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INTEGRATED MULTI-OMICS ANALYSIS REVEALS MOLECULAR INTERACTIONS IN COMPLEX BIOLOGICAL SYSTEMS

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ABSTRACT

The explanation of the molecular processes of complex biological systems is one of the key challenges of contemporary plant biology. Grafting is commonly used to promote the crop production, stress tolerance and fruit quality but the relation biologically at the molecular level of graft union development and rootstock-scion interaction has not been well understood. An integrated multi-omics approach was used in this study to examine the interactions at a molecular level in the development of grafts with a combination of transcriptomic and metabolomic analyses. High-throughput sequencing and metabolic profiling were done using critical periods in graft healing and then analyzed using differential expression and pathways enrichment. The findings showed that there was dynamic reprogramming of gene expression and accumulation of metabolites especially within pathways related to hormone signaling, carbohydrate metabolism, and vascular differentiation. Simultaneous analysis showed that there is marked cross-talk between transcriptional and metabolic networks suggesting that there is concerted regulation during graft union formation. Moreover, network-based analyses revealed essential hub genes and metabolites that may regulate the healing of grafts and successful integration of its functions. These discoveries offer a more systems level-in-depth view of molecular interactions in the development of the graft and make the approach of multi-omics integration an effective discovery of regulatory processes in complex biological systems. The work will help to further the molecular knowledge of graft biology, and provide the basis to enhance the efficiency of grafting and crop performance with specific biological interventions.

KEYWORDS: Multi-omics integration, graft union formation, rootstock–scion interaction, transcriptomics, metabolomics, molecular interactions, regulatory networks, hormone signaling, plant grafting, systems biology.

1. INTRODUCTION

Grafting is an embraced horticultural practice in improving crop productivity, resistance to biotic and abiotic stresses, and optimization of fruit quality. Rootstock-mediated regulation has been demonstrated to affect the yield performance, fruit size and biochemical composition of crops, including watermelon and tomato significantly (Jordana et al., 2023; Ingram et al., 2022; Nie and Wen, 2023; Sun et al., 2023). Although the phenomenon of graft union formation and functional integration between the rootstock and the scion has been widely used in agricultural practices, the underlying molecular processes behind this phenomenon have not been well understood, which restricts the capacity to optimize the grafting process on the molecular level. The concept of graft healing is a very well coordinated biological process of wound response, the formation of callus, reconnection of the vessel and communication between cells. Recent experiments have highlighted the key position of phytohormones, especially auxin, cytokinin, and signaling molecules thereof, in controlling tissue adhesion and vascular regeneration in the course of graft formation (Serivichyaswat et al., 2024; Duan et al., 2023; Kawaguchi et al., 2024). Additionally, it has identified conserved molecular pathways and regulatory genes as important of driving graft development among plant species (Feng et al., 2024). Another important aspect of graft success and regeneration efficiency is the environmental modulation with the help of hormonal signaling and metabolic activity under the influence of such factors as the intensity of light and temperature (Han et al., 2024; Han et al., 2025). The discoveries in high-throughput technologies of omics have made it possible to investigate biological

systems on various levels extensively. Transcriptomic and metabolomic analyses have offered useful information on the dynamics of gene expression and metabolic reprogramming throughout the graft development (Kaleem et al., 2025; Lin et al., 2024; Wang et al., 2023). However, the majority of the existing studies have done single-omics-based analysis, and this limits the capacity to study the intricate, system-wide interactions that underlie graft healing and functional integration.

To address these shortcomings, integrated multi-omics techniques have become potent tools of explaining complicated molecular networks and determining important regulatory elements. With both transcriptomic and metabolomic data, coordinated regulatory processes and interaction networks controlling biological processes can be uncovered. We use the multi-omics integration framework to examine the molecular interaction during the graft development in this research with the goal of obtaining the insight on the system level of understanding the biological response of graft and consequently bypassing the gap between the molecular data and its functional interpretation.

2. RELATED WORK

Recent developments in technology in the area of plant grafting have been drawn more into an interest in the molecular processes that underlie the formation of graft unions using high-throughput omics technology. The transcriptomic investigations have shown changes in gene expression which are dynamic in wound healing, vascular differentiation, and tissue regeneration during graft development (Mo et al., 2024; Xie et al., 2022). Such studies demonstrate that there are conserved regulation pathways that regulate graft healing processes in various plant species. Moreover, co-expression networks have been used to determine gene modules related to graft success, which can be used to understand transcriptional regulation in the context of tissue integration (Xie et al., 2022). Metabolomic studies have also helped in the biochemical re-programming in the formation of grafts. Initial longitudinal metabolite profiling research has revealed the metabolites of primary metabolism and signaling pathways that are important in the development and compatibility of graft union (Loupit et al., 2022). Recent studies that combine metabolomics with sensory and physiological characteristics have shown the way in which metabolic alterations in fruit caused by rootstock can affect the quality and development of fruits (Kaleem et al., 2022). These results indicate that metabolic control is essential in coordinating the graft healing and functional integration. Multi-omics integration has now become an effective way of responding to system interactions. Transcriptomic and metabolomic data studies have also demonstrated the concerted regulation in gene expression with metabolite accumulation in the development of grafts (Kaleem et al., 2025; Lin et al., 2024; Wang et al., 2023). They allow recognizing major pathways in terms of hormone signaling, carbohydrate metabolism, and vascular development, and give a more comprehensive picture regarding graft biology. Moreover, recent studies have also singled out certain regulatory genes and enzymes (fructokinase and auxin transport-related proteins) to promote graft union and efficiency of healing (Kadeer et al., 2025; Wang et al., 2024). Along with the molecular and metabolic regulation, source- sink processes and physiological interactions between the rootstock and the scion have been found to impact the development of the grafts. Investigations of heterografts have shown that signals produced by rootstock do adjust carbohydrate distribution and metabolic equilibrium, thus aiding graft union formation and development (Pu et al., 2024). Hormonal responses and antioxidant activities have also been noted to be important in the regulation of graft healing in various physiological conditions (Shi et al., 2023). Plant metabolism and physiological reactions are also affected indirectly (particularly light intensity and spectral combination) by the environmental factors, which is further known to have an indirect impact on graft success and development (Tang et al., 2022; Utasi et al., 2023; Yang et al., 2023). These results suggest that the process of graft healing is controlled by an intricate interaction of molecular, metabolic and environmental signals.

With these advances, there are still a number of challenges. The majority of studies carried out so far are dedicated to one of the layers of omics or a small set of strategies to integrate, which limits the capabilities of studying cross-talks between the molecular networks. Also, existing methods typically do not provide such a network-scale analysis that could help to distinguish the important regulatory centers and routes of interaction. Hence, systematic multi-omics integration frameworks that integrate transcriptomic and metabolomic data with network-based analysis to unravel coordinated interactions between molecules in graft development are in dire need. In this regard, the current work does not focus on these shortcomings and instead attempts to combine the multi-omics method as a whole to examine the interactions of the molecules in the process of graft healing. This effort will utilize cross-omics integration and network modeling to discover the major regulatory elements and offer a systems perspective of graft union development.

3 3. MATERIALS AND METHODS

3.1 Plant Materials and Experimental Design

A compatible rootstockscion combination was selected according to physiological compatibility, and agronomic relevance was used to make grafted plant systems. Both rootstock and scion had healthy and uniform seedlings to reduce biological variation. A standard splice grafting technique was used to perform grafting under controlled conditions. After grafting, the plants were kept in a healing chamber that was controlled in terms of temperature, humidity, and light to have an optimum grafting union. Care was taken to monitor and maintain the environmental parameters to minimize external variability. The main graft union development experimental design and time-

dependent sampling plan is shown in Fig. 1. The samples were taken at various predetermined time intervals during important phases of graft healing, such as early wound response, callus formation, and vascular reconnection. Graft junction tissue samples were taken at every stage and frozen in liquid nitrogen and stored at -80 C to be used later in carry out molecular analyses. Each time point included the biological replicas to provide the statistical reliability and reproducibility of the results.

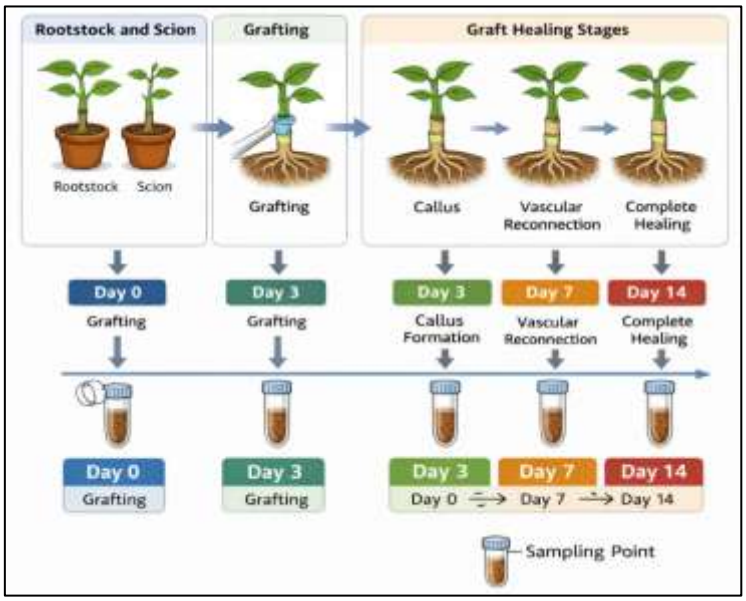

Fig. 1. Experimental Design and Sampling Timeline for Graft Union Development

3.2 Transcriptomic Analysis

An established RNA isolation protocol was used to extract total RNA in graft junction tissues to maintain high purity and integrity. Spectrophotometric and electrophoretic techniques were used to determine the quality and concentration of RNA. Sequencing libraries made of high quality RNA were subsequently sequenced in high throughput using next-generation sequencing platforms. Raw sequencing reads were handled in a bioinformatics pipeline, which included quality control, adapter removal, and low-quality read filtration. Appropriate alignment tool was used to align clean reads to a reference genome and quantify expression levels of genes. Statistical models were used to perform a differential analysis of gene expression to take into consideration the biological variation. Defined thresholds of fold change were used to identify genes that show significant changes in expression, as well as defined thresholds of statistical significance. The functional analysis and enrichment analysis were then conducted to determine biological processes and pathways of differentially expressed genes.

3.3 Metabolomic Profiling

Optimized solvent-based extraction methods were used to extract a wide variety of primary and secondary metabolites. The advanced mass spectrometry platforms, e.g., liquid chromatography–mass spectrometry (LC-MS), were used to analyze extracted samples to provide high sensitivity and accuracy of the metabolite detection. Raw metabolomic data was subjected to procedures of normalization, peak detection and alignment. The identification of metabolites was done through a comparison with reference databases and spectral libraries. The relative metabolite abundances were determined through quantitative analysis in various stages of graft healing. Statistical techniques were employed in determining compounds that had significant variation through a differential metabolite analysis. Pathway enrichment analysis was subsequently conducted in order to associate the changes in metabolites to the biological processes in graft development.

3.4 Multi-Omics Integration

A combination of correlation-based and pathway-driven analysis methods was applied to correlate transcriptomic and metabolomic data to gain a comprehensive picture of the interactions among the molecules, as shown in Fig. 1. To detect significant gene-metabolite associations, pairwise correlation analysis was used to determine significant associations between the expression levels and abundance of metabolites. Integrated pathway analysis has been used to overlay genes and metabolites on to shared biological pathways in order to discover integrated regulatory processes. The main analytical approach was the use of statistical techniques to perform filtering of the significant interactions and filter noise in the combined data. This methodology has helped to identify the cross-talk of transcriptional and metabolic networks, which gave clues into how graft healing processes are co-ordinately regulated.

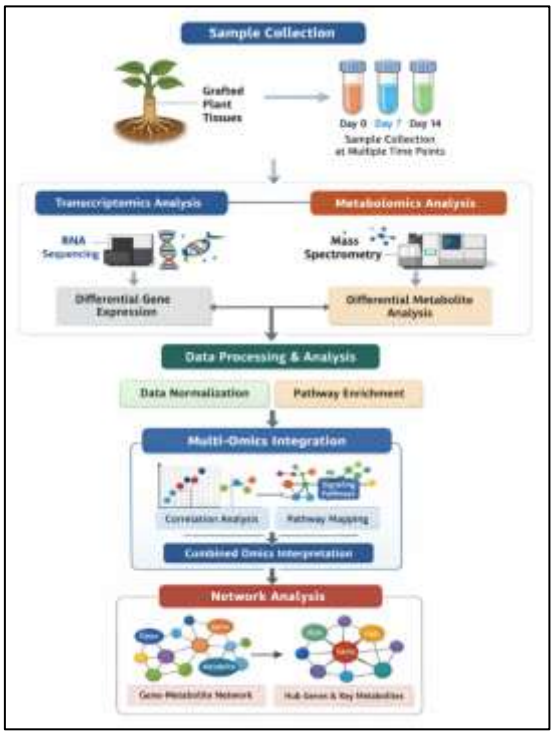

Fig. 2. Integrated Multi-Omics Workflow for Transcriptomic and Metabolomic Analysis of Graft Development

3.5 Network Construction and Topological Analysis

The network of gene-metabolite interaction was created according to statistically significant correlations out of the integrated dataset, in the form of Fig. 3. Network analysis tools were used to analyze the structural properties of the resulting networks and visualize them. The important topological parameters, such as degree centrality, betweenness centrality and clustering coefficients were computed in order to help determine the significance of individual nodes in the network. Using a central position in the network, hub genes and metabolites were identified, which suggests they have a regulatory importance in forming graft unions. The detection of network modules was carried out to determine clusters of significantly interconnected nodes, which are functional modules related to certain biological processes. These studies formed a systems perspective of regulatory architecture of graft formation and pinpointed important components that mediate molecular interactions.

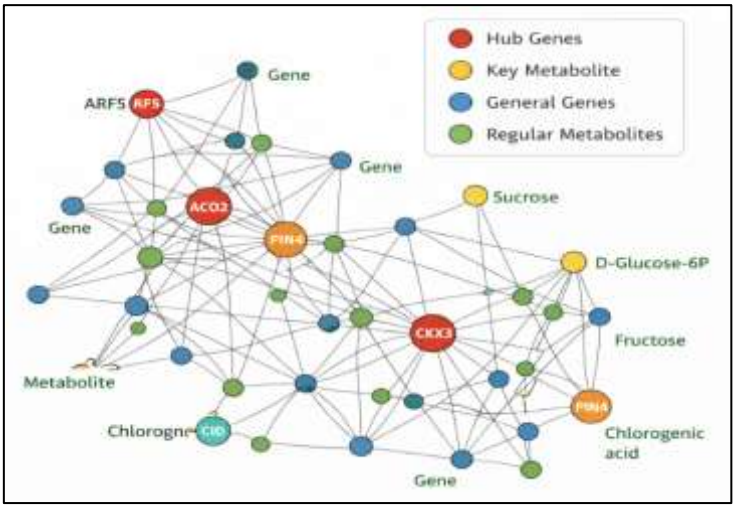

Fig. 3. Gene–Metabolite Regulatory Network Revealing Key Hub Genes and Metabolic Interactions During Graft Development

4. RESULTS

4.1 Differential Gene Expression Analysis

Transcriptomic profiling in various graft developmental phases, revealed a significant re-organization of genes expression patterns, suggesting the presence of active molecular responses during graft healing. Numerous differentially expressed genes (DEGs) were found, and the specific expression patterns were associated with early wound response, formation of the callus, and reconnecting of the vessels. Hormone signaling pathway genes,

especially auxin and cytokinin-related genes, were mainly upregulated, indicating their involvement in important roles in the organization of tissue regeneration. Moreover, genes of stress response and cell wall modification were more active, which indicates the involvement of defense and repair in the process of graft union formation. The analysis of functional enrichment also showed that there were remarkable pathways associated with vascular differentiation and signal transduction. These findings are in line with the previous reports that identify hormone-mediated regulation as a driving force behind the process of graft healing (Serivichyaswat et al., 2024).

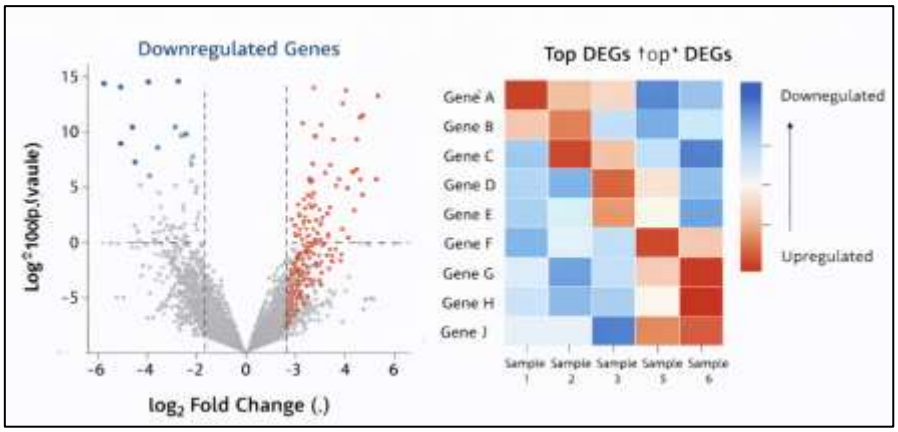

Fig. 4. Differential gene expression analysis

4.2 Metabolomic Changes During Graft Healing

Metabolomic profiling showed changes in metabolite makeup dynamically to develop grafts. Both primary and secondary metabolites showed significant changes, which are indicative of metabolic reprogramming during graft healing. It is important to note that there were significant alterations in the pathways of carbohydrate metabolism, as the later the graft union was formed, the more sugars and energy-related metabolites accumulated. This is indicated by the changes that indicate increased metabolic rates needed to regenerate tissue and develop the vascular network. The identified changes in the metabolite profiles correspond to other works that have reported the importance of sugar metabolism and energy balance in promoting the success of grafts and their development (Kaleem et al., 2022). Moreover, the levels of metabolites that were related to the antioxidant activity were also increased suggesting the ability to reduce oxidative stress when healing.

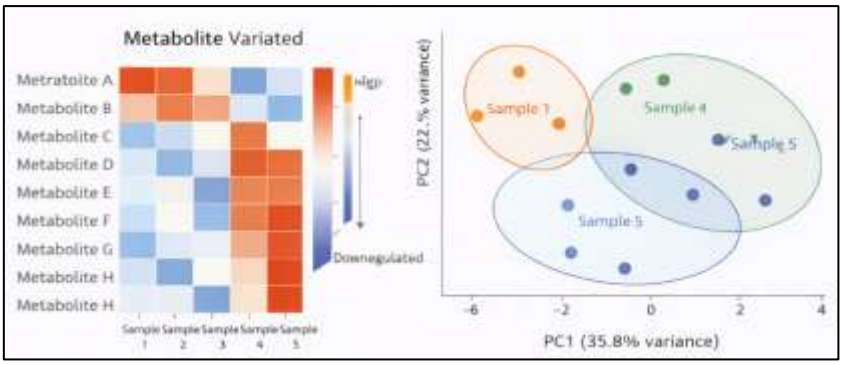

Fig. 5. Metabolomic profiling

4.3 Integrated Multi-Omics Analysis

Combining transcriptomic and metabolomic data sets gave an all-encompassing perspective of molecular interplay in graft development. The analysis was done through correlation analysis which showed that there were strong correlations between the metabolites abundance and the expression of the genes, which is an indication that there are coordinated regulations among the molecular layers. Transcriptomic and metabolomic profiles showed a strong cross-talk with key pathways, such as hormone signaling, carbohydrate metabolism, and vascular development. A pathway integration analysis showed that transcriptional regulation has a direct effect on energy-producing and structural development metabolic pathways. These results demonstrate the significance of multi-layered control in graft repair and demonstrate that individual-omics methods may be insufficient to comprehensively describe the biological processes.

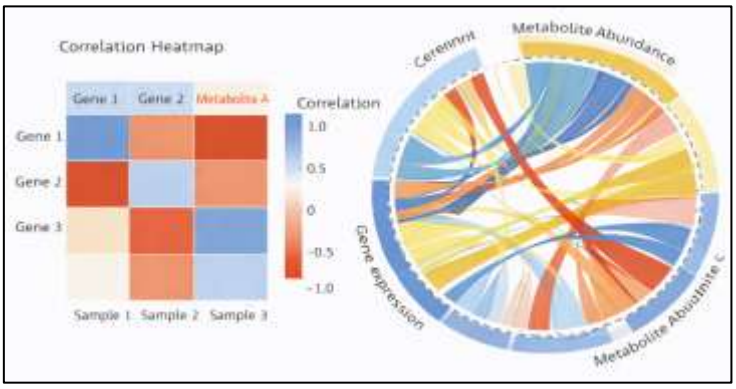

Fig. 6. Multi-omics integration

4.4 Regulatory Network Analysis

Gene-metabolite interaction network analysis demonstrated that there is a well-linked regulatory system that controls the development of grafts. The network constructed (Fig. 3) was made up of various nodes that symbolised genes and metabolites and the connections between them were denoted by significant interactions. Topological analysis of the data revealed some hub genes and metabolites, which are highly central and hints at their essential roles in the process of graft union. These hub elements were mostly linked to hormone signaling pathways, carbohydrate metabolism, and vascular differentiation and support their role in graft healing. Analysis of network modules also identified groups of functionally related genes and metabolites, which suggests coordinated regulatory modules that control particular biological processes. The finding of these important regulators offers good opportunities to be used as future research to enhance graft performance and crop yield.

5. DISCUSSION

The current work gives a holistic systems-level view of the molecular interactions in the development of grafts using multi-omics analysis. Those findings indicate that the graft healing process is a very well-coordinated event that requires transcriptional reprogramming and metabolic changes, as well as network-level control. The subsequent up-regulation of the hormone-related genes, especially the auxin signaling genes, validates the leading role of the phytohormones in transducing the tissue adhesion and vascular regeneration. Such results are in line with past reports highlighting hormone-mediated control in graft union development (Serivichyaswat et al., 2024; Duan et al., 2023). In addition, the energetic variations in carbohydrate metabolism support the metabolic balance and energy supply as a facilitator of cellular mechanisms during graft healing in accordance with the previous metabolomic research (Kaleem et al., 2022). Notably, transcriptomic and metabolomic data integration to show cross-talk between molecular layers is coordinated and cannot be obtained with single-omics analyses. This highlights the benefit of multi-omics methods in the discovery of complicated regulatory systems. The network analysis also revealed the essential hub genes and metabolites that could be the central regulators of graft development, which could serve as the targets of genetic and agronomic manipulations. In comparison to all the past studies, which mostly study individual components of the molecules, this article provides a more global view in merging several layers of data and analyzing the data using networks. Nonetheless, additional confirmation by using functional experiments is necessary to allow the identification of the functions of identified hub genes and metabolites.

On balance, this article contributes to the knowledge about the graft biology and the possibility of multi-omics integration as an effective method to decipher complicated biological processes and enhance crop performance.

CONCLUSION

This paper proposes a multi-omics framework of the aggregate mechanism that explains how molecular interactions during graft development in the context of complex biological systems. The analysis can be used to identify the dynamic reprogramming of gene expression and metabolites patterns during major steps of graft healing, making use of the combination of transcriptomic and metabolomic analyses. The findings emphasize the balanced role of the hormone signaling pathways, carbohydrate metabolism and vascular differentiation pathways in the regulation of graft union formation. Moreover, the network analysis facilitated the identification of key hub genes and metabolites, which offered information on regulatory frameworks of the rootstockscion interactions. The main contribution of the work is its systems level, in which several layers of molecules are considered in order to reveal the coordinated mechanisms of regulation, which cannot be found in single-omics studies. This integrative approach develops the biological insight into graft biology and provides an analytical framework that can be used to understand other complicated biological processes. Secondly, the determination of regulatory elements that are important offers possible areas of genetic enhancement and optimization of grafting in horticultural plants. Although such improvements have been made, additional experimental and functional research is needed to establish the functions of the discovered hub genes and metabolites. Further studies are needed in the future, involving the addition of other layers of omics, including proteomics and epigenomics, in

order to obtain a more detailed picture of regulatory processes. In addition, graft success and trait optimization predictive modeling might be further improved by applying machine learning methods. In general, this work can serve as a scalable basis to further develop molecular research in the plant system and enhance crop performance based on data-driven approaches.

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