film, the temperature was controlled at 22°C, and the light condition was 16 h/8 h light/dark. The leaf disease was checked every day and attention was paid to moisture retention. Onset occurred on the fifth day after inoculation, and the size of the diseased area was measured on the seventh day. The longest (L) and widest (W) diseased

spots were recorded during the measurement (length and width being perpendicular), using the formula A = 1/4 × π × L × W, to count the lesion area. The hazard classification criteria were based on the index of the late blight disease in potato leaves [19], level 1: the area of the lesion was less than 3% or no disease; level 2: the area of the lesion was between 3% and 10%, which meant no chlorosis and water immersion around the allergic dead spots; level 3: the area of the lesion was between 10% and 30%, and the surrounding area was soaked with white mycelia; level 4: the area of the lesion was between 30% and 60%, obvious white mycelia appeared; level 5: the lesion area was greater than 60%, and there was obvious rot. Disease index (DI) = (Σ disease level value × number of disease-level plants)/ (highest disease level value × number of surveyed plants) × 100%.

4.2.4 Transcriptome sequencing after grafting

On August 26, 2009, we took the third blade from the top to the Illumina sequencing platform for transcriptome sequencing with the grafted combination of F/Q, Q/F and ungrafted F and Q which are all consistent and healthy. There were three organisms. RNA extraction and transcriptome sequencing were performed by Beijing NuoheZhiyuan Technology Co., Ltd.

Declarations

Ethics approval and consent to participate

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

Consent for publication

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

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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).

Authors' contributions

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

Acknowledgments

My deepest gratitude goes first and foremost to Professor Degang Zhao, my supervisor, for his constant encouragement and guidance. He has walked me through all the stages of this study. Without his consistent and illuminating instruction, this study could not have reached its present form.

Second, I would like to express others teachers in laboratory, who have instructed and helped me a lot during my study. I also owe my sincere gratitude to my friends and my fellow classmates who gave me their help and time in listening to me and helping me work out my problems during the difficult course of the study.

Last my thanks would go to the 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), for providing financial support. Thanks for Biotechnology Institute of the Guizhou Academy of Agricultural Sciences and Guizhou University/Guizhou Provincial Biochemical Engineering Center, for providing experimental materials.

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Figures

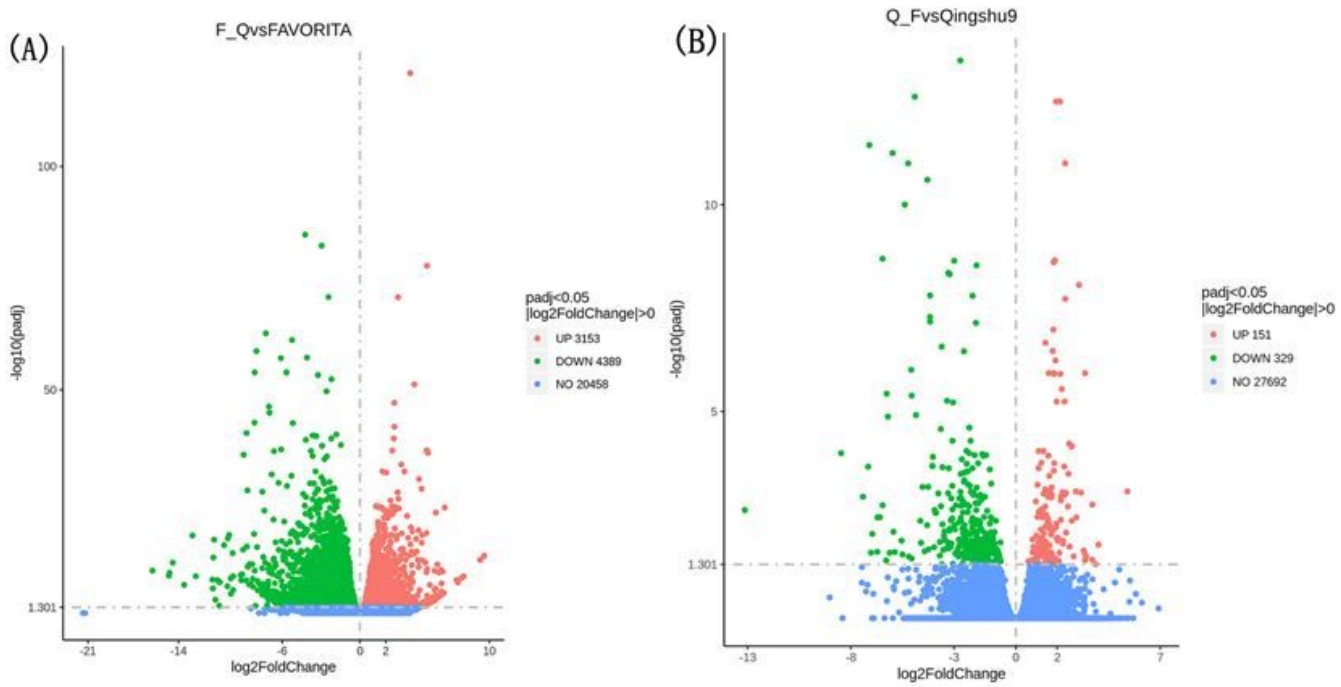

Figure 1

Distribution and expression levels of differentially expressed genes in different grafting combinations of potatoes Note: (A), (B): Distribution of differentially expressed genes

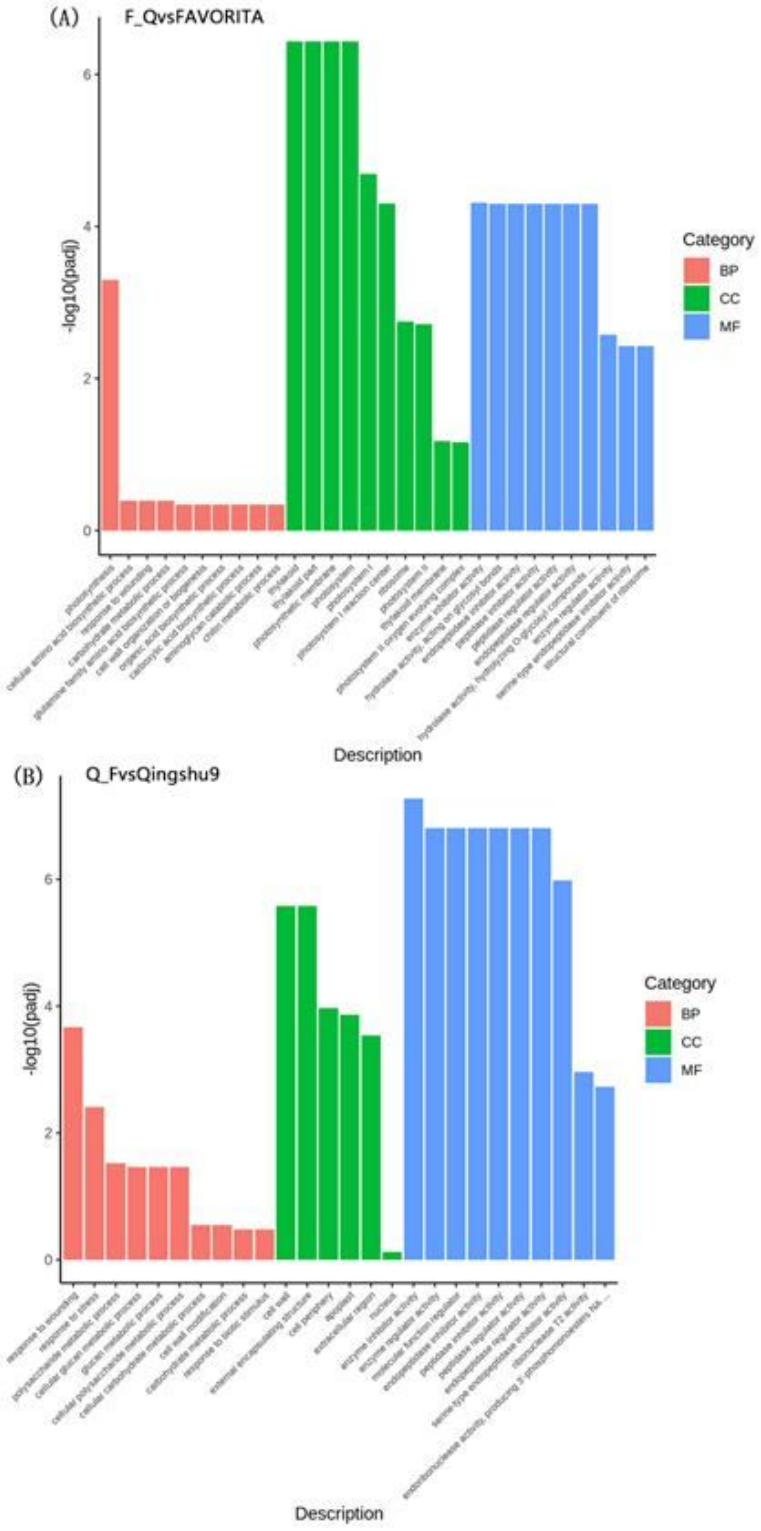

Figure 2

GO classification of differentially expressed genes

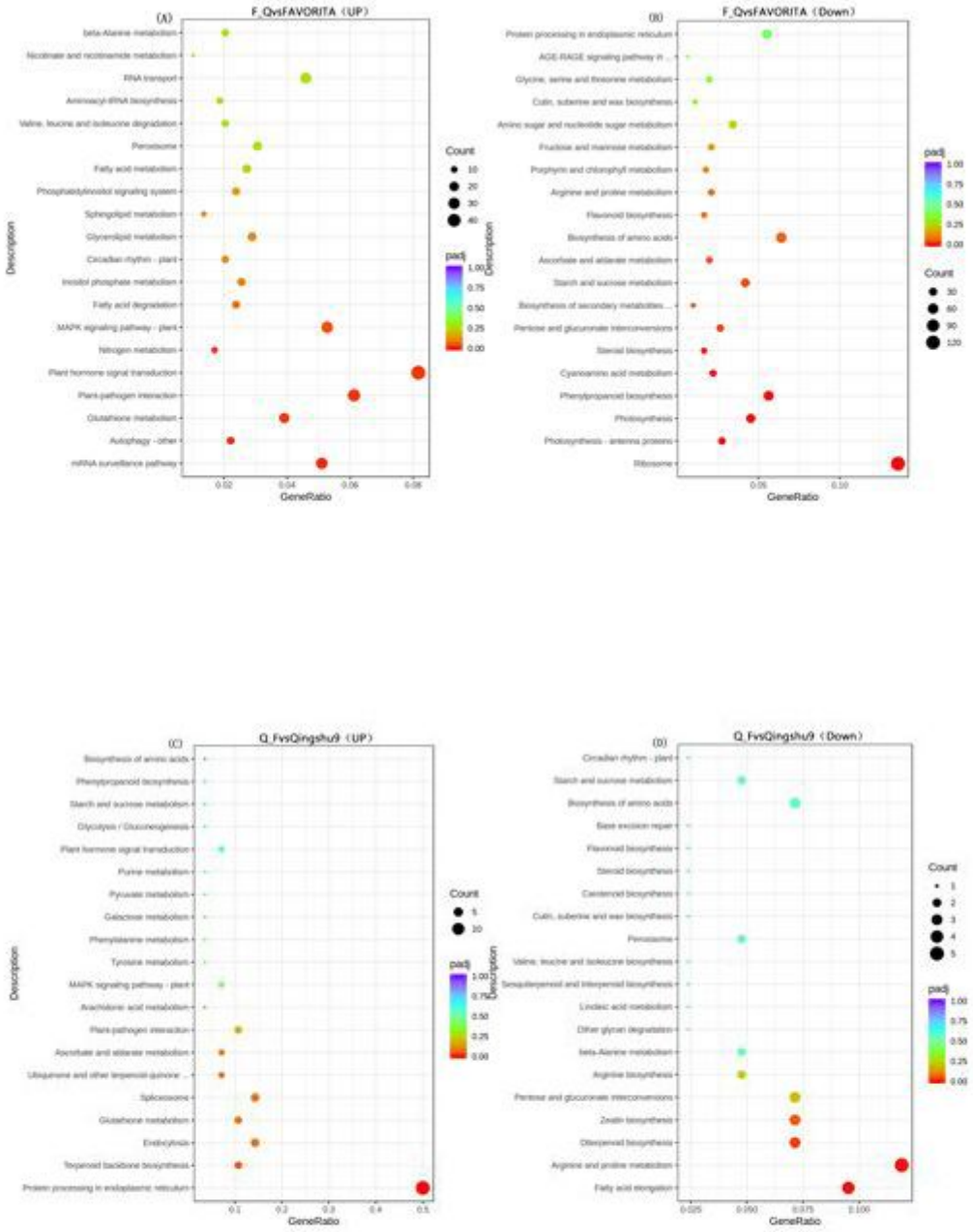

Figure 3

Scatter diagram of KEGG enrichment

Figure 4

Images illustrating the “splice method” of grafting used in this study.

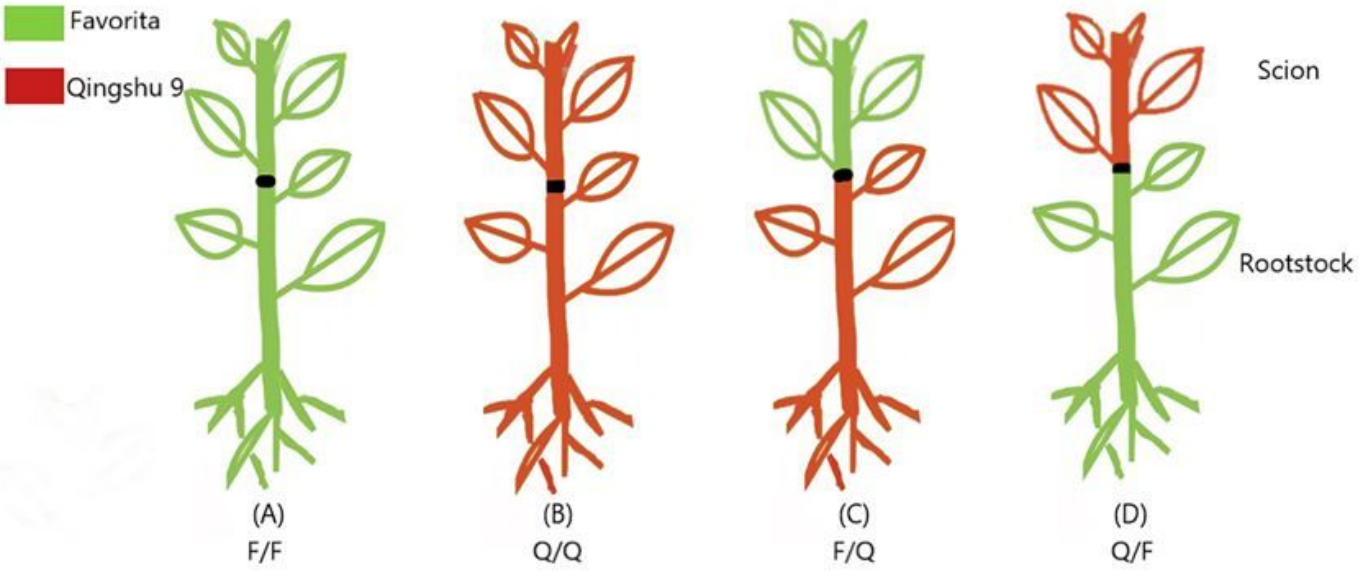

Figure 5

Grafting diagram of Favorita and Qingshu 9 Note: Favorita (F); Qingshu 9 (Q); Scion/Rootstock (A/B). (The same as below)