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GENE REGULATORY NETWORKS IN CROP IMPROVEMENT: INTEGRATING TRANSCRIPTION FACTORS, NON-CODING RNAS AND MULTI-OMICS- A REVIEW

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ABSTRACT

Crop improvement generally needs more and more to know how genes in interaction determines yield, nutritional quality, resource-use efficiency and stress resilience Gene regulatory networks are composed of transcription factors, cis-regulatory elements, microRNAs, long non-coding RNAs and downstream pathways showing how regulatory variations affect gene expression in tissues, developmental stages and environments. This review covers network architecture, critical regulatory components and tools available for the detection and verification of interactions. Integration of genomics, transcriptomics, proteomics, metabolomics and epigenomics analyses of the relationships between molecular variation and crop performance has been facilitated here, with single-cell technologies revealing cell-specific regulators. Network knowledge can be used to breed for higher productivity, quality and climatic adaptation, and regulatory genome editing can change gene expression without harming the essential functions. but gene duplication, regulatory complexity, sensitivity to environment as well as distinguishing fidelity from quality further add practical challenges to apply. For example, interventions can have off-target effects in other tissues, developmental stages and genetic backgrounds. For successful and efficient implementation, the use of a combined molecular data, independent in vitro proof, realistic stress sanitation and assessment of actual performance under diverse conditions in the harvest phase. Future initiatives should make more effort on dynamic, cell-resolved and environment-sensitive networks with an emphasis on predicting uncertainty. Transferring high-performing crop varieties using conventional breeding directed by developmentally, genetically and post-translationally engineered regulatory mechanisms via precise gene editing has the ability to enhance this wild sector of planned agricultural intensification, including food and nutrition security alongside long term enhanced crop resilience.

KEYWORDS: Gene regulatory networks; transcription factors; cis-regulatory elements; non-coding RNAs; multi-omics; genome editing; crop improvement

1. INTRODUCTION

Crop breeding is often trait and associated genetic markers-driven; however, grain yield, flowering time, fruit quality, nutrient-use efficiency, and stress tolerance and other traits are quantitative and are regulated by the coordinated interplay of numerous genes. Such interactions are highly dependent on developmental stages, genetic backgrounds, and environmental conditions (Khan *et al*., 2024; Chaudhary *et al*., 2022). Gene regulatory networks (GRNs) serve as a theoretical framework to understand such interactions as they identify the key regulators, their target genes and conditions modulating regulatory activity. Thus, identifying how molecular interactions lead to favourable traits can aid selection, enabling precise genetic modification and improved crop varieties (Leong *et al*., 2025; Chen *et al*., 2023).

GRN importance is when the beneficial genes are inconsistent in response across varieties, at times and environments. Even within the same plant, a gene that helps keep healthy seedlings may do harm to reproduction, whereas the effect of a favourable allele might shift depending on its collaborator regulators. Context-dependent relationships are needed in identifying what is a functional regulatory relationship versus just an association and what functions to target for fewer side effects (Borowsky and Bailey-Serres, 2024). GRN research has greatly benefited from advances including genetics, genomics, transcriptomics, proteomics, metabolomics, single-cell analysis, and chromatin profiling (Baysoy *et al*., 2023). Genome-editing technologies also allow experimental validation of regulatory interactions. But again, to apply these advances to crop development corrections are necessary used robust experimental designs, integrated multi-omics analysis and functional validation in realistic growing conditions (Nobori, 2025; Chen *et al*., 2023).

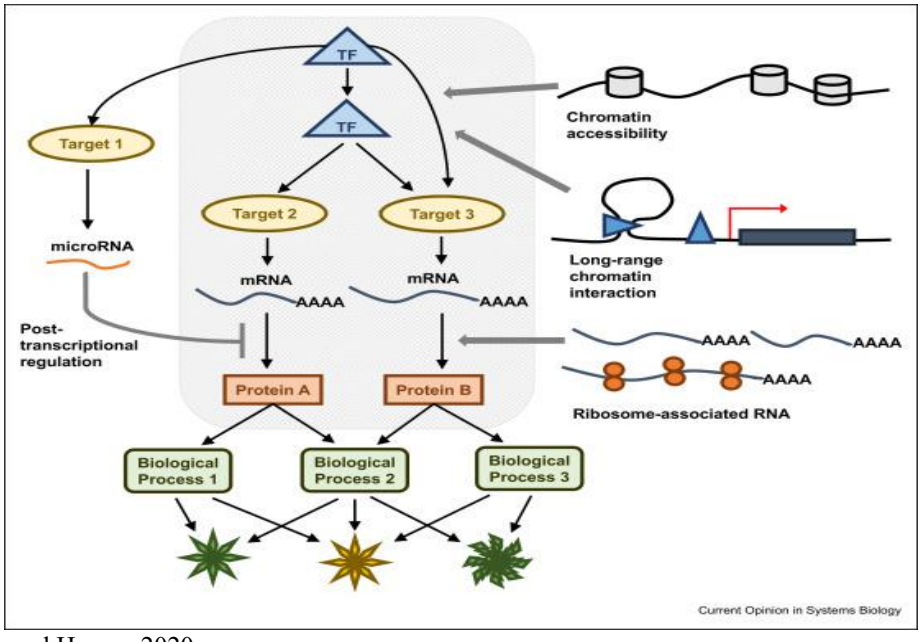
2. Understanding Gene Regulatory Networks

A gene regulatory network (GRN) indicates how a gene's expression is regulated based on the timing, place, and strength of signals it receives (Sinha *et al*., 2020). Transcription factors that bind DNA, cis-regulatory sequences to receive signals from those factors, non-coding RNAs that affect transcripts, and proteins that change how chromatin is wrapped around DNA (Statello *et al*., 2021; Shah *et al*., 2022) are its components. These networks are often shown with nodes for components and edges for their relationships. Some edges in these relationships represent more direct interactions such as a protein binding DNA, experimentally evidenced effects, or only statistical predictions. These meanings are not equivalent. A useful network must separate the learned mechanisms from the theories, otherwise, an elegant and bold representation can imbue uncertainty with an illusion of biological certain (Sumner *et al*., 2026).

Regulation can be thought of as a chain of several linked steps. Cellular signals can activate a transcription factor, which may bind an accessible promoter and alter production of a target RNA (Rambout *et al*., 2018). A microRNA can subsequently reduce that RNA's abundance, and protein modification can alter the activity of the encoded product (Denzler *et al*., 2016; Roundtree *et al*., 2017). These intermediate products possess the tendencies to drive the situation around in the progression of signalling pathways. At every stage there is a possibility for some differences among crop varieties. Protein sequence changes are just one way a trait can be altered; binding site changes, RNA regulation, or tissue-specific chromatin, for example, could also affect a trait. GRNs structure that enables the exploration of these alternatives (Diaz *et al*., 2025).

Gene regulatory networks (GRNs) are dynamic and variable systems across tissues, development and environment. A transcription factor may target different genes in roots and leaves depending on the availability of DNA, its expression may change in response to flower, heat, drought or pathogen infection. This type of context dependent regulation is crucial to crop breeding, where temporary activation of the defence mechanism may be good for stress tolerance but when constantly active may harm yield and production potential (Wen *et al*., 2025). Crop genome complexity also affects regulatory interactions through gene duplication, polyploidy, and genetic variation. In wheat, multiple related genomes contain corresponding genes that may exhibit distinct expression patterns under different conditions (Wang *et al*., 2019; Guerin *et al*., 2019). Functional redundancy among duplicated genes can compensate for individual gene modifications, while simultaneous alterations may produce substantial phenotypic effects. For reliable applications in crop improvement, comprehensive GRN study also requires the tissue-specific expression studies, isolate the copy of particular genes and validation in different genotypes (Wang *et al*., 2025).

Figure 1. Plant Gene Regulatory Network and its Regulation



Source- Qian and Huang, 2020

3. Transcription Factors as Central Regulators

Transcription factors are proteins that bind DNA sequences to control transcription of target genes Major plant families consist of MYB, NAC, WRKY, bZIP, AP2/ERF, bHLH and MADS-box regulators (Hussain *et al*., 2021; Meraj *et al*., 2020). They are involved in growth, flowering, metabolism, defence as well responses to environmental stress. Since a single factor can influence many targets, it can coordinate an entire biological process. Broad actions make transcription factors a strategic target for improvement, but also the bait for off-target effects. Finding a factor linked to a trait of interest is just the start; researchers need to find its targets, partners and activity in the relevant tissues (Bhoite *et al*., 2025).

3.1 Transcription Factor Binding and Specificity

A transcription factor will not bind at every genomic site with its preferred sequence. DNA accessibility, surrounding motifs, partner proteins, and local chromatin determine if binding occurs in a living cell some factors cooperatively work to activate a gene, whilst others compete for the same region or recruit proteins for expression suppression. As a result, a motif revealed by a computational analysis lends itself to being a regulatory site, but does not reaffirm it as an active connection. Greater evidence is corroborated by a synergy of motif details aided by chromatin studies, binding tests, and alterations in target expression when the proposed regulator is experimentally modified (Morales-Cruz *et al*., 2026; Strader *et al*., 2022).

Specificity also depends on timing. The factor could be present in a cell but not active until it gets a hormonal or environmental signal to awaken. Its activity can change due to translocation to the nucleus, interaction with another protein, or chemical modification post-translation. Consequently, measuring the level of its RNA alone may be insufficient. This is important when researchers build networks from transcriptomic datasets alone. Maybe the regulator itself has little change in its own transcript but still exerts a large effect on its targets. By investigating proteomic and functional markers, its possible to ascertain whether or not the proposed regulator was actually in action at the moment of response (Alamos *et al*., 2025; Lai *et al*., 2019).

3.2 Development and Plant Architecture

Developmental transcription factors are important players in the deciphering of processes that can have profound effects upon crop productivity. They are responsible for determining what form a meristem will develop into and, for example, the building of leaves, branches or flowers, the onset of flowering and reproductive organ development (Carrasco and Gutierrez-Marcos, 2026). Branching out can, for instance, increase the number of fruiting sites, but can also create competition for light and assimilates. An earlier flowering can allow a crop to escape late season drought, but also reduces the time to accumulate enough biomass. GRNs provide an understanding of these trade-offs by linking developmental regulators to the programmes they control (Wingler and Soualiou, 2025; Lin *et al*., 2025).

The reproductive transition illustrates the importance of coordinated regulation. A plant must integrate day length, temperature, age and internal nutritional status before flowering at an advantageous time. The pathways linking the transcription factors and mobile signals vary between crop species and within varieties (Thilakarathne *et al*., 2025). Deviation of one regulator may result in helpful adaptation to a given region, but it has to be examined in terms of flowering synchrony, fertility and final yield (Wang *et al*., 2023). Network analysis can tell whether a candidate is at the forefront of the transition or downstream of it. This is important, as a breeder may have to choose between a naturally occurring allele or a more targeted modification (Montez *et al*., 2026).

3.3 Seed and Fruit Development

Seed and fruit development is controlled by the transitions between cell division, expansion, accumulating storage, and maturation (Sripathy and Groot, 2023). This includes changes in regulatory programs across each transition. A single-cell analysis of different endosperm cell types in maize was facilitated by transcription factor binding information (Li *et al*., 2025). Such work helps to demonstrate why pooling expression across an entire seed can mask regulators limited to one tissue. Connecting these cell-specific programmes to traits such as grain weight or composition is the aim of breeding. A candidate factor should be tested at the appropriate stage and in the appropriate tissue before its agronomic value is assumed (Yuan *et al*., 2024).

Complex regulatory questions are also raised for fruit crops. Particular transcription factors (TFs) that influence pigmentation, sugar accumulation, softening, or ripening might affect several quality traits simultaneously (Li *et al*., 2022; Mathiazhagan *et al*., 2021). Edits to improve skin life, particularly, are known to affect flavour or harvest time. Expanding the network explored by researchers may help them anticipate such relationships and make more directed interventions instead of affecting many traits at once. Measurements should be more than only mRNA to metabolites, texture, taste. The most effective regulation is one which produces a desirable mix of traits, rather than optimizing a single lab measurement with no consideration of marketable products (Aydogdu *et al*., 2026).

3.4 Stress Responses and Growth Costs

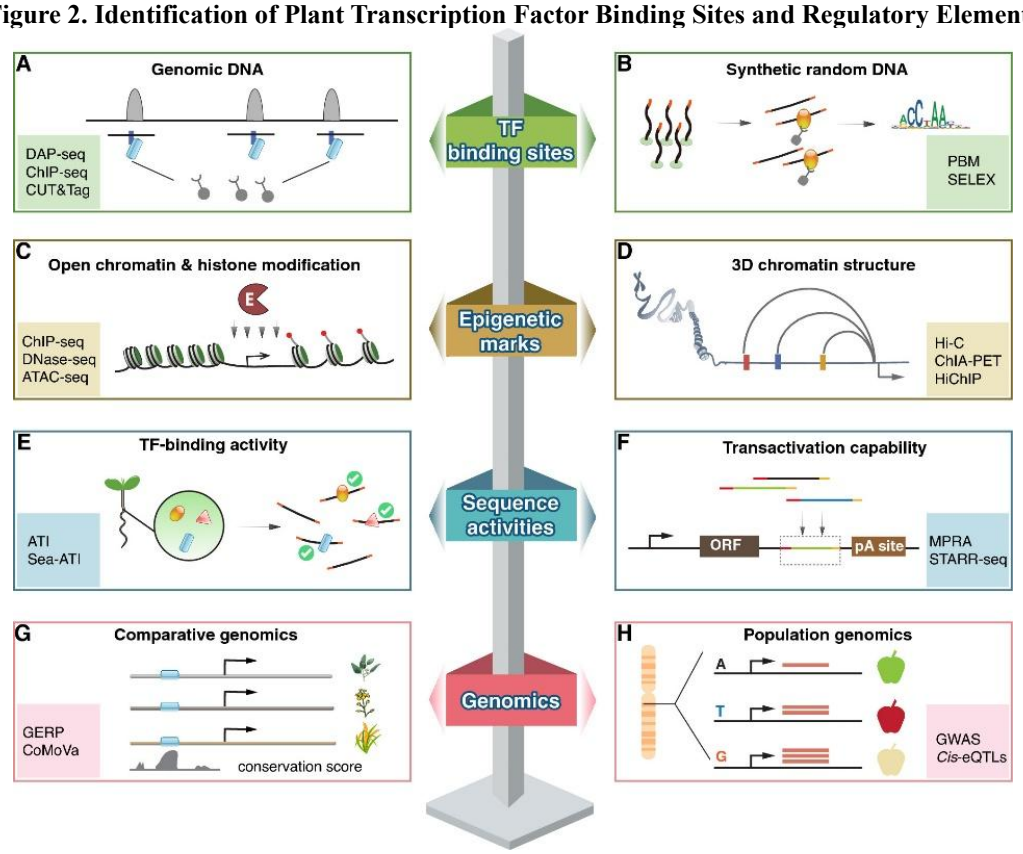
Environmental stress induces regulatory programmes which help to protect cells and maintain plant function. Drought-responsive factors can contribute to water conservation, root development and the build-up of protective metabolites, heat-responsive factors are proposed to preserve protein stability and fertility (Feng and Xia, 2025; Sato *et al*., 2024). However, it is not the case that stronger activation is always better. Using resources towards protection can limit growth when conditions are good, and something that is useful for a short period of abiotic stress may be detrimental if kept for the whole season. In such a case, it is thus, the knowledge of where and when a transcription factor operates which is key to tolerance improvement. Breeding should give sufficient protection with minimal impact on productivity and recovery (Robertson *et al*., 2025).

Cell types respond differently to heat. Elevated temperatures can influence distinct processes and modify dividing root cells, transport systems, and mature tissues even for a single organ. Recent single-cell investigations of maize roots identified cell-specific expression changes and candidate regulatory relationships during heat exposure (Wang *et al*., 2025; Wang *et al*., 2026). This great resolution provides an insight into who might be best helped by a protection strategy. Such an effect, younger roots, in a controlled treatment does not imply greater yield during field heatwaves. Candidate regulators need to be examined across developmental stages, realistic temperature profiles and multiple genetic backgrounds before any of them can be used to develop a consistent improvement strategy (Prado *et al*., 2025).

4. Cis-Regulatory DNA and Chromatin

Non-coding DNA sequences that control gene expression by defining the binding sites of transcription factors and other regulatory proteins are cis-regulatory elements. These elements, which include promoters and other regulatory regions, affect the timing, location, and degree of gene expression without altering the protein sequence (Mattioli *et al*., 2020). Small sequence variations, orchestrate regulatory activity and allow for directed genetic selection of crops while retaining necessary phenotypes (Rodriguez-Leal *et al*., 2017). Regulatory accessibility is limited due to chromatin structure, with tightly packed DNA blocks transcription factor attachment whereas open chromatin promotes regulatory interactions. It has been demonstrated that chromatin accessibility varies between tissues, developmental periods, genotypes and environmental conditions (Isbel *et al*., 2022). Recent studies of the rice regulome have mapped numerous regulatory regions that are accessible, suggesting that non-coding genetic variation is important in dissecting complex crop traits. However, chromatin accessibility alone cannot confirm regulatory function or establish direct relationships between regulatory elements and target genes (Zhu *et al*., 2024).

Figure 2. Identification of Plant Transcription Factor Binding Sites and Regulatory Elements



Source- Li *et al*., 2025

Identifying the true target genes of cis-regulatory elements is notably impervious, as physical proximity is not a clear indication of functional regulation (Chua *et al*., 2022). Such causal relationships might be better identified by integrating gene expression profiles with chromatin interaction data as well as through targeted genetic perturbations. In precision breeding, this level of evidence is especially important, because changing a misassigned regulatory element could lead to failure or suboptimal results (Ali *et al*., 2025). Compared with full gene knockouts, editing regulatory elements allows for controlling target genes while preserving essential biological functions. For example, the alteration of a set of specific transcription factor-binding sites could respond to coping with abiotic stress with no or very little impact on normal plant development. However, unique regulatory elements may affect multiple genes or work over multiple tissues, and therefore the potential for unintended effects. Thus, network-guided regulatory editing requires molecular characterization, functional validation and multi-environment phenotyping for conferring new crops traits with stability and predictability (Li *et al*., 2025; Zrimec *et al*., 2022).

5. MicroRNAs in Crop Networks

MicroRNAs are microRNAs are short sequences of non-coding RNA that targets complementary target transcripts and decreases its abundance or translation. Many of their targets encode transcription factors, placing microRNAs within central developmental and stress-related pathways. One microRNA family may affect several related genes, leading to a coordinated response instead of focal effects. hence, its reach makes the microRNAs major structural elements of networks. It also implies changing the level of a single microRNA can have wider effects than anticipated. Knowing all relevant targets in a crop is crucial before treating a microRNA as a simple improvement candidate (Chen *et al*., 2024; Rabuma *et al*., 2025).

5.1 Developmental Regulation

MicroRNA (miRNA) regulatory modules play a vital role in plant development through the mediation of leaf formation, root growth, flowering and reproductive development genes target gene expression. Their function is dependent on the interaction between miRNAs and their target transcripts, with even the smallest variations potentially altering developmental timing and/or plant form (Shankar and Nath, 2024). These regulatory relationships might vary between crop species, due to differences in target sequences, gene duplication and developmental contexts. Functional interactions and their biological relevance can be examined through a tissue-specific analysis of miRNA abundance and target gene expression. It allows us to manipulate specific regulatory interactions by editing miRNA-binding sites that could improve desirable agricultural traits, without unwanted developmental side effects. However, functional validation is an essential requirement prior to application in precision crop breeding (Ferreira and Reis, 2023).

5.2 Stress Adaptation

MicroRNAs (miRNAs) have emerged as important players in plant responses through the transcriptional regulation of various transcripts involved in the signalling, adaptation, and growth during drought, heat, salinity and nutrient deficiency. Expression can be up or down at stress times, implying protective responses, cellular injury or recovery. Therefore, time dependent expression profiling and comparisons between tolerant and sensitive genotypes is required to unveil functional regulatory relationships (Chen *et al*., 2025). Additionally, they maintain physiological equilibrium through down-regulatory mechanisms to curb stress responsive down-steps which could inhibit growth when conditions become non-stressful. MiRNA feedback loops with transcription factors underlie this regulation, serving to promote stress recovery and enhance yield stability. Yet it takes experimental work to confirm these regulatory relationships in both directions, as well as measuring target response. It is vital to further study these modules in combination stress and realistic, field conditions prior to using miRNA modules in crop improvement programmes (Yang *et al*., 2026).

6. Long Non-Coding RNAs and Other Regulatory RNAs

Long non-coding RNAs are a varied set of RNA molecules that generally do not make proteins. Some help regulate genes by affecting transcription or chromatin, while others act through interactions with proteins or other RNAs. Many are active only in certain tissues, at particular stages of development, or under specific stress conditions, making them promising subjects for studying crop responses. However, their roles can be more difficult to confirm than those of familiar protein-coding genes. Even when one appears near a genetic region linked to a trait, its position and activity pattern alone do not reveal what it does (Chanwala *et al*., 2026; Mattick *et al*., 2023).

6.1 Long Non-Coding RNAs Under Stress

Long non-coding RNAs interact with microRNAs and coding genes in nutrition and stress tolerance in crops. The characterization of stress-responsive Long non-coding RNAs and proposed regulatory networks associated with drought adaptation were reported in wheat and other crops. However, that differential expression alone does not mean they assist the plant in stress tolerance, as they might change as a consequence of a change in developmental state or cell injury (Jha *et al*., 2020; Qiu *et al*., 2025). To determine causative effects, specialized genetic manipulation and physiological testing are needed to functionally validate targets. A large obstacle in revealing levels of lncRNA-dependent action over levels of action associated with their DNA source, because editing the locus may also influence nearby genes. Concomitant approaches that alter RNA abundance independent of local DNA control can help deduce direct pathways. These are related details that need to be appreciated to build trustworthy molecular markers and develop specific genetic interventions to enhance crop drought tolerance (Imaduwage and Hewadikaram, 2024; Shen *et al*., 2026).

6.2 Small Interfering RNAs and Epigenetic Effects

Small interfering RNAs (siRNAs) determine plant gene expression via targeted transcript silencing and epigenetic changes through RNA-directed DNA methylation influencing transposable elements and local genes. They play an especially crucial role in large crop genomes rich in repetitive sequences. Nevertheless, epigenetic states change across tissues, development, and environmental conditions and validation of its maintenance and inheritance is critical for crop improvement (Castel and Martienssen, 2013; Gahlaut and Jaiswal, 2026). coordinated gene regulation resulting from regulatory interactions between siRNAs, long non-coding RNAs (lncRNAs), microRNAs (miRNAs), messenger RNAs (mRNAs), and transcription factors. During think development and environmental stress, these interconnected networks can amplify, stabilize, or counteract molecular responses. Whilst computational analyses have uncovered countless potential interactions, most remain verified. So, the combined use of expression profiling, direct interaction assays, and functional genetic validation is required to identify robust regulatory mechanisms to inform molecular breeding programs (Rathod and Puri, 2026; Feng and Guang, 2024).

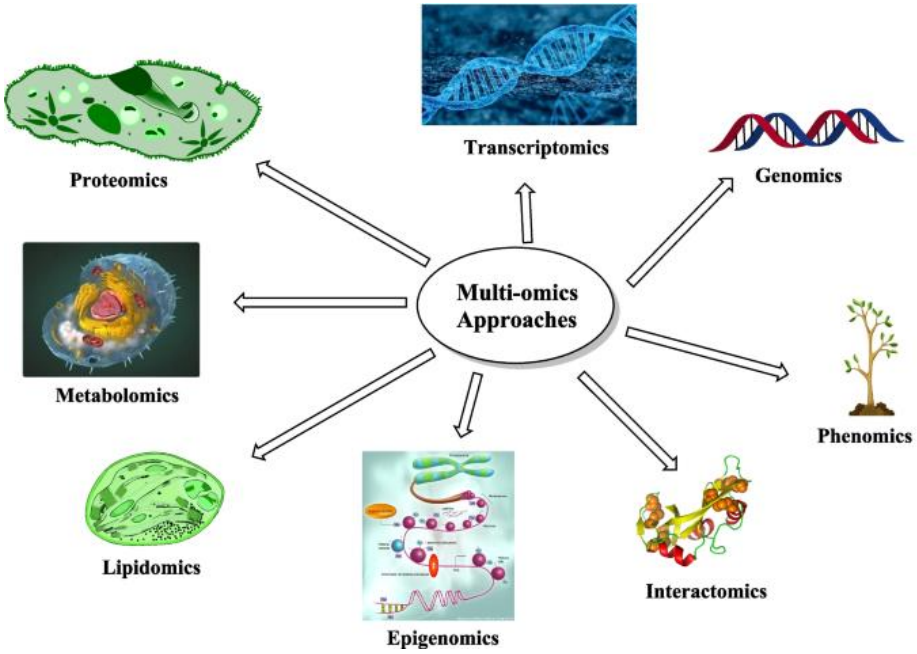
7. Multi-Omics Approaches

Multi-omics is the approach that incorporates several molecular levels for identifying genotype to phenotype transforms. Genomics denotes inheritance, transcriptomics examines RNA, proteomics studies proteins and metabolomics handles the end products of biochemistry. Epigenomics which studies chromatin and DNA modifications, and plant phenomics (Haidar *et al*., 2024). Each dataset provides an answer to a different question. It is only possible to predict the effects of a regulatory variant on an altered expression, which will ultimately affect the yield or quality of crops by integrating them. The difficulty is that these measurements are often collected at different resolutions and represent biological events that occur on different timescales (Kundu and Tanti, 2026).

7.1 Genomics and Genetic Variation

Genome sequencing is the groundwork for the detection of genetic and regulatory variations affecting plant traits, including mutations in coding regions, structural variations, and variation in non-coding regulatory regions. DNA markers associated with preferred traits are identified through population-level association studies, and then fine mapping and functional validation identify causal variants. Pan-genome approaches expose genetic diversity that is not detected in individual reference genomes particularly valuable alleles found in landraces, wild relatives, and diverse breeding populations (Bayer *et al*., 2020; MacNish *et al*., 2026). Identifying regulatory mechanisms is strengthened by incorporating chromatin accessibility, transcription factor binding motifs and gene expression datasets into genetic association data. For instance, a particular variant associated with a large grain weight becomes highly valuable when associated with changes in target gene regulatory activity and gene expression. Though genetic associations can be confounded by linkage among genes, case-specific genome editing enabling positive validation in varied genetic backgrounds is a culmination of such integrated approaches (Mangi *et al*., 2025; Thomson *et al*., 2022).

Figure 3. Multi-Omics Approaches in Plant Research



Source- Sahana *et al*., 2025

7.2 Transcriptomics

Transcriptomics provides a basis for GRN (gene regulatory network) analysis by measuring RNA abundance across developmental and environmental conditions (Jackson *et al*., 2020). Different expression of these genes in comparative transcriptomic studies, while co-expression analysis determines what genes could be involved in the same regulation pathways. However, expression correlation does not automatically mean direct regulatory interactions, since expression can be driven by these differences in development and in tissue composition. Reliable network interpretation is thus dependent on biological replication, standardised sampling and independent verification (Upton *et al*., 2023). Time course transcriptomics improves GRN analysis by identifying the order of regulatory events, from activation of transcription factors to the changes to metabolic, specific tissue or physiological response to an early signal such as transcription factor regulation. Appropriate sampling intervals and recovery-stage observations help distinguish immediate stress responses from long-term adaptation. Integrating temporal expression patterns with functional validation can identify promising regulatory targets for improving crop stress tolerance, recovery and productivity (Illouz-Eliaz *et al*., 2025).

7.3 Proteomics and Metabolomics

Proteomics and metabolomics complement transcriptomics by revealing functional changes in proteins and biochemical pathways, which alone cannot be inferred from RNA content. Likewise, metabolites serve to determine fruit quality, nutritional value and stress adaptation. Protein stability, degradation and post-translational

modifications and molecular interactions affect regulatory activity, while metabolites have important implications for fruit quality, nutrition, and stress response. In developing sorghum grains, transcriptomic and metabolomic studies have uncovered coordinated alterations in growth, metabolism, and storage compound accumulation (Khan *et al*., 2024). Metabolites are also signalling molecules that create feedback interactions between metabolic pathways and gene expression. For instance, sugar accumulation represents carbon allocation, as well as feedback regulation of regulatory networks. Temporal multi-omics analysis and purposeful genetic perturbations that reveal causative regulatory interactions in late-stage responses. Such knowledge can aid breeders in precisely manipulating biosynthetic pathways and regulatory mechanisms with emphasis on get key factors for nutrient accumulation, stress tolerance and crop quality for crop breeding (Fabregas *et al*., 2026).

7.4 Chromatin and Epigenomics

Epigenomic profiling allows us to understand gene regulatory networks by revealing chromatin accessibility, DNA methylation patterns, and histone modifications controlling gene expression. Replacement of These measurements with transcriptomic data is required to identify potential regulatory elements and tissue-specific activity of genes. These examples from studies of the rice regulome have underscored the relevance of chromatin accessibility for detecting regulatory variants for complex agronomic traits (Yan *et al*., 2025). Nonetheless, accessible chromatin is not quite the same as active regulation, and epigenetic changes may be the result of altered expression rather than the cause. Tissue type, developmental stage, and environmental history can also affect interpretation. Consistent, biological replicates and controlled experimental conditions are important for cultures of reliable epigenomics. Linking epigenomic information with genetic variation, expression profile, and functional validation can unravel regulatory mechanisms and assist precise, eco-responsive crop improvement strategies (de Biron and Farrona, 2025).

8. Single-Cell and Spatial Regulatory Maps

Single-cell and single-nucleus sequencing can uncover gene regulatory networks by showing which genes are active in individual cell types details that bulk analyses may miss. When combined with chromatin-accessibility data, these methods can also point to regulatory DNA regions and transcription factors that may shape the behaviour of particular cells. Recent multi-omics research in rice highlights how these tools can reveal cellular diversity and the processes that govern it (Li *et al*., 2026). The approaches are useful for investigating root growth, seed development, and how cells acquire specialized roles. Computational analyses can suggest how cells change over time and identify regulators that may be involved. However, laboratory experiments are still needed to establish whether those regulators have a functional effect (Bian *et al*., 2025).

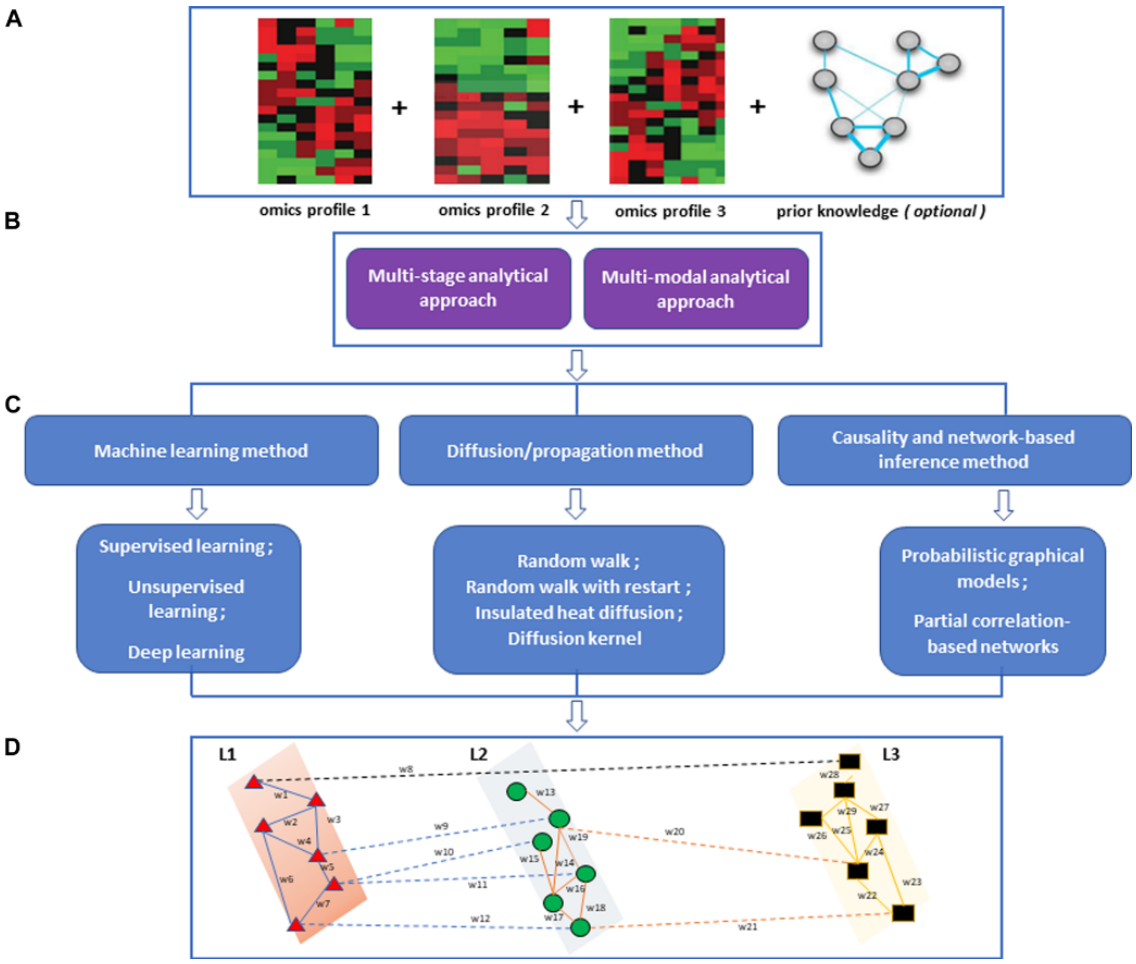
Spatial transcriptomics preserve the gene expression within the plant tissues along with its location within cells added to making it more physical. This approach helps us understand subcellular communication and conditional variation of regulatory activity such as tissue position, nutrient availability, and environmental stress. For instance, transport signals in the vascular tissues can benefit remote organs in a distant regulator context, whereas other regulators act locally. Integrating spatial information with cell-specific gene expression profiles can enhance our understanding of regulatory interactions and developmental processes. But the bottlenecks are technical drawbacks, non-consistent resolution, and complex traits that arise from crop tissues. With the aid of spatial and single-cell multi-omics to validate functional potential, this can enhance the prediction of GRNs and point out potential regulatory targets for precision crop breeding (Adema *et al*., 2024).

9. Network Inference and Computational Integration

Computational approaches and machine-learning techniques facilitate gene regulatory network (GRN) reconstruction by integrating gene expression, transcription factor binding motifs, chromatin accessibility and genetic variation. These techniques predict regulatory interactions, and prioritize candidate genes for crop improvement. Yet, their performance depends on data quality, model assumptions and validation against different genotypes, environments and tissues (Hegde *et al*., 2025). The biggest challenge is to differentiate among direct regulatory interactions, indirect associations and feedback loops. Integrating binding evidence with targeted genetic perturbations improves network reliability and supports the identification of functional regulatory relationships (Gray and Grotewold, 2025).

Multi-omics integration is considered effective with top-level sampling in each tissue under different developmental stages and treatments. The incorrect molecular linking can be caused by a difference in sample collection, stress strength, and handling conditions. Thus, standardized experimental designs, biological repetition and accurate metadata are key for inference of precise networks. Though statistical approaches can assist with some of the missing data, they cannot completely make up for poorly matched biological samples. Independent confirmations, innovative use cases, and open access database strengthen computational GRN reproducibility and predictive performance. These unified approaches can support the discovery of reliable regulatory targets for precision breeding and genetic improvement (Wolters *et al*., 2024).

Figure 4. Multi-Omics Integration and Computational Network Analysis



Source- Agamah *et al*., 2022

10. Experimental Validation of Network Predictions

Validation of predicted interactions and their functional importance, as seen in gene regulatory networks (GRNs), is fundamental for crop improvement. Direct regulatory relationships ie, transcription factor binding to target motif and a change in expression of a target gene whereas microRNA-mediated regulation demands demonstration of consequences on transcript abundance or translation. However, changes in molecular processes don't guarantee agronomic gains so yield, quality and stress resilience evaluation is also vital (Shanthinie *et al*., 2025). Using new genome-editing technologies, scientists can verify predictions for crop improvement by targeting transcription factors, manipulating cis-regulatory domains or microRNA target sites. Several of these approaches are employed to discover functional regulatory interactions, broad-spectrum functional redundancy, and alternative regulatory pathways. Lastly evaluating multiple independently edited lines increases experimental robustness (Zebell *et al*., 2025).

The effectiveness of regulatory modifications also depends on genetic background, developmental stage and environmental conditions. Differences in promoter sequences, interacting proteins and downstream regulatory components may alter the effectiveness of edited genes in different crop varieties. Functional validation requires diverse genetic background, controlled tests and field experiments across multiple environments. The regulatory modifications that depict benefits during seedling development should also be tested through flowering, fruiting, and harvest for their fate on the final yield. Stable, impactful regulatory mechanisms are identified by integrating molecular evidence with physiological readings and agronomic performance. This level of confirmatory evidence is needed to move from GRN discoveries to proven breeding strategies that increase crop productivity, quality and environmental sustainability (Moin *et al*., 2025).

11. Applications to Crop Improvement

Network information can enhance conventional breeding. Knowing the regulatory pathway responsible for a trait enhances the trait value of markers associated with it. A network analysis helps breeders discover and evaluate the presence or absence of the alleles that alter expression in a desirable direction and potential interacting genes in a breeding line. Understanding of trade-offs in biological networks may be able to explain why a marker works in one population and fails to work in another. This does not replace multi-location trials or selection on actual crop performance. This refines the choice of candidates and a mechanism-based biological explanation that may steer further crossing, marker development and validation (Silva and Viana, 2026).

11.1 Yield and Source-Sink Relations

Crop productivity relies on a balanced exchange between the parts of a plant that produce sugars and the organs that use or store them, such as grains, fruits and roots. Gene regulatory networks help control photosynthesis, sugar movement, flowering and storage, so improving these processes together may increase yields. A recent study found that adding heat-responsive control elements to the promoters of cell-wall-invertase genes in rice and tomato boosted gene activity during heat stress and increased yields under the conditions tested (Hu *et al*., 2026). Because the changes activate genes in response to specific conditions, they may improve sugar distribution without keeping gene activity constantly elevated. Their benefits, however, could differ among varieties and growing environments. Further testing should examine effects on plant growth, grain quality, recovery from stress and yield in both challenging and favourable conditions. Combining laboratory checks with field trials will help determine whether these genetic changes offer reliable, practical benefits for crop improvement (Maurya *et al*., 2025).

11.2 Water and Nutrient Use

Water and nutrient-use efficiency depend on coordinated physiological processes, including sensing, uptake, translocation and allocation of resources across plant axes. Gene regulatory networks (GRNs) link root-specific adaptations to plant-wide metabolism, affording insight into regulatory mechanisms that enhance resource use without compromising growth and development. More robust root system may improve water acquisition although it increases carbon costs, and nutrient uptake may not improve grain quality unless a high-efficiency transport and allocation system exists (Holz *et al*., 2024). Precision editing of cis-regulatory elements is an opportunity to extend the expression of nutrient channels and metabolic enzymes, without affecting the function of essential genes. Nonetheless, the efficacy of these interventions depends on the genetic background, availability of nutrients and soil conditions. Consequently, it is vital to thoroughly assess the nutrient accumulation in roots, shoots, and harvested produce along with yield and nutritional quality. Combining molecular regulations with physiological measurements and field trials can facilitate the sustainable development of crops with improved water and nutrient-use efficiency.

11.3 Stress Resilience

Crop resilience refers to the ability to maintain productivity under unfavourable environmental conditions through coordinated physiological and molecular responses. Gene regulatory networks (GRNs) help identify mechanisms that protect root water uptake, reproductive development, pollen viability and grain filling during stress. However, combining protective mechanisms requires careful evaluation because enhanced stress tolerance may increase resource consumption or reduce productivity under favourable conditions (Masoodi *et al*., 2025). Field crops frequently experience combined stresses, including drought, heat, nutrient deficiency and biotic challenges, which may trigger regulatory responses different from those observed under individual laboratory treatments. Therefore, evaluating GRNs under realistic, moderate and combined stresses is essential. Time-course analysis during stress and recovery, combined with multi-environment trials, can identify regulatory alleles that maintain growth, reproductive success and yield stability. Such approaches support the development of climate-resilient crop varieties with improved adaptation and minimal productivity trade-offs (Begna *et al*., 2026).

11.4 Quality and Biofortification

Crop nutritional quality is affected by gene regulatory networks (GRNs) involved in pigment, vitamin, mineral and other bioactive compound synthesis, transport and accumulation. Transcription factors orchestrate metabolic pathways, and non-coding (noncoding) RNAs modulate the expression of associated genes. The multi-omics methodology, by identifying play a role in improving desired nutrition without affecting yield, flavour, storage quality or consumer acceptance (Xue *et al*., 2025). Biofortification is of special concern because higher nutrient absorption does not necessarily result in its accumulation in edible tissues. Regulatory networks controlling mineral transport, storage and nutrition interactions must be looked at in an integrated fashion. The bottlenecks for targeted improvement can be found via tissue-specific expression profiling and genetic variation analysis. However, successful biofortification requires evaluating nutrient concentration, bioavailability and potential accumulation of harmful elements in harvested produce. Multi-environment validation is essential for developing nutritionally enriched crop varieties with stable agronomic performance (Verma *et al*., 2025).

12. Limitations and Future Directions

A major limitation of crop gene regulatory networks (GRNs) is the difficulty of distinguishing causal relationships from statistical associations. Although high-throughput technologies identify numerous potential regulatory interactions, relatively few undergo direct experimental validation. Incomplete annotation of non-coding regions and regulatory RNAs further limits network accuracy, particularly in underexplored crops (Maizels and Briscoe, 2026). Unequal genomic resources across crop species and breeding populations also restrict the identification of valuable regulatory alleles in landraces and wild relatives. Population structure, environmental variation and inconsistent phenotyping can introduce misleading associations. Therefore, improving GRN reliability requires diverse genomic resources, standardized datasets, rigorous functional validation and prioritization of biologically meaningful regulatory interactions (Mylona *et al*., 2026).

Future GRN research should emphasize dynamic, tissue-specific and environmentally responsive regulatory models by integrating time-course multi-omics, single-cell analysis and spatial transcriptomics. Computational approaches can enhance predictions of regulatory interactions, but validation in the right genetic backgrounds and environmental conditions is essential (Zaman and Park, 2026). Just as much to more effectively applied the molecular and functional findings of genome wide interaction networks into crop improvement is to have

interdisciplinary efforts between Molecular Biologists, Geneticists, Physiologists and Plant Breeders. Important lessons are also learned by publish findings of failed trials or context dependent cases which can also improve transparency, predictive accuracy and research reproducibility. Linking molecular findings with typical breeding goals needs more integration, which would help accelerate the development of productive, resilient and nutritionally improved crop varieties (Ramstein *et al*., 2026).

13. CONCLUSION

Gene regulatory networks offer a deeper account of crop traits by connecting regulatory DNA, transcription factors, non-coding RNAs and downstream biological processes. New tools, including multi-omics and single-cell analysis, are making these links clearer, and genome editing can help test whether proposed connections are real. The key is to tell cause and effect apart from patterns that simply reflect a plant's development or its response to the environment. Reliable progress requires careful testing across a wide range of genetic backgrounds and under field conditions. Used carefully, network knowledge can guide breeding toward more precise improvements in yield, quality, nutrient use and resilience, while making the consequences of each intervention easier to anticipate.

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