MULTIOMICS FOR PLANT SYSTEM BIOLOGY

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INTRODUCTION

The incorporation of multi-omics datasets has become a fundamental aspect of recent research in molecular biology, and genetic engineering. The development of technological progress including next-generation sequencing and mass spectrometry coupled with chromatography, has enabled generation of high-throughput data. All omic studies including, transcriptome, proteome, and metabolome are the central datasets in systems biology. Nonetheless, effectively handling and linking these extensive datasets presents challenges. Consequently, there is a demand for a well suited methodological approach for multi-omics integration (MOI), which encompasses the extraction, integration, and meaningful correlation of diverse datasets to unveil intricate biological insights.

MOI has been extensively studied in various domains, including humans, animals, and microorganisms. However, implementing MOI in the context of plants comes with unique challenges due diversity of metabolic processes, the presence of varied genomes, especially in non-model plant species, and intricate interactions with symbiotic organisms. Numerous reviews have addressed the application of plant MOI in fields such as systems biology, precision breeding, and biotechnology. Nevertheless, the rapid proliferation of high-throughput technologies and the generation of large omics datasets can be overwhelming for researchers who are not well-versed in this area, potentially leading to misinterpretations. The process of

selecting suitable software platforms, statistical methods, and modeling techniques is vital but may appear intimidating to those lacking expertise.

Element-Based Integration (Level 1): This unbiased approach includes correlation, clustering, and multivariate analyses to find statistical associations between elements from different datasets.

Pathway-Based Integration (Level 2): This knowledge-based approach focuses on co-expression and mapping-based methods, incorporating prior knowledge of biological pathways.

Mathematical Integration (Level 3): This level involves differential and genome-scale analyses, aiming to generate mathematical models for hypothesis testing and in-depth quantitative understanding of biological systems.

Each of these levels plays a distinct role in the MOI workflow as discussed here based on the article Jamil IN, Remali J, Azizan KA, Nor Muhammad NA, Arita M, Goh HH, Aizat WM. Systematic Multi-Omics Integration (MOI) Approach in Plant Systems Biology. Front Plant Sci. 2020 Jun 26;11:944. doi: 10.3389/fpls.2020.00944.

ELEMENT-BASED APPROACH

Clustering Analysis

Clustering analysis is a valuable method for grouping omics datasets having similar attributes, including expression levels, in order to reveal associations and patterns of genes and proteins. Two main approaches are commonly used for clustering: hierarchical clustering analysis (HCA) and non-hierarchical methods. In the context of integrating multiple omics datasets, non-hierarchical methods are often more applicable, and machine learning algorithms like k-means clustering and random forest have been widely employed for this purpose.

K-means clustering is a technique that groups data points, typically derived from omics expression data, into distinct clusters based on their expression patterns. This approach helps

identify clear and differentiated groupings that reveal various expression patterns. On the other hand, random forest is a machine learning method that applies training data obtained from omics experiments related to genes, proteins, or metabolites. It associates these elements with specific characteristics or traits of interest. Both of these approaches have found widespread application in phyta-multi-omics research.

For example, in a study by Keller and Simm (2018), two modes of protein translation during tomato pollen development were identified by comparing transcriptome and proteome data under control and heat stress conditions. The researchers used the k-means clustering method to categorise expressed transcripts and proteins into developmental phases. This study shed light on the mechanism of protein translation by distinguishing between direct translation, in which transcript-protein pairs correlated significantly at a specific stage, and delayed translation, in which certain proteins showed differential expression in the following stage after their corresponding transcripts in the previous stage. This mechanism helps to explain the observed poor association between transcript-protein pairings at specific stages, notably for proteins involved in carbohydrate and energy metabolism. Furthermore, heat-shock proteins were largely altered at the translational level when subjected to heat stress, indicating a quick plant response to stress conditions.

K-means clustering has also been applied to imtegrate multi-omic data from developing cacao seeds and grapefruits. It effectively identified stage-specific clusters, demonstrating synchronous increases in secondary metabolites such flavonoids as well as upregulation of associated biosynthetic enzymes. Network study of grapefruits revealed substantial time-shift correlations between protein and metabolite pairings, indicating that changes in protein abundance may directly impact metabolic regulation throughout fruit growth and ripening. This highlights the significance of comprehensive multi-omics integration in identifying essential regulatory components in plants.

Acharjee et al. (2016) used a random forest strategy to cluster and link multi-omics datasets with particular phenotypic features of potato tubers, such as colour, shape, starch gelatinization, and peeling discolouration. Remarkably, this study revealed strong associations between different omics data and distinct tuber traits. Colour features, for example, were substantially connected with metabolite data, notably carotenoids, but tuber form was mostly impacted by transcripts related to size. This conclusion implies that various omics platforms are more suited to elucidating the underlying processes of certain phenotypic changes, emphasising the necessity of adopting the best appropriate omics strategy for a given inquiry in order to provide meaningful and descriptive results.

Multivariate Analysis

Multivariate analysis is an efficient way for dealing with huge omics datasets, allowing for greater trial design and metadata analysis flexibility. This technique predicts numerous characteristics and trends within datasets, such as identifying connections in variance or covariance and exploring dynamic linkages and topological networks across transcript, protein, and metabolite components (Weckwerth, 2019). Principal component analysis (PCA), partial least squares (PLS), and orthogonal projection to latent structures discriminant analysis (OPLS-DA) are some of the most often used multivariate approaches. For new users, selecting acceptable multivariate methodologies, ideal parameters, and completing model validation might be difficult. As a result, there are several instructional materials on this topic to aid in learning.

Nonetheless, it is critical to recognise the inherent limits of any MOI technique, especially when working with poorly characterised non-model plant species. Future work should focus on enhancing gene and metabolite annotations suited to individual plant species to improve MOI techniques. Furthermore, the development of user-friendly tools that employ machine learning

techniques can aid in the exact reconstruction of metabolic models, improving the area of multi-omics integration. According to research, *A. fumigatus* secretes a protein known as gliotoxin, which works as a virulence factor and supports the hijacking of p11. Gliotoxin is a fungus-produced secondary metabolite that acts as an immunosuppressive agent. It forms a compound with p11 that changes intracellular calcium dynamics.

Furthermore, researchers discovered correlated discriminatory variables linking the effect of heat treatment to ripening homogeneity in early and middle-season Hass avocados using the multivariate dimension reduction discriminant analysis method DIABLO (Data Integration Analysis for Biomarker Discovery Using a Latent Component Method for Omics studies). After a 1-day heat treatment, both omics datasets revealed substantial variations between early and middle-season avocados. Carbon deprivation induced by reduced glycolysis rates following heat treatment might explain this tendency, which could accelerate protein breakdown to supply amino acids for the TCA cycle.

While the elemental technique is effective for merging plant omics data, these datasets are often statistically evaluated, with minimal consideration given to interacting or co-expressed molecules, in addition to the biological mechanisms that underlie them. As a result, the pathway-based approach, which includes pathway-based integration, is required for a more thorough knowledge of the molecular interaction between distinct omics datasets and their biological response of plants under diverse environmental cues.

PATHWAY-BASED APPROACH

Pathway Mapping

Pathway mapping involves the process of aligning omics datasets, whether they are from transcriptomics, proteomics, or metabolomics, with established metabolic pathway databases. One of the widely used databases for referencing plant metabolic databases is the Kyoto Encyclopedia of Genes and Genomes (KEGG). These databases serve as essential resources for annotating pathways and form the foundation for various software tools designed for Multi-Omics Integration (MOI) at the pathway level. Some commonly used software tools for integrating multi-omics data in plant studies include MapMan and PathVisio. MapMan, for example, enables the integration of all -omics data allowing for the visualisation and reconstruction of metabolic pathways, as demonstrated in a Holm oak research. The integration of these omics datasets into MapMan successfully mapped them onto numerous KEGG pathways, shedding light on enriched pathways like the citrate cycle.

Another software tool, PathVisio, was employed to integrate multi-omics data in a study involving Arabidopsis signaling mutant plants. This work demonstrated the relevance of these software tools in identifying the regulation of metabolic pathways in plant multi-omics investigations by showing the influence of a specific medication on stress-response signalling pathways.

While alternative software tools such as IMPaLA, Paintomics, and InCroMAP are available, their applicability in plant MOI are fairly limited. These tools follow distinct principles for combining multi-omics data, either by totaling differentially expressed components or using statistical methods like Fisher's method. It is feasible to manually recreate integrated biochemical pathways in addition to employing digital tools. This approach entails consulting canonical pathways in

databases like KEGG and modifying them for individual species based on annotated enzymes and metabolites. Manual route reconstruction, while time-consuming, can give useful insights on certain pathways. For example, researchers utilising the KEGG database manually recreated pathways linked to isoflavonoid and phenylpropanoid production in soybean infected with the *Phytophthora sojae* fungus and cyst nematode. Similarly, studies on Persicaria minor and sandalwood successfully reconstructed terpenoid and sesquiterpenoid biosynthetic pathways using available KEGG annotations.

However, it's essential to exercise caution when performing cross-species pathway annotation. Simply accepting the best BLAST hit from model plants without considering the significance of conserved domains or functions can lead to unreliable pathway relationships and erroneous conclusions.To guarantee accuracy, researchers should analyse and curate annotation and route mapping data, as well as conduct specific functional investigations for validation.

Co-expression Analysis

Co-expression analysis is an important tool in Multi-Omics Integration (MOI), focusing on statistical correlations between various omics datasets to assess the significance of links between expressed molecules. These interactions are then converted into weighted networks, which may be visualised using tools like R's Weighted Gene Co-expression Network Analysis (WGCNA) or the Cytoscape tool. This technique has been beneficial in identifying critical clusters, modules, and hubs in plant studies that provide biological insights into specific pathways or regulatory chemicals.

In another maize development study, WGCNA was used to integrate transcriptomics, proteomics, and phosphoproteomics data. In an expression atlas, weighted networks were used to investigate the connections between 23 different maize tissues. Surprisingly, co-expressed

transcripts and proteins constructed from transcriptome and proteome datasets had different strongly connected network hubs. This gap might be explained by the fact that only 46% of proteins from the whole transcriptome list were identified. The unique weighted networks from each omics study were then integrated, creating a consensus network that indicated the crucial functions of numerous transcription factors in maize development.

Integration with pathway databases is another type of co-expression analysis. Researchers used the CitrusCyc2.0 database to uncover pathways with the highest association between transcriptional and metabolic data in an integrated transcriptomics and metabolomics analysis of orange (Citrus sinensis). Cytoscape was used to design the network for upregulated and downregulated components such as transcripts and metabolites. The mutant orange cultivar displayed resistance to fungal infections through changes in fatty acid content and the development of a jasmonic acid (JA)-mediated defensive response, according to this study.

MapMan functional annotation was used in maize development research to decode enriched pathways for highly linked co-expressed hubs. These examples demonstrate the effectiveness of co-expression network analysis combined with route databases in understanding plant growth and stress responses. This method divides omics datasets into highly linked modules and hubs for subsequent analysis.

Although software tools for pathway mapping and co-expression analysis help identify relationships between omics datasets and metabolic pathways, these pathway templates can be static and may not account for changes in experimental conditions or organism-specific variations. To accurately predict metabolic changes in response to specific conditions or treatments for a given species, more advanced mathematical-based integration methods will be discussed in the Mathematical-Based Approach.

MATHEMATICAL-BASED APPROACH

Differential Analysis

Among all integration approaches, mathematical-based MOI is the most sophisticated and complete. It necessitates wide coverage of omics data as well as well-characterized plants. The primary purpose of this technique is to develop well-defined differential equations and models in order to get a systems-level knowledge. Identifying system components, establishing system regulation and topology, generating suitable mathematical equations, and lastly choosing and optimising parameters are typical phases in this study.

Differential analysis has been employed in a wide range of plant and fruit research, which may be classed as non-targeted or focused route studies. The creation of a differential equation for protein density during tomato ripening is a non-targeted approach method. This is performed by integrating transcriptomics and proteomics data from nine tomato developmental stages to establish rate constants for translation (kt) and degradation (kd). According to the findings, the equation can predict the expression of more than half of the 2,400 transcript-protein pairs tested, with protein levels mostly governed by translation rates rather than degradation.

Differential analysis may be used in focused pathway analysis to predict the metabolic flow and dynamics of a particular route. The ODE technique, for example, was used to successfully model lignin production in poplar (*Populus trichocarpa*). 21 target genes targeted for RNAi in the monolignol pathway were produced in this work, followed by transcriptomics and targeted proteomics. To describe the impact of gene silencing, a transcript-protein equation was created, and ODEs were utilized to establish chemical kinetics for forecasting levels and fluxes of metabolites. This model accurately predicted the impact of gene mutations on lignin content and

wood characteristics, providing important insights for breeding programmes involving this tree species.

Arabidopsis plants exposed to cold shock were used in another focused pathway study. Microarray data reflecting transcriptomics spanning four cold eras were linked to AraCyc and KEGG metabolic pathways. Differentially Expressed Genes (DEGs) were connected to corresponding metabolites using Matlab's Reporter Metabolic Centric Algorithm, and metabolite and pathway scores were computed. Under cold treatment, a tripartite network model covering genes, metabolites, and pathways identified stress-modulated networks related to carbon, redox, and signal metabolisms.

Furthermore, constraint-based modelling, such as Flux Balance Analysis (FBA), which is frequently performed in Matlab using the COBRA (Constraint-Based Reconstruction and Analysis) toolbox, has been used in mathematical modelling. For example, data from metabolomics, proteomics (enzymatic activity), and growth studies were used to successfully simulate anthocyanin production in grapes (Vitis vinifera). The results showed that anthocyanin metabolic flux was greatly increased during nitrogen deficiency as a way of utilising surplus energy.

These examples demonstrate the value of differential analysis for MOI in plants, whether targeting specific pathways or using a non-targeted approach. As long as enough molecular information is available, this degree of integration can be used in model and non-model species. Several resources for mathematical and flow studies are available, including E-flux and Metabolic Adjustment by Differential Expression, which have been thoroughly evaluated by Fukushima et al. (2014). Differential analysis is also an important component of future genome-scale omics integration.

Genome-Scale Analysis

Historically, differential analysis was primarily associated with the development of stoichiometric equations for specific goals, such as assessing translation rates or metabolic flux inside isolated systems or pathways. To build functioning mathematical models, this top-down technique depended on experimental data.Genome-Scale Modelling (GSM), on the other hand, takes a bottom-up strategy, seeking to build a genome-scale model first through intensive curation before experimental validation. This method aims to thoroughly map metabolic pathways at the organism and cellular levels, taking into account each individual response for a comprehensive mathematical evaluation. The development of genome-scale metabolic reconstructions is divided into four primary steps: drafting a reconstruction using annotated genomes, refining pathways using experimental data, translating the network into a mathematical representation, and lastly verifying and iterating the model for correctness.

Developing GSMs for plants and other eukaryotes is notably more complex than for prokaryotes due to extensive compartmentalization, size, polyploidy, and the presence of numerous diverse secondary metabolic pathways. PlantSEED, a simplified GSM database based on 10 well-annotated plant genomes, is a significant resource for annotating metabolic processes in novel plants in the context of plant metabolic reconstruction. This database is notably valuable for genome-scale reconstructions of primary metabolism. Manual curation, on the other hand, is still required for specialised plant secondary metabolism, which is both rich and species-dependent.

GSM tools like PlantSEED offer researchers a versatile platform for studying metabolic processes across various scales, from single cells to entire plants. PlantSEED, for example, was used to simulate Arabidopsis' complicated root system, combining transcriptomics and metabolomics data. The metabolic flow of indole-3-acetic acid, a key growth regulator in Arabidopsis roots, was

accurately predicted across diverse tissues in this work. It is important to note, however, that PlantSEED's metabolic responses are mostly based on C3 plants such as Arabidopsis and may not include C4 model species such as maize and foxtail millet (Setaria italic). To address this, for these specific circumstances, a C4 genome-scale model (C4GEM) was constructed, followed by the integration of diverse data sets for functional analysis.

Moreover, GSMs have been applied to *Glycine max* using tools like Plant/Eukaryotic and Microbial Metabolomics Systems Resource (PMR) and MetNetDB. This multi-omics data is being used to better understand mechanisms such as starch utilisation and fatty acid buildup in legumes. Rapeseed (Brassica napus), another plant known for its seeds, also underwent metabolic reconstruction. The Bna572+ database update enabled the development of a model using Flux Variability Analysis (FVA), a method within Flux Balance Analysis (FBA).

Despite these advancements, mathematical-based integration, a unique feature of GSMs, can reliably predict species-specific changes or perturbations when supported by extensive database annotations and validated models based on experimental evidence. This includes MFA which employs isotopic labeling techniques to quantify cellular flow states. However, plants present unique challenges due to their diverse cellular and tissue types, as well as organelle compartmentalization. Consequently, plant flux investigations are often limited to homogeneous cellular or tissue samples and those with long metabolic steady states, such as seeds. Researchers must carefully consider these challenges before conducting integrated omics experiments or validating models using mathematical-based integration techniques.

CHALLENGES AND OVERALL VISION

Several problems confront the integrative multi-omics method, including disparities in data, changes in data structure, and noise across technology platforms. These difficulties occur as a

result of the heterogeneous type of data collected by various omics platforms. The bias caused by differences in the identification of molecules such as transcripts, proteins, and metabolites is frequently underestimated.

Furthermore, when dealing with datasets that lack repeatability, give only qualitative information, contain false positives or negatives, and lack adequate metadata to explain observed phenotypic changes, multi-omics integration might be difficult. These are general issues with multi-omics integration, and techniques for dealing with them are described in the literature.

A comparatively simple technique is element-based multi-omics integration, which covers correlation, grouping, and multivariate analytics. Each of these strategies, however, has its own set of restrictions. Correlation analysis, for example, may have limited breadth and insights, and particular correlation approaches may be vulnerable to outliers. Future advancements in this field may include the completion of gene and metabolite annotations for individual species, as well as the investigation of alternate correlation approaches to lessen the influence of outliers.

Pathway-based integration gives biologists intuitive tools, but it may need intermediate to expert-level programming knowledge. Incompletely defined routes for non-model species, as well as ID mismatches between databases, provide obstacles in this type of integration. Future endeavours should focus on standardising data formats for co-expression analyses and supporting research into enzymatic processes and protein interactions.

Metabolic flow simulations and perturbation predictions are possible using a mathematical-based method that includes differential and genome-scale analysis. These alternatives, however, need considerable programming and mathematical abilities. Subjective model selection and parameter choices, the occurrence of redundant chemical components, and modelling regulatory effects are

all challenges. Future enhancements might include the creation of more user-friendly algorithms and comprehensive datasets for model development.

It is crucial to remember that post-translational modifications and environmental variables might have an influence on cellular and biochemical processes, and these elements can be difficult to anticipate based only on metabolic reconstructions. Integrating other omics data, including epigenomics, post-translational modification proteomics, and phenomics, via a "PANOMICS" platform might give a more complete knowledge of plant molecular control. Machine learning, especially deep learning technologies such as artificial neural networks, is predicted to play a critical role in the future integration of various omics data.

While multi-omics techniques have a lot of promise in plant research, analysing large-scale information may be difficult. Collaborative efforts combining varied research teams are critical, particularly when dealing with the complexities of plant organisms. COST Action FA1306 aims to foster collaborative effort and provide methodological recommendations for multi-omics research in plants.

CONCLUSION

Recent advances in omics technology have resulted in the creation of massive databases. As a result, effective and methodical data integration methodologies are required to extract valuable insights and connect data with research objectives. This review presents a three-tiered architecture for Multi-Omics Integration (MOI) that includes three components. These levels range from relatively simple element-based integration approaches like correlation, grouping, and multivariate analysis to more complex route-based techniques like pathway mapping and co-expression analysis. Finally, the most difficult integration requires mathematical methods,

notably differential and genome-scale studies. This complete paradigm is explained within the context of current literature, emphasising its practical applicability in plant MOI investigations.

Nonetheless, it is critical to recognise the inherent limits of any MOI technique, especially when working with poorly characterised non-model plant species. Future work should focus on enhancing gene and metabolite annotations suited to individual plant species to improve MOI techniques. Furthermore, the development of user-friendly tools that employ machine learning techniques can aid in the exact reconstruction of metabolic models, improving the area of multi-omics integration. According to research, *A. fumigatus* secretes a protein known as gliotoxin, which works as a virulence factor and supports the hijacking of p11. Gliotoxin is a fungus-produced secondary metabolite that acts as an immunosuppressive agent. It forms a compound with p11 that changes intracellular calcium dynamics.

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