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Network biology to uncover functional and structural properties of the plant immune system

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Abstract

In the last two decades, advances in network science have facilitated the discovery of important systems' entities in diverse biological networks. This graph-based technique has revealed numerous emergent properties of a system that enable us to understand several complex biological processes including plant immune systems. With the accumulation of multiomics data sets, the comprehensive understanding of plant–pathogen interactions can be achieved through the analyses and efficacious integration of multidimensional qualitative and quantitative relationships among the components of hosts and their microbes. This review highlights comparative network topology analyses in plant–pathogen co-expression networks and interactomes, outlines dynamic network modeling for cell-specific immune regulatory networks, and discusses the new frontiers of single-cell sequencing as well as multiomics data integration that are necessary for unraveling the intricacies of plant immune systems.

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Keywords

Multiomics, Integration, Network topology, Systems biology, Host–microbe interaction, Plant immunity.

List of abbreviations

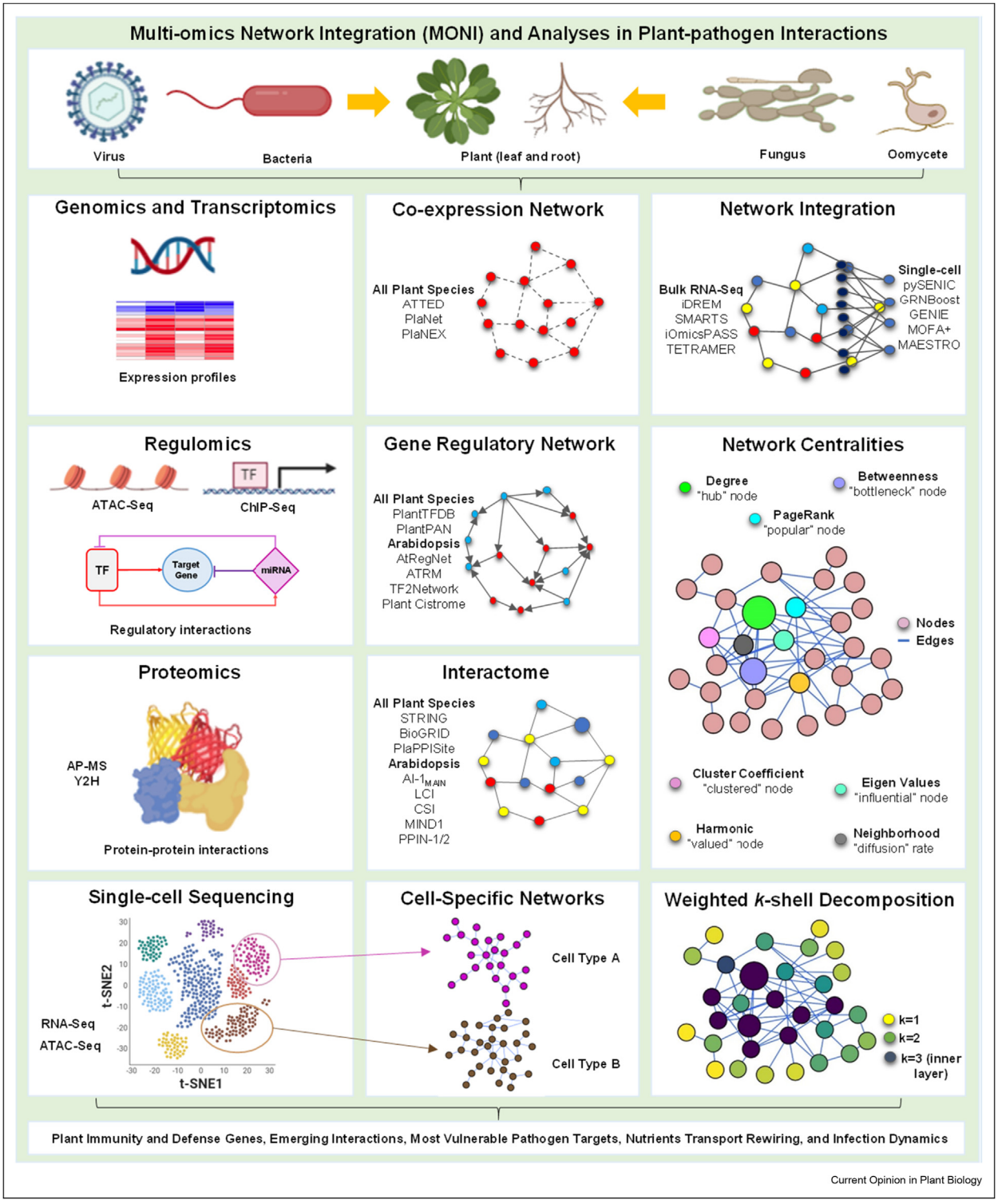
Coseq, Co-expression analysis of sequencing data; CEMiTool, co-expression modules identification tool; CSI, cell surface interactome; GCN, gene co-expression network; GENIC, GEne Network Inference with Ensemble of trees; GRN, Gene regulatory network; iDREM, Interactive dynamic regulatory event miner; MONI, Multi-omics network integration; ncRNA, noncoding RNA; PMRR, powdery mildew resistance regulated; PPI, protein–protein interaction; SARS-CoV, severe acute respiratory syndrome; SARS-CoV-2, Severe acute respiratory syndrome - 2; scATAC-Seq, single-cell ATAC sequencing; scRNA-Seq, single-cell RNA sequencing; SENIC, single-cell regulatory network inference and clustering; WGCNA, weighted gene co-expression network analysis; W *k*-shell, weighted k-shell decomposition.

General introduction

The graphical representation is a convenient, abstract description of nodes' relationships (i.e. edges) in general. Because it is an abstraction, the physical and/or functional nature of the relationships could be fundamentally different in different types of networks despite their similar appearances as graphs [1,2]. Such nodes in a biological network including genes, RNAs, proteins, and metabolites interact with each other and provide a cumulative effect required for the desired functional outcome [1,2]. These interactions include protein–DNA, noncoding RNA–RNA, protein–protein, and metabolite–metabolite interactions that are coordinated through gene co-expression (Figure 1). [3]. These networks can be undirected or directed based on the network type, that is, if the network edges do not have a direction of interaction in the context of biological relation among nodes, then it is termed as an 'undirected network' (e.g. gene co-expression network [GCN]), whereas the network with directed edges representing the direction of biological relation is termed as 'directed network' (e.g. gene regulatory network (GRN), signaling pathways, and metabolic networks). Similarly, the protein–protein interaction (PPI) networks are 'undirected network' that encompass the physical or predicted interactions of different proteins to execute a biological function.

The GCN is conceptually the same as conventional gene clustering methods. That is to reduce (or summarize) information in large transcriptome data to aid human interpretations: classifying genes into groups of the genes with similar expression patterns and learning relationships among the gene groups. Advantages of the GCN over conventional methods are gained by imposing graphical representation of the genes, which allows representation of higher–dimensional relationships among genes or gene groups, consistent combinations of information from multiple conventional methods, and local adjustments of parameter values. Because the information content of GCN is often still too high for efficient human interpretations, various graph analytic techniques are used to further reduce the information. Furthermore, the information about the type of interaction within (positive/negative regulation) the networks like GRNs and functional modules of GCNs and PPIs are crucial to decipher the course of activities under steady-state cellular conditions [4].

Figure 1



MONI and network organization analyses for a comprehensive understanding of various plant pathosystems. Phytopathogens (virus, bacteria, fungus, oomycetes, and others) infect numerous flowering plants thorough leaves and roots to manipulate the genetic and functional circuitry apparatus. These alterations have a global effect on genomics, transcriptomics, regulomics, and proteomics levels on pathogen infection. These multiomics data sets and their interactions can unravel the comprehensive understanding of the plant immune system. In addition, new developments in single-cell sequencing have enabled us to integrate the multiomics single-cell sequencing (RNA-Seq and ATAC-Seq) in plants with the existing complexity of cellular heterogeneity in plant–pathogen interactions. Finally, the identification of potential plant immune and defense-related modules and genes, most vulnerable pathogen targets, the rewiring of nutrient transport, and infection dynamics at different stages of plant immunity can be determined by several network integration techniques and multiple centrality analyses.

These functional modules can be influenced by diverse internal and external stimuli, including biotic pathogens, to rewire the flow of information for appropriate biological responses. This includes an immune system capable of recognizing microbial-associated molecular patterns as well as the activities of pathogenic effectors and initiates microbial-associated molecular pattern—triggered immunity (MTI) and effector-triggered immunity (ETI), respectively [5,6]. However, the effectors of pathogenic microorganisms (bacteria, viruses, oomycetes, and fungi) can manipulate host cellular networks for microbial propagation and ultimately induce effector-triggered susceptibility (ETS) (Figure 1). Therefore, network biology—based analyses are essential to comprehensively understand these cross-species sophisticated and multifaceted interactions encompassing several biomolecules (Figure 1) [7]. In this short review, we will highlight the current progress and new avenues of network biology techniques and their applications to study the structural organization and functional iteration of plant immune systems.

Network topology and structural centralities in phytopathology

The network structure can be mined to identify structurally special nodes, which make good candidates for biologically special nodes [1]. Generally, there are three groups of structural centralities based on the network topology: (i) neighborhood-based centralities (e.g. degree, coreness, and LocalRank) calculate the influence of nodes based on their surrounding nodes, (ii) path-based centralities (e.g. shortest path length, betweenness, information, closeness, and Katz centrality) compute the influence of nodes based on the distance among them, and (iii) iterative refinement centralities (e.g. eigenvector centrality, PageRank, and LeaderRank) compute the influence of nodes based on the mutual effect of node neighbors and their influence in a network [8]. In plant—pathogen interaction, network-based centrality methods have been extensively exploited to comprehend the most vulnerable proteins in both global-scale co-expression networks and PPIs or interactomes [9]. Given the size and complexity of the PPI network, the application of network analytics has proven highly effective for human understanding. The strongly clustered modules and subnetworks not only compress the network topological features but also highlight the strongly clustered proteins interacting and/or forming complexes to participate in the same functional pathway [10]. Furthermore, hubs (local and/or global) of the subnetwork or PPI network are pivotal for the alteration in the performance of functional pathways. These hubs can be computed by not all but some of network analysis algorithms (shortest path length, betweenness, eigenvector, information, Katz, harmonic, and other frequently used centralities) which consider the edge weight through the NetworkX python package [11]. Most of the biological interactomes studied earlier follow scale-free network topology revealing the structural power-law distribution, suggesting only a few nodes are connected to almost the entirety of the network [12]. Recently, a comprehensive data-centric study by Broido and Clauset [13] demonstrated that not all networks follow scale-free topology; however, the study reported that most of the biological and technological networks possess the strongest scale-freeness as compared with social networks. These abundantly connected proteins are termed as 'hubs', and they are considered the most vulnerable nodes during any biological stress, specifically pathogen infections [14], whereas nodes with high betweenness centrality are termed as 'bottlenecks' which are extremely important because these nodes act as bridges between two subnetworks while traversing the whole network [15]. Both hubs and bottlenecks commonly represent nodes of functional significance in biological networks and are often targets of diverse plant pathogens including bacteria, viruses, oomycetes, photobionts, and other holobionts (Figure 1) [16—19]. Specifically, hubs and bottlenecks have prioritized the proteins among thousands that are targets of bacterial effector proteins [9]. Based on the protein's co-evolution among host and pathogen, the genes encoding pathogens' targets evolve faster than nontargets. In addition, it has been reported that immune function proteins are enriched in different network centralities and possess conditional phenotypes than nonimmune functions with morphological and essential phenotypes. In addition, these centralities have been used to identify emergent immune players (genes) in several plant pathogen—infected co-expression networks (Figure 1) [20—22]. However, co-expression network analysis in seven flowering plants has revealed that nonimmune hub and bottleneck genes are negatively correlated with the rate of evolution, which supports the hypothesis that central nodes are evolutionarily conserved [23]. These studies suggest that the rule is quite different for immune and nonimmune functional genes; the latter is not under pathogen pressure, thus might evolve slower than others. Beyond hubs and bottlenecks, other measures of centrality have also been used to uncover novel facets of immune signaling networks. For example, the clustering coefficient has been used to discover shared and different immune signaling in five crop and model plants [21]. Likewise, the shortest path length has been used for integrated proteomics and transcriptomics to evaluate that *Arabidopsis*-pathogen effector targets are closer to differentially expressed genes (DEGs) than other genes (non-DEGs) [10]. These centralities are a great source to identify superspreaders (proteins responsible to transmit the information most effectively) and significantly affected modules in the interactome.

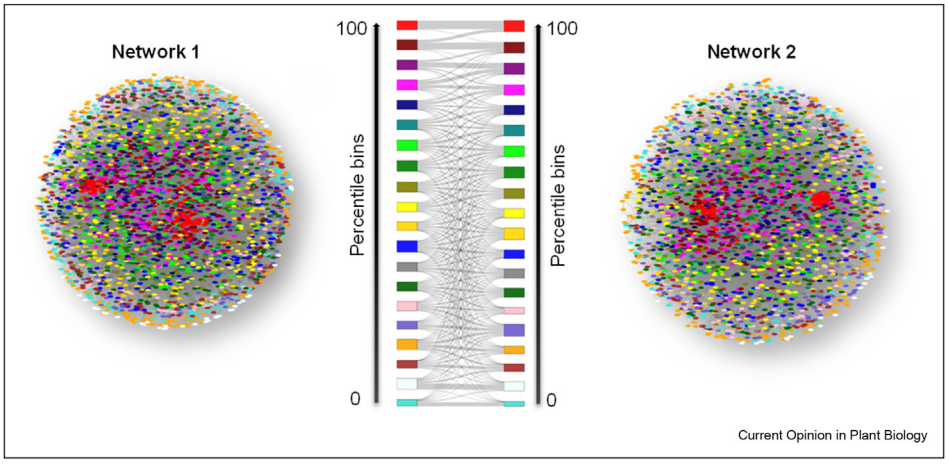
It is well described that network topological arrangements are equivalent in social, human, or plant

networks, where scale-free topology exists, and hubs are the most vulnerable nodes during any stress [1,24]. Given the significance of the aforementioned network centralities, additional topological centrality indices can also be insourced from social and eukaryotic network studies to interpret the network topology features in plant–pathogen interactions and pathogen-infected networks (Figure 1) [25,26]. The PageRank algorithm, previously used by the Google Search tool to rank websites, has been used to explore the impact of receptor-like kinase extracellular domains in the cell surface interactome [27]. Moreover, a new network analysis method 'weighted k-shell decomposition' has been used in social networks to prune the organized structural layers (shells) and identify the core and most influential portions of the network [28,29]. This method has been modified and applied to biological systems in two different host–pathogen interactomes to identify the core nodes which are most vulnerable during biotic stress [9,30]. The first study successfully discovered 40% of pathogen effector targets in *Arabidopsis* that were not predictable by hubs and bottlenecks [9], whereas the second study highlighted the proteins targeted by SARS-CoV and SARS-CoV-2 infection [30]. In addition, this study emphasized the significance of emerging network centrality indices to understand the pathogenies of other viruses and bacteria in human and other biological systems. Correspondingly, LocalRank, structural holes, coreness, network proximity, eccentricity, Katz centrality, and closeness centrality have been used in several human interactome studies to highlight significant genes associated with several human diseases and cancers that were not highlighted by conventional network centralities such as degree and betweenness [31,32]. These network features can be extremely helpful in the identification of novel pathogen effector targets, emerging immune players, and significant proteins in several functional pathways and signaling cascades (Figure 1) [33]. It is important to note that diverse pathogens target strategic nodes within a network that correspond to gene products involved in a wide spectrum of biological processes including hormonal pathways, immune responses, energy metabolism, photosynthesis, and translation. Henceforth, exploiting this plethora of network structural and topological architectures is imperative to identify emerging players/clusters in plant–pathogen interactions.

Module detection and comparative immune network analysis

The analysis of transcriptomic data sets such as RNA-Seq and microarray has empowered us to have a very close insight into the transcription regulatory mechanism of a genome [34]. The ultimate aim in interpreting intricate biological processes is the discovery of causal genes and governing mechanisms regulating those biological processes [8,35]. Using these multivariate transcriptomic data sets, a GCN can be constructed that enables system-level evaluation of organisms that lack information such as interactomes [36]. Furthermore, GCN analysis facilitates the characterization of modules of co-expressed genes that may

Figure 2



Comparative plant immune network analysis. Simulated gene co-expression networks (network 1 and network 2) representing interspecies/intra-species plant–pathogen interaction. The different colored modules demonstrate different clustered gene subnetworks based on weighted *k*-shell decomposition percentile. The Sankey plot of weighted *k*-shell decomposition percentile bin overlaps between both networks. Shell numbers were normalized to percentile and binned into 20 buckets represented as color blocks in the Sankey plot. The first red block represents 100–95 percentile, whereas the bottom percentile bucket in cyan represents 5–zero percentile shell distribution. The arrow represents the increase in the bucket shell percentile from zero to 100. The node in both networks is representing the bin color from the Sankey plot.

share biological functions [37]. The multivariate approach can be influenced by parameters including but not limited to distance matric, distance threshold cutoff, statistical methods (correlation, Bayes, entropy, and generalized linear model), and the tests (z-test, modulation, and permutation) [38,39]. Furthermore, the graphical representation of a network is often enigmatic. Edges are typically assigned if measures of expression correlation are above the certain predefined cutoff, such as Pearson's correlation coefficient. The definition of correlation thresholds is a nontrivial problem for which there is no standard approach [40]. To resolve this issue, the weighted node connectivity score method has been proposed [41]. In this method, the weight between one pair of the node reflects the strength of the connection between them, for example, the absolute value of Pearson's correlation coefficient between a gene pair. Based on the permutation-based test, it is also possible to get the p-value for statistical significance to help a researcher to integrate the significance of connections between a gene pair [39]. Some of the widely used GCN analysis packages are weighted gene co-expression network analysis (WGCNA), co-expression modules identification tool (CEMiTool), and co-expression analysis of sequencing data (coseq) [42–46]. Out of these three packages, CEMiTool is most efficient in terms of computation, whereas WGCNA is most widely exploited in biological systems including plant–pathogen interaction studies [43]. The CEMiTool is an unsupervised gene filtering method and is able to give reproducible results as compared with another method such as the coseq. The automated parameter selection is one of its advantages over widely accepted WGCNA [47]. Moreover, the CEMiTool uses an algorithm designed over the Cauchy sequences, to select the optimal β parameter. WGCNA and CEMiTool use hierarchical clustering, whereas the coseq is based on the *k*-means clustering method [43]. Before *k*-means clustering, the coseq uses log centered log ratio transformation to transform RNA-seq expression data. This combined approach ensures tights and distinct clusters of genes [45]. Overall, CEMiTool is a user-friendly method to automatically generate a gene-reproducible co-expression network. However, the coseq pipeline is also user-friendly and does not require high computational power but its nonreproducibility is its major disadvantage.

GCN analysis is generally the preferred method to detect similar network modules from (i) two related plant species, (ii) two conditional samples from the same plant model, and (iii) two developmental samples from the same plant model (Figure 2). To perform these tasks, several methods have been developed and used over the years in several biological systems. Recently, a differentially co-expressed module detection method named DiffCoEx was built on WGCNA to analyze samples from multiple conditions [48]. The main logic behind this method is to group two genes (i.e. differentially co-expressed genes) together when they have different correlation patterns with the same sets of genes in two conditional samples. Unlike the traditional approach, DiffCoEx can make unbiased comparisons of more than two data sets in an unsupervised manner. In another approach, s-core decomposition, a gene in the GCN of two different species, was ranked to categorize conserved or diverged groups of genes using indicators of centralities [49]. The s-core decomposition–based method works perfectly even if the networks to be compared are highly connected and complex. Moreover, the s-core method is quite flexible toward a wide variety of networks. Similarly, the Eigen-decomposition method has been used on GCNs obtained from two different samples to identify modules [50]. This is a similar approach as WGCNA is for module detection but less sensitive for module comparisons. In addition, the PhytoNet database contains expression profiles, interspecies GCN topology comparison, and module detection of 19 phytoplankton and land plants [51]. The GCNs available in PhytoNet were used to identify gene clusters related functionally in cyanobacteria, green algae, and land plants. Recently, CoCoCoNet, a comparative co-expression analysis method, was used for the identification of autism-related conserved gene modules in 14 different animal and plant species through comparative GCN analysis [52]. Furthermore, the 'signedKME' function from the WGCNA package was used to calculate module membership (kME) scores for a wide variety of flowering plants to study the development of floral form [53]. The kME score reflects the correlation between gene expression level and the module Eigen-gene values. These comparative network analysis methods along with our suggested approach in the following can be applied to explore the conserved modules between two plant–microbe interaction transcriptomic co-expression studies. Assuming comparable GCNs would have identical indicators of centralities (degree distribution, closeness centrality, betweenness centrality, etc.), then the functionally related genes would possess similar ranks. Furthermore, ranks based on a particular centrality can be binned to make centrality-based modules.

In addition, for more robustness, several centralities could be combined to give genes a comprehensive rank to create centrality-based host–microbe interaction modules (Figure 2). In a recent attempt to compare two GCNs of human tissue samples infected with SARS-CoV vs. SARS-CoV-2 virus, the weighted *k*-shell decomposition method was used by assigning the shell numbers to each gene followed by the rescaling of shells (layers) into percentiles and binning them into the optimal number of buckets [30]. The aforementioned approach can be applied to study the conserved and distinct interspecies/intraspecies modules involved in plant–microbe interactions.

System-wide network modeling in plant–microbe interactions

Biological networks represent the behavior of their components in different functional processes in any given condition. In the last five years, several transcriptome-side studies have been performed to model the transcriptional response and reprogramming at different stages of plant immunity during plant–pathogen interactions. Lewis et al. [54] deciphered that most plant defense genes were induced at the early stage (before pathogen manipulation) of *Pseudomonas syringae* pv *tomato* (*Pto*) DC3000 pathogen infection. Furthermore, the role of phytohormones and their cross talk have been extensively studied to define the alterations in the signaling cascades through network rewiring and pathway integration for robust cellular function [55–57]. Another such study used a comprehensive genetics approach using combinatorial mutants pertinent to jasmonate, ethylene, phytoalexin-deficient 4, and salicylate signaling pathways and demonstrated the transcriptional rewiring for the robust regulatory response to pathogen stimulus [58]. These studies also highlighted the dynamic regulatory landscape of pathogen infection and the strategies of plants to counter the infection. To model these dynamic relationships and the regulatory response of phytohormones, few attempts have been successful [59,60]. Naseem et al. [59] uncovered the role of auxin, salicylic acid, and cytokinin and their regulatory cross talk in plant growth and defense by the standardized qualitative dynamical systems approach to simulate the dynamics of the static GRN. However, in another study, the role of jasmonic acid on the transcriptional reprogramming and dynamic regulatory behavior of several transcription factors (TFs) was elaborated by using network-fueled integrative temporal transcriptome and TF promoter motif analysis in plant growth and defense signaling networks [60]. Nobori et al. [56] provided the in-planta bacterial pathogen *Pto* DC3000 temporal transcriptional profile of several combinations of mutants for plant defense signaling cascades to identify the conserved and distinct transcriptome signatures influenced by plant immunity.

The undirected interactions, specifically GCNs, have been used before to establish the emerging gene co-regulation by the same transcriptional regulatory program, as well as for the identification of novel proteins and their interacting complexes altered during several plant–pathogen interactions (Figure 1) [22,34]. The 'guilt by association' concept provides a possibility for exploration of expression-based clustered neighbors, which are more expected to participate in the same functional pathway or regulate the identical biological processes [15]. Recently, several studies have exploited the GCNs to unravel the novel players at different stages of plant immunity through network rewiring during pathogen attack in both model systems and cash crops [20,22]. One such study in wheat identified the powdery mildew resistance regulated genes are highly correlated with *Blumeria graminis f. sp. tritici* (*Bgt)* resistance genes as hubs in wheat [22]. A similar study also highlighted the enrichment of hubs in stress-specific functional pathways in fungal (*Bgt*)-infected wheat samples [20].

Subsequently, the protein–DNA interactions or GRNs, which are directed interactions, modeled to control (activation or inhibition) the gene transcription by TFs when needed to regulate the cellular function (Figure 1). With the advancement in molecular biology techniques, several regulators (TFs, proteins, microRNAs (miRs), or other small RNAs) have been identified responsible to regulate the candidate genes [18,61,62]. Generally, the regulation dynamics is determined by multilevel parameters including changes in connections of a regulator, the strength of each connection, target gene expression correlation, binding affinity, and modeling approaches (Boolean, differential equation, hidden Markov models) [63–66]. However, hybrid modeling strategies along with several parameters have been implemented for comprehensive dynamic GRN construction [63]. However, another GRN inference algorithm uses Random Walk with Restart with a focus on the local network topology rather than global network topology [67]. Few of these hybrid methods as well as multiomics strategies have been successful to model the dynamics of stress/disease response in both animal and plant model systems [65–67]. In addition, some GRN modeling techniques highlight the top regulators based on the parameter's combinatorial ranking [64]. During pathogen infection, the GRN regulators (specifically TFs) are hijacked by the pathogen effectors to express the genes encoding several nutrients required for pathogen propagation [54,65]. In addition, miRs fine-tune the rewired GRN by the RNA silencing system for an additive pathogen infection effect [68]. However, these small RNAs including long-noncoding RNAs are crucial for a comprehensive understanding of GRN cross talk and rewiring in different hormone and stress response pathways [61,69–71].

Subsequently, proteins organize themselves in conjunction with other proteins through PPIs or interactomes to accomplish functional pathways and signaling cascades [12,72]. Numerous interactomes have been generated over the years to study the plant–pathogen interactions as a whole or/and immune receptor networks or immune signaling networks (Figure 1) [14,27,73–75]. These interactome studies provide a systematic understanding of physical PPIs between plant–pathogen or in-planta interactions structurally participating in the reconstruction of the macromolecular complexes associated with the molecular machinery of cells in the plant immune system [9].

In addition, these large-scale interactome studies advocate the notion that diverse pathogen (bacteria, virus, fungi, and oomycetes) effector proteins target one type of proteins that are highlighted by the hubs and bottlenecks in the host protein network [74]. However, there are other pathogen target proteins that possess low centralities such as the pathogen-associated molecular pattern interacting pattern recognition receptors (PRRs). Moreover, a recent study mapped an extensive interactome by identifying the mutant-specific interactions through the yeast two-hybrid technique of 10 phytohormones [55]. The study established hundreds of emergent interactions and communities previously not reported in signaling pathway cross talk. Notably, current PPI approaches pertain to identify static interactions. Integration of transcriptome data pertaining to the above-described phytohormones and pathogen infection with interactome data sets will make a large contribution toward comprehensive understanding of functional pathway cross talk in plant–pathogen interactions and immunity [57]. One of such integrated multiomics studies highlighted the power of network biology to decipher the static and dynamics of disease-, defense-, and susceptibility-related protein complexes in plant–pathogen interaction [10]. Interestingly, the study reported that pathogenic effector targets are super information spreaders and reside in close proximity to DEGs. In addition, the study revealed that one percent of DEGs are effector targets at any time of pathogenic infection and pathogen alters the expression of approximately 71% of effector targets and their interactors in the *Arabidopsis* interactome. A similar approach can be implied to elucidate the static nature of PPIs and GRNs in plant–pathogen interactions. The immune response in a plant is achieved by the extensive transcriptional reprogramming, which results in changes in the protein interaction partners of a complex and regulated gene by TFs. The changes in PPI partners are based on the expressed proteins at an instant and their roles in biological processes. However, the GRN size varies based on the expression of genes, TFs, and the changes in the TF binding motifs to genes [54,56]. These result in the rewiring of PPIs and GRNs during different stages of plant immunity to perform specific functions.

In addition, new developments in next-generation sequencing platforms, that is, single-cell RNA sequencing (scRNA-Seq) and single-cell ATAC (assay for transposase-accessible chromatin) sequencing (scATAC-Seq) used for genome-wide chromatin accessibility, have opened new avenues for cell-specific gene expression, GRN construction, and analyses to identify master regulators or TFs for cell fate transition as well as protein complex dynamics at different stages of plant development and pathogen infection (Figure 1) [76–78]. Rich-Griffin et al. [77] used two plant immune elicitors, flg22 and Pep1, to study the GRNs in epidermis, cortex, and pericycle cells of *Arabidopsis* roots. They identified the differences in the immunity GRN of each cell type and emphasized the recruitment of cell-specific GRNs based on the functional ability of each cell type. Similarly, Zhang et al. [78] described 24 putative cell clusters along with cell-specific marker genes in the *Arabidopsis* root at different stages of plant development. They also highlighted different levels of ion assimilation and hormonal responses in each cell cluster of roots. The cell-specific GRN construction is a challenge in the emerging field of plant–microbe interaction. However, we need this technology-intensive platform to decipher the cell-specific immunity GRNs in different tissues (leaves, roots, and shoots) infected by the phytopathogens to understand the role of each cell in inducing immunity [79]. Recent developments in fast and most efficient GRN inference algorithms such as GRNBoost2, GENIE3, and SENIC workflow have provided an amazing resource for cell-specific GRN inference (Figure 1) [80–82]. In addition, python-based implementation of the SENIC algorithm, pySENIC, is lightning fast to be implemented in plant–pathogen interactions [83]. However, the cellular heterogeneity provides immense challenges in single-cell–derived network interpretation and unbiased candidate gene prioritization for perturbations in functional pathways [84]. Therefore, a comprehensive network centrality–based pipeline is needed for meaningfully augmenting the root or shoot during plant–pathogen interactions [79].

Multiomics network integration for network rewiring and pathway dynamics

The enormous collection of high-throughput data sets from different experimental techniques has provided an excellent opportunity to network systems biologists for unbiased analyses and interpretation of data for a comprehensive understanding of molecular activity during a biological response. As such, the data sets generated by genomics, transcriptomics, proteomics, and metabolomics are massive, and multiomics network integration (MONI) remains the bottleneck of big data research in biology [85,86]. In the last couple of years, several MONI strategies have been exploited in other model systems including, humans, mice, and microbes (Figure 1) [87–89]. These strategies use element-, pathway-, and mathematical-based approaches to highlight the most significant modules/components of the biological system under study [90]. The element-based approach is dependent on correlation, clustering, and multivariate analysis. On the other hand, the pathway-based approach is dependent on pathway mapping and co-expression analysis, whereas the mathematical-based approach is dependent on differential analysis and genome-scale analysis. However, there is an existential challenge in the multiomics study design to evaluate the scope and restrictions of

study, sample strength, and statistical power [91]. Several factors can influence the statistical power in multiomics studies including but limited to the type of study (randomized or observational), sample allocation (balanced or unbalanced), high sample size, high effect size, hypothesis testing (parametric or nonparametric), significance level, number of hypothesis testing, sample variation, and confounders that can introduce bias [92]. On a positive note, these comparative parameters can be assessed at the one-stop shop 'MultiPower', which recommends a suite of harmonized figures of merit as a quality metric for different types of multiomics studies [91]. These multimodel approaches can be used in high-throughput MONI to unravel the emerging and most relevant players in plant–pathogen interactions and different stages of plant immunity [85]. However, MONI in plants is poorly studied and reviewed because of the inadequate genome annotations, complex symbiont interactomes, and metabolic diversity [8,85,90]. Thus, integration becomes a challenge specifically in crop plants and nonmodel plant systems. In addition, new tools are being developed for MONI to identify significant subnetworks, dynamics of gene regulation, and master regulators in different functional pathways and signaling cascades [93,94]. These master regulators are the most vulnerable components of GRNs and interactomes to spread the expression information (regulation) with high connectivity and centrality. The pathogen manipulates the host TFs to regulate the target genes of functional pathways for plant immunity as well as the nutrient synthesis/transport. The functional networks are very scarce; thus, TFs with high connectivity and centrality in the structural network are *a priori* interactions for any hypothesized functional hubs [33]. These approaches have also been applied in plant–pathogen interactions to explore the subnetworks associated with both ETI/MTI and ETS [10]. In addition, dynamic gene regulatory event mining is a resourceful tool to investigate the significant regulators (TFs/miRs) at different stages of plant-pathogen infection [64,95]. One such tool 'iDREM' (Interactive Dynamic Regulatory Events Miner) reconstructs the dynamic GRNs by integrating temporal transcriptomics, epigenomics, and proteomics along with static GRNs, PPIs, and miR-target gene networks (Figure 1) [64]. The tool identifies the significant regulators (TFs, miRs) responsible for the regulation of the gene signature enriched in functional pathways over time by an unsupervised hidden Markov model. This provides a unique prospect to identify the transcriptional output that is dissimilar at different stages of plant–pathogen interaction and plant immunity, that is, from MTI and ETS and regulators (TFs/miRs) that are the target of pathogen effectors. In addition, this technique can infer the transcriptional amplitude difference in MTI, ETS, and ETI conditions of target genes for specific regulators during plant–pathogen interaction. These analyses can pinpoint the specific components accurately to work with during the fight against pathogens and manipulation using advanced gene editing techniques for crop improvement and disease resistance [33,95].

With recent advancements in computational techniques, several tools have been developed and exploited for scRNA- and scATAC-Seq data and network integration to unravel the new frontier of MONI [96]. However, these integration techniques have not been used in plant science that often [79]. A recent study in the *Arabidopsis* root is the first of its kind in scRNA- and scATAC-Seq integration in plants [97]. The study highlighted the significance of an integrated approach to identifying the significant TFs associated with the gene regulatory events underlying epidermis development. A similar approach can be exploited to explore the cell-specific plant–pathogen interactions in the root or leaf by MONI to comprehend the pathway dynamic and network rewiring at different stages of plant immunity [77].

Moving forward, the next frontier in system-wide network analysis will be the identification of master regulators in reconstructed cell-specific regulatory networks from scRNA-Seq and scATAC-Seq for cellular development, pathogen infection, and hijacked nutrient transport in plants (Figure 1) [84]. The upcoming technical studies have reduced the challenges of single-cell–derived GRNs due to cellular heterogeneity for comprehensive network centrality analyses. In summary, conventional and new network centralities have an enormous impact on discovering emergent modules and players in multifaceted plant–pathogen interactions and plant immunity.

Conclusions

With the accumulation of high-throughput sequencing data sets, the interpretation of biomolecular adaptation and response in plant–microbe interaction needs the methodical multiomics data and multiomics network integration (MONI) (MONI) techniques for a comprehensive understanding of biological events. These diverse system-wide studies and emerging network centralities have been propagated widely in several host–pathogen interactions and disease studies along with plant–pathosystem studies. Network biology specifically, MONI, and centrality analyses can assist us to decipher the unmapped regions in plant–microbe interactions. In addition, the prediction and prioritization of significant genes and proteins involved in plant immunity and defense, most vulnerable pathogen targets, nutrient transport, and infection dynamics can accelerate the experiments from controlled to field environments. Furthermore, these techniques can be transcribed from model plant systems to several crop plants to counteract the pathogen- and other biotic- or abiotic-stressed crop loss.

Author contribution

M.S.M. conceived the idea and designed the review structure. B.M. and N.K. wrote the first draft of the manuscript. All authors discussed, critically reviewed the manuscript, and approved the final version for submission.

Declaration of competing interest

The authors declare no conflict of interest.

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