

Systems Biology of Plant Secondary Metabolism: Integrating Multi-Omics to Decipher Biosynthesis, Regulation, Ecological Functions, and Metabolic Engineering

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Abstract

Plant secondary metabolites are structurally diverse bioactive compounds that play essential roles in plant growth, development, defense, ecological interactions, and environmental adaptation while serving as valuable resources for pharmaceutical, nutraceutical, and agricultural applications. The integration of multi-omics and computational biology has revolutionized the understanding of specialized metabolism by connecting genes and transcripts with complex regulatory networks governing metabolite biosynthesis, transport, and accumulation. From a systems biology perspective, this review aims at giving a comprehensive account of the diversity, biotic functions, genomic organization, transcriptional regulation, interactions with the plant proteome and metabolome, as well as regulatory networks and evolutionary dynamics of plant secondary metabolism. Moreover, it emphasizes the role of AI, network biology, synthetic biology, metabolic engineering and precision breeding in discovery of pathways, functional gene annotation, predictive metabolic modeling, and crop improvement. The applications of systems biology in developing climate-resilient crops, in enhancing resistance to pest and diseases, in biofortifying crops for nutrition and in sustainable production of high-value phytochemicals are also discussed. Lastly, current challenges, such as the integration of multi-omics, computational challenges, functional annotation and experimental validation are critically discussed and future opportunities based on pan-genomics, single-cell and spatial multi-omics, AI-assisted breeding and next-generation metabolic engineering are highlighted. This review, which combines recent advances in molecular, computational and biotechnological fields, shows that systems biology is a powerful tool for understanding and dissecting plant secondary metabolism and its relevance for sustainable agriculture, plant biotechnology and the discovery of natural products from plants.

Keywords: Plant secondary metabolism; Systems biology; Multi-omics integration; Metabolic engineering; Sustainable agriculture.

1. Introduction

Plant secondary metabolites, also known as specialized metabolites, are a wide variety of low-molecular weight compounds that are produced from primary metabolic intermediates, but are not specifically needed for normal plant growth and development. Rather these metabolites have essential ecological and physiological roles such as protection from herbivores and pathogens, protection from UV radiation and oxidative stress, attracting pollinators and seed dispersers, allelopathic interactions, and adaptation to fluctuations in the environment. While all of these classes are important in natural products, they constitute one of the largest sources of bioactive natural products used in medicine, agriculture, cosmetics, and nutrition, and are collectively referred to as major classes of plant secondary metabolites (PSM).^[1-5] The remarkable structural and functional variation of plant secondary metabolites has resulted from what is arguably a complex genomic and biochemical process, in which genes were duplicated, parts of the genome were duplicated, plant enzymes have diverged, biosynthetic gene clusters have been formed and natural selection has acted. The evolutionary mechanisms have facilitated an uninterrupted increase of their metabolisms and their ability to occupy new ecological niche while improving their tolerance to biotic and abiotic stresses. Thus, specialized metabolites are now known to play an important role in plant fitness and serve as promising sources for drug discovery, functional foods, industrial biotechnology and sustainable agriculture.^[2,3,6,7] Although extensive research has been conducted, the molecular regulation of plant secondary metabolism is still not sufficiently understood, due to the highly interconnected nature of the regulatory network controlling the biosynthesis of metabolites, which spans many biological levels. Specialized metabolites are synthesized and accumulated through dynamic interplay of genomic variation, epigenetic modifications, transcription regulation, protein levels, post-translational modifications, metabolite fluxes, hormone signaling, cellular compartmentalization and environmental factors. Gene or enzyme-based studies have made inroads in understanding particular biosynthetic pathways, but a reductionistic perspective is not always sufficient to account for the dynamic nature of regulatory interactions that shape metabolic complexity.^[8-10] The advent of systems biology has transformed the study of plant secondary metabolism by enabling the investigation of biological systems across multiple molecular layers through genomics, transcriptomics, proteomics, metabolomics, phenomics, and computational biology. Advances in next-generation sequencing, mass spectrometry, single-cell and spatial omics, artificial intelligence, machine learning, and network biology have provided increasingly comprehensive insights into biosynthetic genes, transcriptional regulators, protein interaction networks, metabolic pathways, and regulatory networks that collectively govern specialized-metabolite biosynthesis. Integration of these complementary datasets has facilitated pathway reconstruction, functional gene

annotation, and prediction of novel bioactive compounds.^[11-13] The availability of advances in genome editing, synthetic biology and artificial intelligence has also advanced the rational engineering of plant specialized metabolism at a rapid pace. The new technologies like genomics editing, multi-omics pathway engineering and machine learning-based pathway prediction can now be used to precisely modify biosynthetic pathways to produce higher levels of phytochemicals, make crops more stress tolerant, more climate resilient and more nutritious and medicinal. The innovations present a new class of opportunities for taking basic research into fields and applications that will contribute to solving global problems of food security, climate change, biodiversity conservation and sustainable bioresource utilisation.^[14,15] The current review provides an overview of recent developments in the field of systems biology for secondary metabolism in plants, especially concerning the integration of multi-omics, computational biology, artificial intelligence, and metabolic engineering. Specific attention has been given to the molecular control mechanisms of specialized metabolites biosynthesis, multi-omics integration, and new computational tools.^[16,17]

2. Diversity and biological functions of plant secondary metabolites

Secondary metabolites (also called specialized metabolites) are a large and chemically diverse group of natural products that are synthesized by specialized biosynthetic pathways from primary metabolism. These substances are not essential for plant growth and reproduction, but they are essential for plant survival, environmental adaptation and resilience. The remarkable structural diversity of specialized metabolites produced by plants has been discovered and is estimated to exceed 200,000, and is one of the largest sources of bioactive natural compounds for pharmaceutical, nutraceutical, agricultural and industrial use.^[18-21] Based on their biosynthetic origin and chemical properties, plant secondary metabolites fall into general categories of alkaloids, terpenoids, phenolic compounds, flavonoids, glucosinolates, lignans, saponins, cyanogenic glycosides and many other metabolites specific to their lineage. Alkaloids have high pharmacological activity, terpenoids are involved in defense, signalling and volatile communication, phenolics and flavonoids are responsible for antioxidant activity, UV protection, pigmentation and pathogen resistance. The evolutionary diversification of specialized metabolism in plants is clearly reflected in the development of glucosinolates and cyanogenic glycosides as chemical defense compounds against herbivores and pathogens.^[22,23] Secondary metabolites are very tissue-, organ-, development- and species-specific. Roots tend to accumulate phenolics and flavonoids in defense against oxidative stresses, UV radiation, and prevention of plant pathogens, while roots produce coumarins, phytoalexins, and other metabolites related to rhizosphere activity and nutrient acquisition. Flowers and fruits contain pigments and volatile terpenoids that aid in pollination and seed dispersal and the seeds contain protective alkaloids and

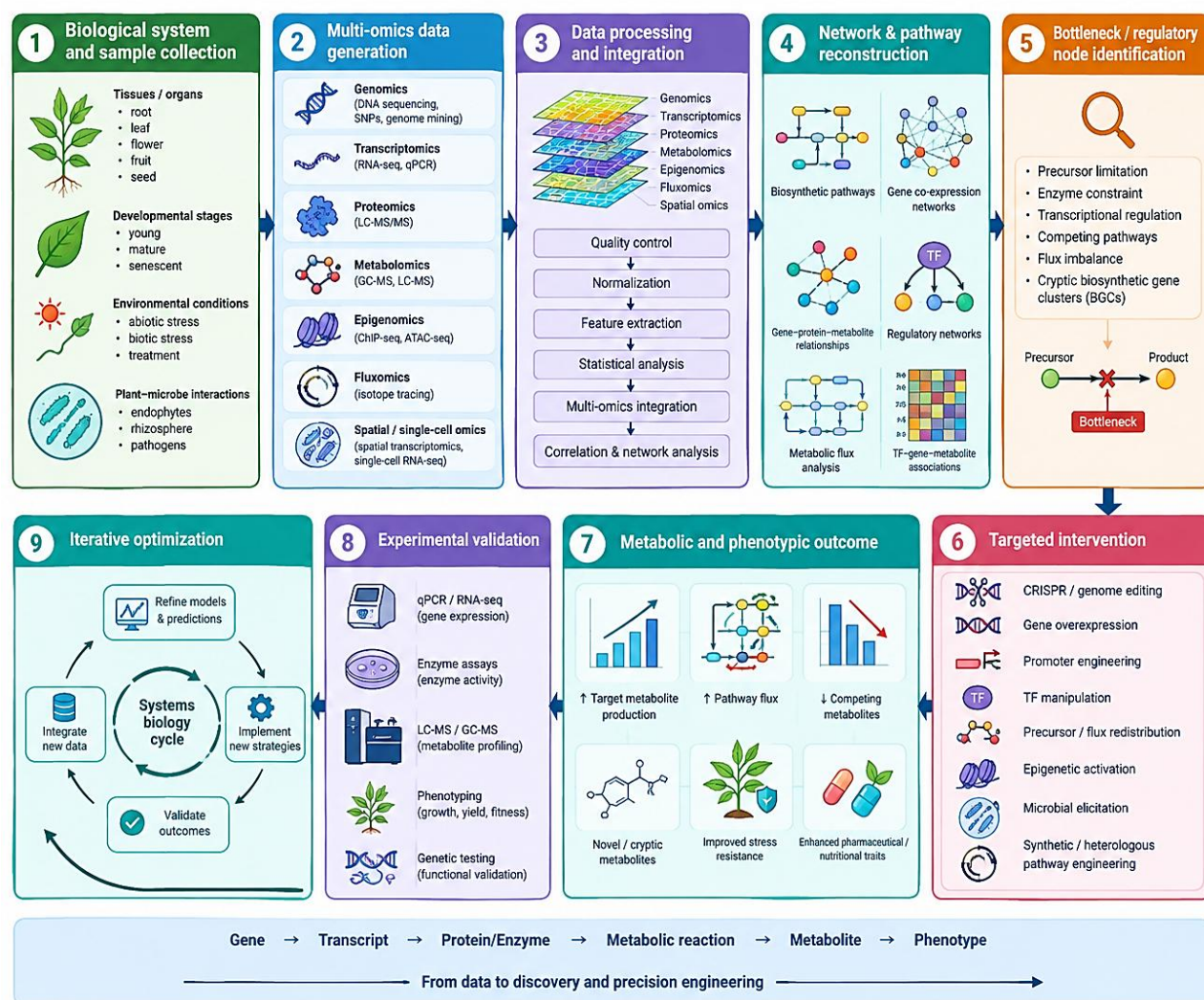


Fig.1: Mechanistic systems biology pipeline for deciphering and engineering plant secondary metabolism.

tannins. Glandular trichomes, resin ducts, oil glands, laticifers, and secretory cavities are specialized structures that are important for metabolite biosynthesis, storage, and secretion, which help prevent autotoxicity and increase defense efficiency.^[24,25] Diversity and biological functions of plant secondary metabolites are shown in Fig.1. Beyond their structural diversity, secondary metabolites perform multiple ecological and physiological functions that contribute to plant fitness. They function as chemicals to repel insects and fungi, as well as to deter nematodes, bacteria and viruses; regulate plant-microbe and plant-plant interactions; participate in allelopathy; attract pollinators; and increase drought, salinity, extreme temperature, heavy metal and ultraviolet radiation tolerance. Their biosynthesis is dynamically regulated in response to environmental signals and phytohormones allowing plants to quickly adjust their metabolic profile under changes in environmental conditions, thereby adapting to different ecosystems.^[26-29] However, the recent developments in multi-omics technologies, artificial intelligence, and metabolic engineering have led to the rapid identification of novel phytochemicals and their biosynthetic pathways, as well as engineering high-value metabolites. Consequently, knowledge about the biodiversity and biological activities of plant secondary metabolites is vital for systems biology, sustainable agriculture, and

plant biotechnology.^[30-32]

Fig. 1 shows mechanistic systems biology pipeline for deciphering and engineering plant secondary metabolism. Biological materials representing different tissues, developmental stages, environmental conditions, or plant-microbe interactions are subjected to complementary omics analyses, including genomics, transcriptomics, proteomics, metabolomics, epigenomics, fluxomics, and spatial or single-cell profiling. Following quality control, normalization, feature extraction, and computational integration, multi-omics datasets are used to reconstruct biosynthetic pathways, gene regulatory networks, gene-protein-metabolite associations, and metabolic flux distributions. Integrated network analysis enables identification of pathway bottlenecks, regulatory nodes, precursor limitations, competing metabolic routes, and cryptic biosynthetic potential. These targets can then be manipulated through CRISPR-based genome editing, gene overexpression, promoter engineering, transcription-factor modulation, precursor or flux redistribution, epigenetic activation, microbial elicitation, or heterologous pathway engineering. The resulting changes in metabolite production, pathway flux, stress responses, and phenotypic traits are experimentally validated using molecular, biochemical, metabolomic, and phenotypic approaches, establishing

an iterative systems biology–engineering–validation cycle.

3. Systems biology framework

A revolutionary way to understand the complexity of plant secondary metabolism is to combine several levels of biological information into a system-based approach, known as systems biology. Systems biology provides a holistic method of studying the dynamic interactions between genome, transcriptome, proteome, metabolome and phenome to understand the regulatory networks responsible for the biosynthesis of specialized metabolites as opposed to the traditional reductionist studies of individual genes or enzymes. Combination of high-throughput omics technologies and computational modeling can be used for comprehensive characterization of biosynthetic pathways, regulatory mechanisms, metabolic fluxes, and plant response to environment, leading to systems-level understanding of plant metabolism.^[33-37]

3.1 Multi-Omics integration

In multi-omics data integration, multiple molecular data are used in a synergistic manner to unravel the molecular mechanism and regulation of plant secondary metabolism. Information from genomics and transcriptomics data helps in finding out biosynthesis-related genes and regulators whereas proteomics data help in understanding the quantity, post-translational modification, and protein interactions. Data from metabolomics show alterations in the metabolism pathway and phenotype data reveal the changes in the morphology of plants. Integration of all these data helps in reconstructing pathways and understanding regulatory pathways and biomarkers.^[38,39]

3.2 Computational biology

Computational biology provides the analytical foundation for interpreting complex multi-omics datasets generated from systems biology studies. Advanced bioinformatics algorithms, network biology, mathematical modelling, machine learning, and artificial intelligence allow bio synthetic pathways to be reconstructed, functions of genes to be predicted, regulatory hubs to be identified, and metabolic fluxes to be simulated. These computational methods enable the integration of heterogeneous biological data on a large scale, fast identification of new specialized metabolites, and predictive metabolic engineering for crop improvement and sustainable agriculture.^[40]

3.3 Bioinformatics resources

Public bioinformatics resources are crucial in systems biology research, offering comprehensive data repositories of genomic, transcriptomic, proteomic and metabolomic data. Ensembl Plants, Phytozome, Gramene, NCBI GEO, Expression Atlas, UniProt, STRING, KEGG, PlantCyc, Databasem of Metabolites (DBM), MetaboLights and KNApSACk are among the databases used for genome annotation, gene expression analyses, protein characterization, pathway reconstruction and metabolite identification. These resources can be integrated with analytical tools such as Cytoscape, MetaboAnalyst, OmicsNet, and Galaxy to visualize and interpret biological networks, integrate multi-omics, and provide systems-level interpretation of plant secondary metabolism, which speeds up functional genomics and metabolic engineering research.^[41-44]

4. Genomic and transcriptional regulation of plant secondary metabolism

Table 1: Multi-omics approaches, their applications, and representative bioinformatics tools/platforms in plant secondary metabolism

Omics approach	Major information generated	Application in secondary metabolism	Representative bioinformatics tools/platforms
Genomics	Genes, genome variation, and biosynthetic gene clusters	Identification of biosynthetic genes and pathway evolution	Ensembl Plants, PLAZA, Gramene
Transcriptomics	Gene-expression profiles	Identification of pathway genes, transcription factors, and regulatory modules	Galaxy; WGCNA
Proteomics	Enzymes, regulatory proteins, and protein abundance	Functional validation of biosynthetic pathways and enzyme regulation	STRING; Cytoscape
Metabolomics	Metabolite profiles and abundance	Metabolite identification, pathway reconstruction, and biomarker discovery	MetaboAnalyst
Phenomics	Observable plant traits	Linking molecular changes with stress responses and agronomic traits	Galaxy; computational phenotyping platforms
Spatial omics	Cell/tissue-specific molecular distribution	Localization of biosynthesis, transport, and metabolite accumulation	OmicsNet; Cytoscape
Integrated multi-omics	Cross-layer biological relationships	Systems-level reconstruction and prediction of specialized metabolism	OmicsNet; Cytoscape; MetaboAnalyst; Galaxy

The biosynthesis, accumulation and diversification of specialised metabolites are regulated by complex interactions between genomic architecture and transcriptional control. Advances in whole genome sequencing, transcriptomics, comparative genomics, functional genomics, and the discovery of biosynthetic gene clusters, gene duplication, and polyploidization, as well as the identification of transcription factors, phytohormones, and gene regulatory networks, have shown that these are integrated in a coordinated fashion to control metabolic pathways. Their combination with genome-editing technologies and systems biology has helped to identify important biosynthetic genes, metabolic pathway reconstruction, and precision engineering of high-value phytochemicals for sustainable agriculture and plant biotechnology.^[45-48] Multi-omics approaches, their applications, and representative bioinformatics tools/platforms in plant secondary metabolism are shown in [Table 1](#).

4.1 Biosynthetic gene clusters

Biosynthetic gene clusters (BGCs) are linear (co-linear) clusters of genes that encode enzymes, transporters and regulatory proteins that are involved in the biosynthesis of specialized metabolites. Plant BGCs, in contrast to primary metabolic genes, tend to be recruited from other gene families, tandem duplicated and reshaped by chromosome rearrangements, followed by adaptive selection, so as to coordinate the expression of complex biosynthetic pathways. In recent years, genomic research has shed light on the evolution and regulation of metabolic diversity in plants through the identification of BGCs that are responsible for the biosynthesis of terpenoids, alkaloids, benzoxazinoids, cyanogenic glycosides, steroidal glycoalkaloids, and triterpenes.^[47,48]

4.2 Gene duplication and polyploidy

Two key evolutionary processes that contribute to the evolution of plant secondary metabolism are gene duplication and whole genome duplication (polyploidy). Tandem, segmental, and dispersed duplications produce extra copies of genes that later acquire new functions (neofunctionalization) or new constraints (subfunctionalization) to give rise to larger enzyme families like the cytochrome P450 monooxygenases, the terpene synthases, the methyltransferases and the UDP-glycosyltransferases. Polyploidization also contributes to metabolic innovation through dosage effects and boosts metabolic diversification and plant adaptation to environmental stress.^[49,50]

4.3 Genome editing

Precise modification of genes that are involved in the biosynthesis of specialised metabolites is revolutionising functional genomics and metabolic engineering using genome-editing technologies. CRISPR/Cas systems are the most popular genome editing platform used for programmable manipulation of biosynthetic genes and regulatory elements, while precision base editing and prime editing allow the highly accurate and precise substitution of nucleotides without the need to generate

double-strand DNA breaks. These technologies have enabled improvements in the production of medicinal metabolites, enhancement of nutritional quality, improvement of stress resistance and development of climate resilient crops by targeted metabolic engineering.^[51,52]

4.4 Transcriptomics

Understanding of transcriptional regulation of plant secondary metabolism has significantly benefited from transcriptomic technologies. Comprehensive analyses of gene expression, at the tissue-, organ- and cell-specific level, are possible by high throughput RNA sequencing (RNA-seq), single-cell transcriptomics and spatial transcriptomics. The genetic and regulatory analysis of specialised metabolism has been further advanced by integrating transcriptomic profiles with metabolomic and proteomic data, which has allowed the identification of biosynthetic genes, regulatory modules and tissue-specific metabolic pathways, improving pathway reconstruction and functional annotation of specialised metabolism.^[53-57]

4.5 Transcription factors

Transcription factors are involved in controlling the biosynthesis of specialized metabolites and coordinate the expression of the metabolic enzymes and regulatory genes that control specialized metabolism, thus acting as master regulators. In response to developmental and environmental signals, flavonoid, alkaloid, terpenoid, glucosinolate and phenylpropanoid biosynthesis is controlled by major transcription factor families such as MYB, bHLH, WRKY, NAC, AP2/ERF and bZIP. These transcription factors communicate with each other to create complex regulatory units that coordinate metabolic, hormonal and stress responsive signaling pathways in order to maximize the production of metabolites.^[58-62]

4.6 Hormonal regulation

Phytohormones are key players in the crosstalk with transcription factors and signaling pathways that coordinate the biosynthesis of specialized metabolites. Biosynthetic pathways involved in pathogen resistance, abiotic stress tolerance and developmental regulation are regulated by salicylic acid, ethylene, abscisic acid, and auxin, while jasmonic acid is the key regulator of defense-related secondary metabolism. Under the regulation of hormonal signals, metabolic pathways can be dynamically reconfigured, with the result that metabolic pathways are plastic and can be adapted to environmental conditions.^[11,63-65]

4.7 Gene regulatory networks

Gene Regulatory Networks (GRNs) represent an organizational scheme that links transcription factors, signal molecules, biosynthesis genes, epigenetic factors, and environmental inputs into a network to control specialized metabolism. The development of integrated omics and network computational technologies has facilitated GRN modeling and identification of regulatory

hubs as well as metabolic outcomes predictions. This will facilitate functional genomics, precision metabolic engineering, and sustainable crop improvement through rational manipulation of specialized plant metabolism.^[12,66-69]

5. Proteome–Metabolome Integration

By integrating the metabolome and proteome, the study can present a full picture of the secondary metabolisms of plants by relating plant proteins to metabolites. Proteomics can be used to detect functional enzymes, regulatory proteins and protein post-translational modifications that regulate biosynthetic pathways, while metabolomics can detect the dynamic accumulation of specialized metabolites, which are the end products of cellular regulation. The addition of these complementary layers of omics allows metabolic pathways to be reconstructed, key regulatory enzymes identified, metabolic fluxes quantified and molecular responses to developmental and environmental cues elucidated. In recent years, the ability to study the biosynthesis of specialized metabolites and metabolic engineering for sustainable crop improvement has gained significant ground with the development of high-resolution mass spectrometry, quantitative proteomics, metabolomics and computational systems biology.^[70,71]

5.1 Proteomics

Proteomics offers direct information regarding the abundance, location, activity and interactions of proteins contributing to the biosynthesis of specialized metabolites. Label-free quantitative proteomics, tandem mass tag (TMT) analysis and data-independent acquisition (DIA) are modern mass spectrometry-based methods which allow for large-scale identification of biosynthetic enzymes, transporters and regulatory proteins. Complementary to genomic and transcriptomic studies of plant specialized metabolism, proteomic analyses have provided insight into the structure of enzyme complexes, regulation of metabolic pathways, and protein response to developmental and environmental factors.^[70-72]

5.2 Metabolomics

Metabolomics describes the entire set of small molecules generated in plant tissues, and is the most closely defined molecular phenotype of cellular metabolism. Targeted and untargeted profiling of specialized metabolites are made possible by advanced analytical platforms like liquid chromatography–mass spectrometry (LC–MS), gas chromatography–mass spectrometry (GC–MS), capillary electrophoresis–mass spectrometry (CE–MS) and nuclear magnetic resonance (NMR) spectroscopy. Metabolomics combined with proteomics facilitates the reconstruction of metabolic pathways, discovery of biomarkers, functional annotation of unknown metabolites and in-depth study of metabolic responses to environmental stresses.^[73-75]

5.3. Post-Translational modifications

In specialized metabolite biosynthesis, post-translational

modifications (PTMs) are essential mechanisms regulating the stability, localization, enzymatic activity and signaling of proteins. The mechanisms of phosphorylation regulate enzyme activation and signal transduction in a rapid manner, ubiquitination is responsible for selective degradation of proteins via the ubiquitin–proteasome pathway, and glycosylation regulates protein folding, transport, stability and enzyme function. The dynamic regulation of biosynthetic enzymes and transcriptional regulators by reversible modifications has been demonstrated by quantitative PTM proteomics under developmental programs and environmental stresses, highlighting the ability of these modifications to fine-tune specialized metabolism.^[76,77]

5.4 Metabolic flux analysis

Metabolic flux analysis (fluxomics) gives quantitative information on the flow of carbon, nitrogen and energy in interconnected metabolic pathways, which is not obtained by measuring metabolite abundance. The stable isotope labelling and the use of mass spectrometry and computational modelling allow the estimation of the activities of the pathways, identification of the rate-limiting reactions, as well as the analysis of metabolic reprogramming under the different physiological conditions. Combination of fluxomics data with proteomic and metabolomic data has led to a better understanding of pathway regulation and the optimization of specialized metabolite production for metabolic engineering applications.^[78-81]

5.5 Spatial metabolomics

Spatial metabolomics is an approach that involves combining metabolite profiling with mass spectrometry imaging to visualize the spatial distribution of tissue/cell-specific metabolome specializations, while simultaneously maintaining the spatial organization of metabolites. Newly introduced imaging technologies such as MALDI-MSI, DESI-MSI and SIMS allow metabolites to be localized in reproductive organs, roots, vascular tissues and glandular trichomes, which are unique tools for unprecedented insights into tissue-specific biosynthesis, transport and storage. Spatial metabolomics, combined with spatial transcriptomics, spatial proteomics, and artificial intelligence, is revolutionizing plant systems biology, allowing for detailed mapping of metabolic networks and elucidation of the spatiotemporal regulation of specialized metabolism.^[15,82-85]

6. Regulatory networks and environmental responses

The molecular networks that control secondary metabolism are highly interconnected and integrate metabolic pathways, cell organization, developmental programmes and environmental signals. Primary metabolism, hormonal regulation, transcription control and environmental factors all regulate the biosynthesis, transport and accumulation of specialized metabolites, a process that does not function in isolation. With recent developments in multi-omics technologies, network

biology, and systems biology, dynamic interactions between genes, proteins, metabolites, and environment have been discovered as a means by which plants quickly reprogram specialized metabolism when encountering developmental and ecological fluctuations. These integrated regulatory networks are ultimately responsible for plant adaptation, defense and metabolic plasticity.^[86-90]

6.1 Primary-secondary metabolism crosstalk

Primary and secondary metabolism are closely coupled and regulated by the allocation of carbon, nitrogen, sulfur and energy. Key intermediates for alkaloid, terpenoid, phenylpropanoid, flavonoids and other specialized metabolite biosynthesis are provided by central metabolic pathways such as glycolysis, the tricarboxylic acid (TCA) cycle, pentose phosphate pathway and shikimate pathway. Plants can use this dynamic regulation of metabolic flux between primary and secondary metabolism to ensure optimal use of resources in growth, development, and stress adaptation, which occurs at the transcriptional level, through enzyme activity, and via hormonal signaling.^[30,31,91]

6.2 Cellular regulation

Synthesis of specialized metabolites is very compartmentally organized and regulated at the cellular and tissue level. Specialized cells such as glandular trichomes, secretory cells, vascular tissues, root hairs, resin ducts, and oil glands are involved with the synthesis, transport, and storage of metabolites. Metabolic channeling, reduction in autotoxicity and increased biosynthetic efficiency are achieved by cellular compartmentalization in the plastid, ER, cytosol, vacuole and peroxisome. The recent progresses in single-cell transcriptomics, spatial transcriptomics, and spatial metabolomics have given rise to an unprecedented insight into tissue-specific metabolic regulation and cellular heterogeneity associated with specialized metabolism.^[16,92,93]

6.3 Abiotic stress regulation

Plants secondary metabolism is significantly affected by abiotic stresses such as drought, salinity, temperature extremes, high light intensity, high intensity of UV radiation, heavy metals, elevated levels of atmospheric CO₂. Reactive oxygen species (ROS) accumulation under stress triggers a complex signaling pathway mediated by jasmonic acid, abscisic acid, salicylic acid, calcium signaling and mitogen-activated protein kinase (MAPK) cascades, leading to the activation of transcription of specialized metabolite biosynthesis. Increase in the synthesis of flavonoids, phenolics, terpenoids, alkaloids and other antioxidants metabolites lead to a better protection of cells, redox homeostasis, osmotic adjustment and stress tolerance.^[94-97]

6.4 Biotic stress regulation

Rapid metabolic reprogramming in response to plant-pathogen, plant-herbivorous insect and plant-parasitic organism interactions for increased chemical defense.

Pathogen-associated molecular patterns (PAMPs) and elicitors derived from herbivores trigger immune signaling pathways leading to the synthesis of phytoalexins, glucosinolates, terpenoids, alkaloids, phenylpropanoids and volatile organic compounds. The specialized metabolites serve roles as antimicrobial agents, insect repellents, signaling molecules, and indirect defense molecules that attract natural enemies of herbivores, thereby enhancing plant fitness and immunity.^[26-28]

6.5 Microbiome interactions

The plant microbiome contributes significantly to specialized metabolism by interacting with beneficial microorganisms that live in the rhizosphere, phyllosphere and endosphere of the plant. Plant growth-promoting rhizobacteria (PGPR), mycorrhizal fungi, and endophytic microbes are involved in the regulation of phytohormone signaling, nutrient availability, and defense pathways, making them valuable tools for increasing the production of secondary metabolites that are beneficial for humans and agriculture. Integration of microbiome research with genomics, metabolomics, and systems biology is providing new opportunities to exploit beneficial microbial communities for sustainable crop improvement, enhanced stress resilience, and increased production of valuable phytochemicals.^[2,14,54,55,98-101]

7. Evolutionary dynamics of plant secondary metabolism

Plants have undergone millions of years of evolution and adaptation, yielding an amazing range of specialized metabolites that help plants to thrive in different environments. Lineage-specific biosynthetic pathways have evolved from metabolic pathways through gene duplication, whole genome duplication, enzyme functional divergence, natural selection and environmental pressures. Specialized metabolism is highly dynamic and continually influenced by ecological interactions, which enable plants to optimize both defense and reproduction together with environmental adaptation, as shown by recent advances in comparative genomics, phylogenomics, metabolomics and systems biology. The knowledge of the evolution of secondary metabolites will be useful for understanding metabolism innovations and for discovering new bioactive metabolites in agriculture, biotechnology and medicine.^[102-106]

7.1 Evolution of metabolic pathways

Specialized metabolic pathways have been recruited from an ancestral primary metabolic network by acquiring genes, by changing enzymes to be promiscuous, by rearranging pathways, and by clustering biosynthetic genes together. These functional diversifications of enzyme families have led to the production of structurally diverse alkaloids, terpene, acyl transferases, methyltransferases, glycosyl transferases, and cytochrome P450 monooxygenases, which are responsible for the synthesis of structurally diverse products such as terpenoids, phenylpropanoids,

flavonoids, glucosinolates, and other alkaloids. Comparative genomic studies are also beginning to show that innate metabolic diversity within the plant kingdom is the result of repeated innovation and pathway expansion.^[46,107-109]

7.2 Diversification

Metabolic diversification is achieved by ongoing modification of biosynthetic genes by duplication, mutation, recombination, and regulatory evolution. Metabolites are species-specific and result from enzyme neofunctionalization and gene family expansion. Metabolic diversification is one of the most rapidly evolving biological systems, driven by environmental heterogeneity, co-evolution with pollinators, herbivores, pathogens and beneficial microbes.^[110,111]

7.3 Natural selection

Specialized metabolism is an important factor in the evolution of plants and has been driven by natural selection, which favors the metabolites that enhance plant fitness under certain ecological constraints. Positive selection has led to the evolution of defense compounds against herbivores and pathogens, while balancing and diversifying selection have caused metabolic variation to be maintained in natural population. Plants adaptively evolve biosynthetic enzymes, transcription factors, and pathways for more efficient chemical defenses, reproductive success, and environmental tolerance in ever-changing ecosystems.^[13,112,113]

7.4 Domestication

The composition and diversity of secondary metabolites have been greatly modified by plant domestication, with artificial selection for agronomic, nutritional, medicinal and sensory properties. Numerous biosynthetic pathways have been changed in domestication, such as the selection of a strain with better yield, less bitterness, enhanced flavor, or decreased toxicity, and may have lost metabolic diversity relative to their wild relatives. The modern genomics, pan-genomics and genome-wide association studies (GWAS) and genome editing now enable the reintroduction and optimization of useful specialized metabolites from wild germplasm into cultivated plants for precision breeding and sustainable agriculture.^[114-118]

8. Artificial intelligence and network biology in plant secondary metabolism

In order to integrate and analyze large amounts of multi-omics data in plants, artificial intelligence, machine learning, and network biology are becoming more and more widely used. Using such computational methods makes it possible to construct gene regulatory networks, biosynthetic pathways, discover novel metabolites, and model metabolic responses to changing environmental factors. Due to integration of heterogeneous molecular and phenotypic data sets, AI-based methods will help to perform functional annotation, precision breeding, and metabolic engineering of crops.^[110,119-121]

8.1 Gene co-expression networks

Gene co-expression network analysis can be used to determine clusters of co-expressed genes that are involved in specific metabolic pathways. Research on genome-wide expression data using the network-based approach, e.g. weighted gene co-expression network analysis (WGCNA), can identify biosynthetic genes, transcription factors, regulatory modules, and metabolic hubs for secondary metabolite production. Co-expression networks have been integrated with metabolomics and comparative genomics to significantly improve the reconstruction of pathways and functional gene annotation in medicinal and crop plants.^[21,122,123]

8.2 Machine learning

The analysis of high-dimensional plant omics data is now heavily reliant on machine learning (ML). Supervised and unsupervised learning algorithms enable metabolite classification, prediction of gene functions, pathway reconstruction, discovery of metabolite biomarkers, and prediction of accumulation of specialized metabolites under various developmental and environmental conditions. The applications of ML models also enhance genomic selection and precision breeding, accounting for complex genotype-phenotype relationships.^[124]

8.3 Deep learning

Plant systems biology has gained a tremendous boost from deep learning algorithms such as convolutional neural networks (CNNs), recurrent neural networks (RNNs), graph neural networks (GNNs), and transformer-based models that have been able to detect complex patterns in genomic sequences, transcriptomic profiles, metabolomic spectra, and phenotypic images. These methods offer more accurate identification of metabolites, prediction of enzyme function, disease types, high throughput phenotyping and biosynthetic pathway prediction than traditional computational approaches.^[98,125-127]

8.4 Predictive metabolic models

The integration of multi-omics data with computational simulations into predictive metabolic models describes metabolic fluxes, regulatory interactions, and pathway dynamics. The metabolic modeling of the constraint-based approach, genome-scale metabolic models (GEMs), and flux balance analysis (FBA) can predict metabolic responses to various environmental conditions and direct metabolic engineering efforts in a rational way. The use of these models has been indispensable to optimizing the production of secondary metabolites and designing climate-smart crops.^[2,128,129]

8.5 Digital phenotyping

Digital phenotyping employs advanced imaging techniques, remote sensing, robotics, unmanned aerial vehicles (UAVs), hyperspectral imaging, thermal imaging, LiDAR and artificial intelligence (AI) to measure plant growth, physiology, stress response and metabolite-associated phenotypes in a high throughput manner. Combination of digital phenotyping with genomics,

transcriptomics, metabolomics and environmental data facilitates accurate association studies of genotype–phenotype; it contributes to the speed of crop improvement; and helps precision agriculture in the context of changing climatic conditions.^[5,129]

9. Synthetic biology and metabolic engineering

In the past few years, synthetic biology and metabolic engineering approaches have proven to be feasible methods of reprogramming the metabolic pathways in plants to enhance the biosynthesis of useful secondary metabolites. Combined omics analysis along with systems metabolic modeling can help identify bottlenecks and modify biosynthetic genes, promoters, and metabolic pathways. The progress made in the field of genome editing using CRISPR technology, design of synthetic promoters, and pathway reconstruction is helping in the creation of disease-resistant, nutritional, and climate-resistant crops.^[8,130,131]

9.1 Pathway engineering

Pathway engineering is the strategic modification of biosynthetic pathways to enhance the yield of specialized metabolites, or to produce new bioactive compounds. This approach involves the overexpression of enzymes in biosynthetic pathways, the inhibition of alternative pathways, the optimization of pathway flux, and the rewiring of entire pathways in plants or alternative hosts. Multi-omics approach and computer modelling can be used to identify metabolic bottlenecks and to guide rational pathway engineering for producing high-value phytochemicals (alkaloids, terpenoids, flavonoids, phenolics, and others) at higher levels.^[7,93]

9.2 Gene stacking

Gene stacking allows for the more efficient pathway to be engineered and the more prolific metabolite production to be achieved by simultaneously introducing or tuning multiple biosynthetic genes into a metabolic pathway. Coordinate control of complex metabolic networks, with the reduced incidence of unwanted pleiotropic effects, is made possible by the modern multigene assembly technologies, CRISPR multiplex editing, and synthetic gene circuits. Gene stacking has been extensively used for enhancing the nutritional content, disease resistance, stress tolerance, and production of pharmaceutical compounds in economically important crops.^[132,133]

9.3 Promoter engineering

Promoter engineering is a means of controlling expression of a gene with a precisely modified promoter sequence, or with a synthetic promoter that has a desired spatial, temporal, and stress-responsive activity. Tissue-specific, inducible and synthetic promoters allow biosynthetic genes to be finely-tuned to enhance metabolic efficiency, with less burden on plant growth. Promoter engineering, in combination with CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi), provides highly flexible regulation of specialized metabolic pathways and is a key technique for precision metabolic engineering.^[2,6]

9.4 Precision breeding

Precision breeding is a technology platform that combines genome editing, genomic selection, pan-genomics, artificial intelligence and multi-omics technologies to boost the development of crops with improved specialized metabolite profiles. CRISPR/Cas systems, base editing and prime editing allow for quick manipulation of biosynthetic genes and regulatory elements without added foreign DNA, which can be used to improve medicinal plants, functional foods, and climate-resilient crops. Systems biology and precision breeding are expected to be key technologies in sustainable agriculture, crop biofortification and in the future in plant-based pharmaceuticals.^[134,135]

10. Case studies: Systems biology-driven dissection and engineering of plant secondary metabolism

The application of systems biology in plant secondary metabolism is best seen when complex biological information from multiple levels is condensed to the level of a single metabolic limit, regulatory point or cryptic biosynthesis pathway and then used to guide experimental intervention. Unlike methods that look at genes or metabolites in isolation, systems approaches take into account both pathway structure and metabolite content, enzyme activities, regulatory mechanisms, environment and metabolic flows. The three case studies described below show different ways this can be done: rerouting precursor flow through a terpenoid limit, combining transcriptomics and metabolomics to solve the mystery of plant-fungus metabolism interaction, and using metabolomics to perturb epigenetic mechanisms of cryptic secondary metabolism.

10.1 Case study 1: Rerouting terpenoid flux to overcome a diterpene biosynthetic bottleneck

An illustrative case of metabolic engineering guided by systems is the engineering of the rice momilactone pathway into *Nicotiana benthamiana*. Momilactones are unique diterpenoids that mediate rice allelopathy; however, understanding of the entire biosynthesis of the compound has been hindered due to low accumulation of its pathway intermediates and products when heterologously expressed. To circumvent this limitation, de La Peña and Sattely understood low accumulation of intermediates and final product as the key hurdle in multistep diterpene biosynthesis and shifted the supply of the diterpene precursors from the chloroplast into the cytosolic mevalonate pathway. Here is an example of how pathway-level knowledge was utilized for a rational engineering strategy. Instead of the consecutive optimization of individual downstream enzymes, the researchers changed the metabolic environment of the whole pathway first. Increased precursor availability led to the accumulation of pathway intermediates and facilitated the rapid screening of biosynthetic enzymes candidates. Then the improved system allowed the reconstitution of the entire momilactone biosynthesis pathway in *Nicotiana benthamiana*, and the level of metabolites produced exceeded the corresponding values obtained in rice by more than ten times. The same

flux-rerouting strategy resulted in better production of other diterpenoid compounds such as forskolin and taxadiene; thus, in this case, the limitations caused by precursor availability and subcellular compartmentalization turned out to be significant system-level constraints.^[136] In this example, it is clearly demonstrated that a metabolic bottleneck can be located upstream of the enzyme catalyzing the target metabolite formation. Therefore, it is advisable to consider not only the optimization of individual biosynthetic genes but also pathway reconstruction, analysis of precursor pool, compartmentalization, and flux distribution prior to the latter. In the field of plant secondary metabolism, it provides a way of rational approach to solving the problem.

10.2 Case Study 2: Multi-Omics dissection of a plant-fungal interaction in *Ephedra sinica*

Plant-fungus interactions also constitute yet another application of systems biology, as variations in the process of secondary metabolism occur due to coordinated regulation of fungal signals, transcription factors, biosynthetic genes, and metabolites accumulation. In particular, an analysis of interaction of *Ephedra sinica* with an endophytic fungus *Didymella keratinophila* conducted through an interaction culture approach identified an active fungal strain which could induce changes in the secondary metabolism of the host plant. This study further analyzed a regulatory network of TF-gene-metabolites in order to examine the molecular mechanism underlying the metabolites accumulation triggered by fungi. The key advantage of such an approach consists in its ability to correlate a biological stimulus with the various levels of molecular information, whereas the metabolite accumulation is not considered in isolation. The interaction of the fungus with the host was accompanied by variations in the secondary metabolites, while transcriptome analysis allowed identifying transcription factors and genes which may be related to these changes. Combining all these data made it possible to reveal the relationship among fungal stimulation, transcription regulation, biosynthesis genes, and metabolite accumulation.^[137] As far as metabolic engineering is concerned, the system *E. sinica*-*D. keratinophila* demonstrates the potential for using plant-associated microorganisms as biological elicitors. Furthermore, the phenotype that emerges as a result of such treatment can be studied via the means of integrated regulatory networks, which can help in identifying the candidates for transcription factors or biosynthetic genes to test them functionally. In particular, the use of this methodology might provide a way to improve the accumulation of pharmaceutically valuable plant metabolites by manipulating the regulation of the metabolic pathway rather than engineering each enzyme involved.

10.3 Case Study 3: Metabolomics guided activation of cryptic secondary metabolism in an endophytic fungus

The promise of systems-level metabolomics is

highlighted further by recently published research investigating the endophytic fungus *Xylaria papulis* Lloyd from the plant *Andrographis paniculata*. Endophytic fungi are known to have biosynthetic gene clusters that are poorly expressed or silenced in laboratory environments, presenting a significant obstacle for tapping into their true biosynthetic potential. Vishwakarma et al. investigated whether chemical epigenetic modification can solve this problem by exposing *X. papulis* to the histone methyltransferase inhibitor UNC1999. The researchers conducted a test with multiple doses of UNC1999, comparing the treated and untreated samples by means of metabolomics. Treatment with 250 nM of UNC1999 changed the secondary metabolome and improved the antioxidant and antibacterial effects of the crude extract. By using GC-MS and UHPLC-HRMS/MS methods, the researchers were able to detect the secondary metabolites that had been induced or regulated with the help of epigenetics, which provided evidence for the existence of metabolic potential that was previously not accessible due to silencing. This study, therefore, presents an example of a systems-level approach that combines an upstream regulation perturbation with chemical analysis. The significance of this particular experiment stems from the fact that the metabolic bottleneck was not associated with the availability of substrates or an inactive biosynthetic enzyme in the pathway. On the contrary, the limiting factor was connected with the expression status of the secondary metabolism machinery. Epigenetics alteration was employed as the experimental tool, whereas untargeted metabolomics was used as the measurement method to monitor the chemical diversity. Another critical feature that should be emphasized is that for systems biology, although metabolomics is capable of demonstrating activation of chemical pathways, alterations in the concentration of metabolites do not provide any information regarding the exact genes responsible for the process.^[69] Taken together, the described case studies present three unique approaches through which systems biology can compensate for gaps in the secondary metabolism. In the momilactone pathway, compartment and pathway level manipulation provided the solution by diverting the flux towards the formation of an intermediate. In the example of *E. sinica* – *D. keratinophila* association, the regulation was integrated to identify the effect of the fungus on the transcription and metabolic processes. For *X. papulis*, it was metabolomics that guided the epigenetic perturbation to uncover the hidden secondary metabolism of this plant. All the above case studies prove that systems biology is most useful when multidimensional data are used to pinpoint a specific biological constraint and then linked to an experimentally testable intervention.

11. Applications of systems biology in plant secondary metabolism

The use of plant secondary metabolism has been revolutionized with the combination of multi-omics technologies, artificial intelligence, genome editing, and

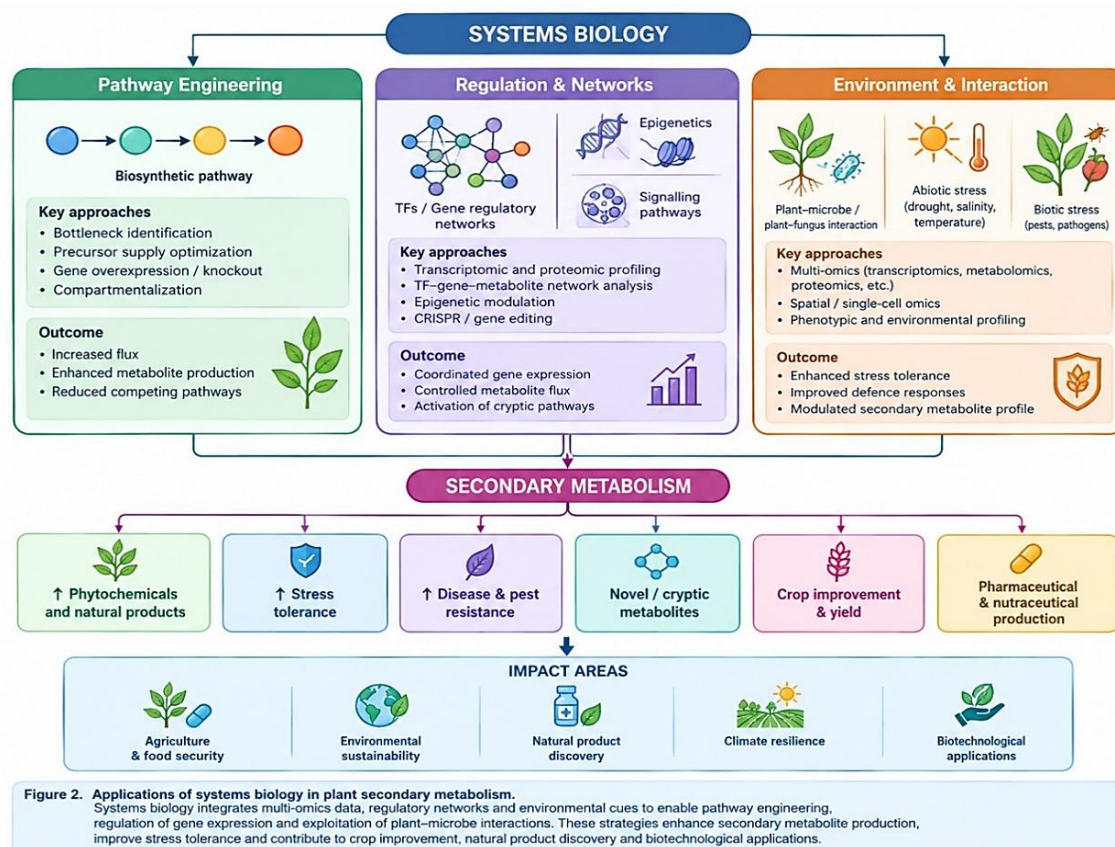


Fig. 2: Applications of systems biology in plant secondary metabolism.

metabolic engineering that has been brought together in systems biology for enhanced productivity, resilience, and phytochemical quality of crops. Biosynthetic pathway and regulatory network have been used to develop crops of higher nutritional value and higher levels of resistance to biotic and abiotic stresses and to produce pharmaceutically valuable metabolites. Precision breeding and innovative plant biotechnology are contributing to sustainable agriculture and the bio-based economy.^[13,115,138] Applications of Systems Biology in Plant Secondary Metabolism is shown in Fig. 2. Fig. 2 illustrates applications of systems biology in plant secondary metabolism. Systems biology integrates multi-omics datasets, regulatory networks, pathway engineering, and environmental or plant-microbe interactions to identify and manipulate secondary-metabolism pathways, resulting in enhanced phytochemical production, stress tolerance, disease and pest resistance, discovery of novel or cryptic metabolites, crop improvement, and pharmaceutical and nutraceutical production. These outcomes support applications in agriculture and food security, environmental sustainability, natural-product discovery, climate resilience, and biotechnology. By using systems biology, genes, regulatory networks, and metabolic pathways involved in agronomically important traits can be identified, and high-yielding crops of improved quality and increased production of beneficial secondary metabolites can be developed. The integration of genomics, transcriptomics, metabolomics, and genome editing has in turn led to the development of precision breeding and the functional validation of candidate genes, which have enhanced crop performance and

nutritional value.^[118,128,129] Table 2 describes applications, current challenges, future perspectives, and representative outputs of systems biology in plant secondary metabolism.

11.1 Climate resilience

The secondary metabolites of plants play crucial roles in adaptation to climate change by increasing drought, salinity, heat, cold, UV and oxidative stress tolerance. Stress-related genes, signaling pathways and metabolic networks have been identified by systems biology approaches which control the accumulation of protective compounds like flavonoids, terpenoids, alkaloids, phenolics etc. These insights will help develop climate-resilient crops that will continue to produce in adverse conditions.^[7,18,132,133]

11.2 Pest and disease resistance

Specialized metabolites are an integral part of plant's defense against herbivorous insects and pathogens. Regulatory networks controlling the biosynthesis of phytoalexins, glucosinolates, terpenoids, alkaloids, phenolics and volatile organic compounds that enhance innate immunity have been identified through multi-omics analyses. However, the use of systems biology, genome editing and precision breeding has helped to create disease-resistant crops without relying on chemicals for crop protection, a practice that has been dubbed sustainable crop protection.^[4,6,15,19,21]

11.3 Biofortification

The goal of biofortification is to boost the accumulation of vitamins, antioxidants, carotenoids, flavonoids and

Table 2: Applications, current challenges, future perspectives, and representative outputs of systems biology in plant secondary metabolism

Area	Current application	Major challenge	Future opportunity	Representative systems-biology output
Crop improvement	Identification of beneficial metabolic traits	Complex genotype–phenotype relationships	AI-assisted precision breeding	Candidate genes, metabolic biomarkers, and predictive genotype–phenotype relationships
Climate resilience	Understanding stress-responsive metabolites	Dynamic responses to changing environmental conditions	Predictive climate-smart metabolic models	Stress-responsive regulatory networks and metabolic signatures
Pest and disease resistance	Identification of chemical defense pathways	Complex plant–microbe interactions	Targeted metabolic engineering	Defense-related metabolites, regulatory hubs, and plant–microbe interaction networks
Biofortification	Enhancement of nutritional metabolites	Maintaining yield and metabolic balance	Precision metabolic engineering	Target pathways, metabolic bottlenecks, and nutritionally relevant biomarkers
Pharmaceutical production	Production of valuable phytochemicals	Pathway bottlenecks and low yields	Synthetic and heterologous biosynthesis	Reconstructed biosynthetic pathways, optimized flux, and high-value metabolite production
Multi-omics integration	Systems-level pathway reconstruction	Data heterogeneity and standardization	Integrated multi-omics platforms	Gene–protein–metabolite relationships and pathway-level regulatory models
Functional annotation	Identification of candidate genes	Large annotation gaps	AI-based gene-function prediction	Candidate biosynthetic genes and predicted gene functions
Spatial biology	Cell- and tissue-specific metabolic mapping	High-resolution data complexity	Single-cell and spatial multi-omics	Spatially resolved biosynthetic pathways and cell-specific metabolic networks
Metabolic engineering	Pathway optimization	Unintended metabolic effects	Next-generation predictive engineering	Identified bottlenecks, redesigned pathways, and optimized metabolic flux
Digital plant biology	Phenotyping and predictive modeling	Integration of real-time datasets	Plant digital twins	Predictive models linking molecular, phenotypic, and environmental responses

other bioactive metabolites in crops. The application of genomics, metabolomics, synthetic biology and metabolic engineering has allowed for precise manipulation of biosynthetic pathways in order to improve the nutritional value of plants without impacting growth. All of these contribute to reducing micronutrient deficiencies and contribute to food security worldwide.^[1,2]

11.4 Pharmaceutical production

Therapeutic secondary metabolites, such as alkaloids, terpenoids, flavonoids, phenolics and glycosides, extracted from plants are beneficial in the treatment of cancer, antimicrobial, anti-inflammatory and antioxidant agents. Pathway optimization, heterologous biosynthesis and high-yield production of useful phytochemicals in plants and engineered microbial hosts have been enabled by system, synthetic and metabolic engineering approaches. These technologies can be considered sustainable options to manufacture plant-based drugs

and contribute to the rise of natural bioactive compounds.^[1]

12. Critical comparison and evidence-based assessment of systems biology approaches

Although omics technologies have substantially expanded the ability to characterize plant secondary metabolism, their individual contributions differ in resolution, biological interpretation, cost, and experimental validation requirements. A critical assessment is therefore necessary because increased data generation does not necessarily translate into improved mechanistic understanding. The value of a systems biology strategy depends on whether complementary datasets can be integrated to establish biologically meaningful relationships and whether computationally predicted associations are subsequently validated experimentally.

12.1. Strengths and limitations of individual omics

approaches

Individual omics approaches provide distinct but incomplete views of secondary metabolism. Genomics offers relatively stable information on genes, genome variation, biosynthetic gene clusters, and pathway organization, but the presence of a biosynthetic gene does not necessarily demonstrate its expression or metabolic function. Transcriptomics provides dynamic information on gene expression and is particularly useful for identifying candidate biosynthetic genes and regulatory modules; however, transcript abundance may not correlate directly with protein abundance, enzyme activity, or metabolite accumulation. Proteomics provides a closer functional connection to enzymatic processes, but protein abundance alone cannot fully represent enzyme activity because post-translational modification, substrate availability, compartmentalization, and metabolic feedback may influence pathway output. Metabolomics provides a direct readout of metabolic phenotypes and is therefore highly valuable for pathway discovery and biomarker identification, but metabolite annotation, structural isomer discrimination, and causal assignment to specific biosynthetic genes remain challenging. Phenomics links molecular changes with observable traits but generally provides limited information about the molecular mechanisms responsible for those traits. Consequently, no individual omics layer can independently provide a complete description of secondary-metabolite biosynthesis. Single-omics studies remain valuable for hypothesis generation and identification of candidate pathways, but their conclusions should be interpreted within the limitations of the biological layer being measured.

12.2. Single-Omics versus Multi-Omics strategies

Single-omics approaches are comparatively simpler to design, generate smaller and more tractable datasets, and can provide high-resolution information for a specific biological layer. They are therefore particularly useful when a clearly defined biological question is being addressed. However, single-layer analyses can produce incomplete or potentially misleading interpretations when relationships between transcription, protein abundance, enzyme activity, and metabolite accumulation are assumed rather than experimentally demonstrated. Multi-omics integration provides a broader systems-level perspective by connecting molecular layers and allowing relationships between genes, transcripts, proteins, metabolites, regulatory factors, and phenotypes to be examined simultaneously. This increases the possibility of identifying pathway bottlenecks, regulatory hubs, precursor limitations, and coordinated responses that may remain undetected in individual datasets. Nevertheless, multi-omics integration introduces additional challenges, including differences in data dimensionality, sampling times, normalization procedures, statistical assumptions, missing values, batch effects, and difficulties in distinguishing correlation from causation. Thus, multi-omics should not be considered inherently superior to single-omics; its advantage depends on appropriate

experimental design, temporal and spatial matching of datasets, and biologically meaningful integration.

12.3. Conventional approaches versus ai-assisted approaches

Conventional statistical, pathway-based, and network-analysis approaches remain important because their assumptions and outputs are generally more interpretable and can be directly connected to established biological mechanisms. Methods such as differential expression analysis, correlation analysis, pathway enrichment, co-expression analysis, and network reconstruction are particularly useful for generating testable hypotheses from omics datasets. AI and machine-learning approaches can process substantially larger and more heterogeneous datasets and may improve prediction of gene functions, metabolite classes, biosynthetic pathways, genotype-phenotype relationships, and metabolic responses. However, AI-assisted approaches also introduce important limitations. Their performance depends strongly on the quality, size, diversity, and annotation of the training data. Models trained on incomplete or biased datasets may produce apparently accurate but biologically incorrect predictions. In addition, complex machine-learning and deep-learning models can be difficult to interpret mechanistically, creating a risk that predictive performance may be mistaken for biological explanation. Therefore, AI should be viewed primarily as a complementary predictive framework rather than a replacement for experimentally grounded systems biology. The most informative strategy is to use AI or machine learning to prioritize candidate genes, pathways, metabolites, or regulatory relationships and subsequently test those predictions experimentally.

12.4. Computational predictions versus experimental validation

A major distinction should be made between computational association, computational prediction, and experimentally demonstrated biological function. Co-expression networks, molecular docking, pathway enrichment, machine-learning models, and genome-scale metabolic models can identify candidate relationships, but these approaches do not independently establish causality. Experimental validation may include gene overexpression or knockout, CRISPR-based editing, promoter analysis, enzyme assays, metabolite quantification, isotope-labeling experiments, mutant complementation, or targeted biochemical characterization. When computational predictions are supported by such experiments, confidence in the proposed biosynthetic or regulatory mechanism is substantially increased. Conversely, predictions that have not been experimentally tested should be presented as hypotheses rather than established mechanisms. This distinction is particularly important in plant secondary metabolism because correlations between transcript abundance and metabolite accumulation may arise from coordinated responses rather than direct pathway regulation. Similarly, predicted gene-metabolite

associations or AI-generated pathway annotations may require functional confirmation before they can be used for metabolic engineering.

12.5. What is established, what remains uncertain, and what is needed?

Several aspects of plant secondary metabolism are now well established. Multi-omics datasets can identify candidate biosynthetic genes, transcription factors, metabolic pathways, and metabolite signatures, while network-based approaches can reveal coordinated regulatory modules. Increasing evidence also demonstrates that environmental signals, phytohormones, plant-microbe interactions, and developmental processes influence secondary metabolism through interconnected regulatory networks. However, important uncertainties remain. The causal relationships between many candidate genes and specialized metabolites have not been experimentally established, particularly for poorly characterized plant species and low-abundance or structurally complex metabolites. The relationship between transcript-level changes and actual metabolic flux is also not always predictable. Spatial and temporal heterogeneity further complicates interpretation because molecular changes may occur in specific cell types, tissues, or developmental stages. In addition, computational models and AI-based predictions may be limited by incomplete reference databases, insufficiently annotated metabolites, and datasets that do not adequately represent natural environmental variation. Future research should therefore prioritize experimentally validated multi-omics studies with coordinated sampling, standardized data-processing pipelines, spatial and temporal resolution, and quantitative measurements of metabolic flux. Integration of computational prediction with genetic, biochemical, and phenotypic validation will be essential for moving systems biology from descriptive association toward causal understanding and predictable metabolic engineering.

13. Challenges and future prospects

Despite the increasing use of multi-omics techniques, their combination poses difficulties, including different structures of data sets, different resolutions, different dimensionalities, and different temporal dynamics. These factors can impede the process of correct modeling of regulatory networks and metabolic pathways. Furthermore, non-standardization of experimental protocols, annotations of metadata, and analyses limits the reproducibility and comparability of results. Despite the growing number of sequenced genomes of plants, a lot of the genes responsible for specialized metabolism are not yet characterized. Additionally, omics technologies produce large heterogeneous data sets, which necessitate scalable computational capacity for storing, combining, and analyzing the data. Besides, present-day models poorly describe dynamic environmental response, metabolic feedback, and spatial organization. As a result, predictions based on combined data sets need to be experimentally validated using

functional genomics, metabolomics, and genome editing methods. New technologies, including those yet to be developed, are expected to incorporate more and more accurate and predictive technologies to understand plant specialized metabolism. These approaches will enable the discovery of genetic diversity and novel biosynthetic genes, while single-cell and spatial multi-omics will uncover cell-specific metabolic regulation and tissue-level biosynthetic networks at an unprecedented level of resolution. By harnessing vast quantities of biological data and compiling them into predictive models, AI and machine learning will also improve pathway reconstruction, metabolite prediction, gene annotation and precision breeding. New ideas like 'Digital Twins of plants', which integrate real-time multi-omics, phenomics and environmental data, will be used to model plant growth, stress responses and metabolite production across a range of environmental conditions. Together with precision agriculture, synthetic biology, CRISPR-based genome editing, and AI-driven metabolic engineering, these will help bring crops to market that are resilient to climate change, nutritionally superior cultivars, and efficient production platforms of premium phytochemicals. Together, they will put in place a predictive, data-driven toolkit for plant systems biology that will greatly advance sustainable agriculture, precision breeding and plant-based pharmaceutical production.

14. Conclusion

The plant secondary metabolism is a highly dynamic and complex biological system which is essential for the adaptation, defense and production of valuable bioactive compounds of plants. Systems biology has greatly advanced our understanding of the molecular networks controlling the biosynthesis, regulation, and evolution of specialized metabolites through the integration of complementary omics, computational, and phenotypic information. Advances in AI, machine learning, synthetic biology, genome editing, and precision breeding have rapidly progressed in the discovery of pathways, metabolic engineering, and engineering of crops for better resistance, nutrition, and phytochemicals. These developments are providing new possibilities for sustainable agriculture, pharmaceutical production, and climate-smart crop development. While significant advances have been made, there are still issues of multi-omics integration, functional annotation, data standardization, computational modeling, and experimental validation. By breaking these barriers with interdisciplinary teamwork and sophisticated metabolic models, more precise prediction and control of plant metabolisms can be achieved. In summary, the integration of systems biology into new technologies offers a strong basis for understanding plant secondary metabolism and will be a key driver in the development of plant science, sustainable agriculture, biotechnology and future production of high-value natural products from plants.

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CRedit Author Contribution Statement

Hitesha Gole: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Writing-Original draft, Writing-Review and editing. **Sudarshan Rajput:** Validation, Writing - Review & editing. **Anushri Deulkar:** Visualization, Writing - Review & editing. All authors have read and approved the final version of the manuscript for publication and agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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No data were generated or analyzed during the current study. Therefore, data sharing is not applicable to this article.

Conflict of Interest

There is no conflict of interest.

Artificial Intelligence (AI) Use Disclosure

The authors declare that artificial intelligence (AI)-assisted tools were used only for language refinement, grammar improvement, and manuscript structuring purposes during the preparation of this work. All technical content, experimental implementation, results, and interpretations were independently developed and verified by the authors.

Supporting Information

Not applicable.

References

- [1] R. A. Dixon, A. J. Dickinson, A century of studying plant secondary metabolism—from “what?” to “where, how, and why?”, *Plant Physiology*, 2024, **195**,48–66, doi: 10.1093/plphys/kiad596.
- [2] R. D. Payet, A. Aoudate, R. Casson, A. Houghton, M.T. Pham, A. Osbourn, A million shades of green: understanding and harnessing plant metabolic diversity, *The EMBO Journal*, 2025, **44**,4409–4418, doi: 10.1038/s44318-025-00496-z.
- [3] J. Palazon, M. A. Alcalde, Secondary metabolites in plants, *Plants*, 2025, **14**,2146, doi: 10.3390/plants14142146.
- [4] M. Wang, S. Zhang, R. Li, Q. Zhao, Unraveling the specialized metabolic pathways in medicinal plant genomes: a review, *Frontiers in Plant Science*, 2024, **15**,1459533, doi: 10.3389/fpls.2024.1459533.
- [5] C. Liu, H. Xu, Z. Li, Y. Wang, S. Qiao, H. Zhang, Application and progress of genomics in deciphering the genetic regulation mechanisms of plant secondary metabolites, *Plants*, 2025, **14**,1316, doi: 10.3390/plants14091316.
- [6] Y. Gao, C. Zhao, Development and applications of metabolic models in plant multi-omics research, *Frontiers in Plant Science*, 2024, **15**,1361183, doi: 10.3389/fpls.2024.1361183.
- [7] L.-V. Méteignier, H.-W. Nützmann, N. Papon, A. Osbourn, V. Courdavault, Emerging mechanistic insights into the regulation of specialized metabolism in plants, *Nature Plants*, 2023, **9**,22–30, doi: 10.1038/s41477-022-01288-7.
- [8] Q. Yan, G. Zhang, X. Zhang, L. Huang, A review of transcriptomics and metabolomics in plant quality and environmental response: from bibliometric analysis to science mapping and future trends, *Metabolites*, 2024, **14**,272, doi: 10.3390/metabo14050272.
- [9] Y. Liu, S. K. Singh, S. Pattanaik, H. Wang, L. Yuan, Light regulation of the biosynthesis of phenolics, terpenoids, and alkaloids in plants, *Communications Biology*, 2023, **6**,1055, doi: 10.1038/s42003-023-05435-4.
- [10] S. Manickam, V. R. Rajagopalan, R. Kambale, R. Rajasekaran, S. Kanagarajan, R. Muthurajan, Plant metabolomics: current initiatives and future prospects, *Current Issues in Molecular Biology*, 2023, **45**,8894–8906, doi: 10.3390/cimb45110558.
- [11] C. Rich-Griffin, A. Stechemesser, J. Finch, E. Lucas, S. Ott, P. Schäfer, Single-cell transcriptomics: a high-resolution avenue for plant functional genomics, *Trends in Plant Science*, 2020, **25**,186–197, doi: 10.1016/j.tplants.2019.10.008.
- [12] D. Ilyaskina, S. Altves, L. Dong, H. Bouwmeester, S. El Aidy, Adaptive and metabolic convergence in rhizosphere and gut microbiomes, *Microbiome*, 2025, **13**,173, doi: 10.1186/s40168-025-02179-7.
- [13] W. Yang, H. Feng, X. Zhang, J. Zhang, J. H. Doonan, W. D. Batchelor, L. Xiong, J. Yan, Crop phenomics and high-throughput phenotyping: past decades, current challenges, and future perspectives, *Molecular Plant*, 2020, **13**,187–214, doi: 10.1016/j.molp.2020.01.008.
- [14] H. Li, N. Chen, H. Zhang, D. Xu, Multidimensional regulation of transcription factors: decoding the comprehensive signals of plant secondary metabolism, *Frontiers in Plant Science*, 2025, **16**,1522278, doi: 10.3389/fpls.2025.1522278.
- [15] L. J. Sweetlove, R. G. Ratcliffe, A. R. Fernie, Non-canonical plant metabolism, *Nature Plants*,

- 2025, **11**,696–708, doi: 10.1038/s41477-025-01965-3.
- [16] A. Khan, F. Kanwal, S. Ullah, M. Fahad, L. Tariq, M. T. Altaf, A. Riaz, G. Zhang, Plant secondary metabolites—central regulators against abiotic and biotic stresses, *Metabolites*, 2025, **15**,276, doi: 10.3390/metabo15040276.
- [17] Z. Ma, L. Hu, Y. Zhong, Structure, evolution, and roles of MYB transcription factors proteins in secondary metabolite biosynthetic pathways and abiotic stresses responses in plants: a comprehensive review, *Frontiers in Plant Science*, 2025, **16**,1626844, doi: 10.3389/fpls.2025.1626844.
- [18] M. Erb, D. J. Kliebenstein, Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy, *Plant Physiology*, 2020, **184**,39–52, doi: 10.1104/pp.20.00433.
- [19] S. Alseekh, A. R. Fernie, Metabolomics 20 years on: what have we learned and what hurdles remain?, *The Plant Journal*, 2018, **94**,933–942, doi: 10.1111/tpj.13950.
- [20] A. R. Fernie, E. Pichersky, Focus issue on metabolism: metabolites, metabolites everywhere, *Plant Physiology*, 2015, **169**,1421–1423, doi: 10.1104/pp.15.01499.
- [21] F. Zhu, W. Wen, Y. Cheng, S. Alseekh, A. R. Fernie, Integrating multiomics data accelerates elucidation of plant primary and secondary metabolic pathways, *Abiotech*, 2023, **4**, 47–56, doi: 10.1007/s42994-022-00091-4.
- [22] A. R. Fernie, S. de Vries, J. de Vries, Evolution of plant metabolism: the state-of-the-art, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 2024, **379**, 20230347, doi: 10.1098/rstb.2023.0347.
- [23] G. Polturak, A. Osbourn, The emerging role of biosynthetic gene clusters in plant defense and plant interactions, *PLoS Pathogens*, 2021, **17**, e1009698, doi: 10.1371/journal.ppat.1009698.
- [24] H.-W. Nützmann, A. Huang, A. Osbourn, Plant metabolic clusters—from genetics to genomics, *New Phytologist*, 2016, **211**, 771–789, doi: 10.1111/nph.13981.
- [25] A. Jaczkowski, A. Bültemeier, B. Seligmann, B. Pucker, J. Franke, Emerging technologies for the discovery of biosynthetic genes in plants, *Natural Product Reports*, 2026, Advance Article, doi: 10.1039/D6NP00011H.
- [26] J. Leong, R. L. Last, Promiscuity, impersonation and accommodation: evolution of plant specialized metabolism, *Current Opinion in Structural Biology*, 2017, **47**, 105–112, doi: 10.1016/j.sbi.2017.07.005.
- [27] J.-K. Weng, R. N. Philippe, J. P. Noel, The rise of chemodiversity in plants, *Science*, 2012, **336**, 1667–1670, doi: 10.1126/science.1217411.
- [28] E. Pichersky, R. A. Raguso, Why do plants produce so many terpenoid compounds?, *New Phytologist*, 2018, **220**, 692–702, doi: 10.1111/nph.14178.
- [29] D. Elser, D. Pflieger, C. Villette, B. Moegle, L. Miesch, E. Gaquerel, Evolutionary metabolomics of specialized metabolism diversification in the genus *Nicotiana* highlights allopolyploidy-mediated innovations in N-acylnornicotine metabolism, *Science Advances*, 2023, **9**, eade8984, doi: 10.1126/sciadv.ade8984.
- [30] T. Isah, Stress and defense responses in plant secondary metabolites production, *Biological Research*, 2019, **52**, 39, doi: 10.1186/s40659-019-0246-3.
- [31] R. Akula, G. A. Ravishankar, Influence of abiotic stress signals on secondary metabolites in plants, *Plant Signaling & Behavior*, 2011, **6**, 1720–1731, doi: 10.4161/psb.6.11.17613.
- [32] L. Yang, K.-S. Wen, X. Ruan, Y.-X. Zhao, F. Wei, Q. Wang, Response of plant secondary metabolites to environmental factors, *Molecules*, 2018, **23**, 762, doi: 10.3390/molecules23040762.
- [33] S. Alseekh, A. Aharoni, Y. Brotman, K. Contrepolis, J. D'Auria, J. Ewald, J. C. Ewald, P. D. Fraser, P. Giavalisco, R. D. Hall, M. Heinemann, H. Link, J. Luo, S. Neumann, J. Nielsen, L. Perez de Souza, K. Saito, U. Sauer, F. C. Schroeder, S. Schuster, G. Siuzdak, A. Skirycz, L. W. Sumner, M. P. Snyder, H. Tang, T. Tohge, Y. Wang, W. Wen, S. Wu, G. Xu, N. Zamboni, A. R. Fernie, Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices, *Nature Methods*, 2021, **18**, 747–756, doi: 10.1038/s41592-021-01197-1.
- [34] R. Fernie, A. Aharoni, L. Willmitzer, M. Stitt, T. Tohge, J. Kopka, A. J. Carroll, K. Saito, P. D. Fraser, V. DeLuca, Recommendations for reporting metabolite data, *The Plant Cell*, 2011, **23**, 2477–2482, doi: 10.1105/tpc.111.086272.
- [35] O. Fiehn, Metabolomics by gas chromatography–mass spectrometry: the combination of targeted and untargeted profiling, *Current Protocols in Molecular Biology*, 2016, **114**, 30-4, doi: 10.1002/0471142727.mb3004s114.
- [36] G. J. Patti, O. Yanes, G. Siuzdak, Metabolomics: the apogee of the omics trilogy, *Nature Reviews Molecular Cell Biology*, 2012, **13**, 263–269, doi: 10.1038/nrm3314.
- [37] B. B. Misra, C. Langefeld, M. Olivier, L. A. Cox, Integrated omics: tools, advances and future approaches, *Journal of Molecular Endocrinology*, 2019, **62**, R21–R45, doi: 10.1530/JME-18-0055.
- [38] W. Weckwerth, Green systems biology—from single genomes, proteomes and metabolomes to ecosystems research and biotechnology, *Journal of Proteomics*, 2011, **75**, 284–305, doi: 10.1016/j.jprot.2011.07.010.
- [39] P. G. Dominguez, I. N. Abreu, T. Moritz, I. G. F.

- Budzinski, Systems biology as a tool to uncover interdisciplinary links within the complex forest tree system, *Monitoring Forest Damage with Metabolomics Methods*, 2024, 71–108, doi: 10.1002/9781119868750.ch4.
- [40] A. Razzaq, B. Sadia, A. Raza, M. K. Hameed, F. Saleem, Metabolomics: a way forward for crop improvement, *Metabolites*, 2019, **9**, 303, doi: 10.3390/metabo9120303.
- [41] D. M. Bolser, D. M. Staines, E. Perry, P. J. Kersey, Ensembl Plants: integrating tools for visualizing, mining, and analyzing plant genomic data, *Plant Genomics Databases: Methods and Protocols*, 2016, 1–31, doi: 10.1007/978-1-4939-6658-5_1.
- [42] M. Van Bel, F. Silvestri, E. M. Weitz, L. Kreft, A. Botzki, F. Coppens, K. Vandepoele, PLAZA 5.0: extending the scope and power of comparative and functional genomics in plants, *Nucleic Acids Research*, 2022, **50**, D1468–D1474, doi: 10.1093/nar/gkab1024.
- [43] M. K. Tello-Ruiz, S. Naithani, P. Gupta, A. Olson, S. Wei, J. Preece, Y. Jiao, B. Wang, K. Chougule, P. Garg, J. Elser, S. Kumari, V. Kumar, B. Contreras-Moreira, G. Naamati, N. George, J. Cook, D. Bolser, P. D'Eustachio, L. D. Stein, A. Gupta, W. Xu, J. Regala, I. Papatheodorou, P. J. Kersey, P. Flicek, C. Taylor, P. Jaiswal, D. Ware, Gramene 2021: harnessing the power of comparative genomics and pathways for plant research, *Nucleic Acids Research*, 2021, **49**, D1452–D1463, doi: 10.1093/nar/gkaa979.
- [44] I. Papatheodorou, P. Moreno, J. Manning, A. M.-P. Fuentes, N. George, S. Fexova, N. A. Fonseca, A. Füllgrabe, M. Green, N. Huang, L. Huerta, H. Iqbal, M. Jianu, S. Mohammed, L. Zhao, A. F. Jarnuczak, S. Jupp, J. Marioni, K. Meyer, R. Petryszak, C. A. Prada Medina, C. Talavera-López, S. Teichmann, J. A. Vizcaino, A. Brazma, Expression Atlas update: from tissues to single cells, *Nucleic Acids Research*, 2020, **48**, D77–D83, doi: 10.1093/nar/gkz947.
- [45] Y. Perez-Riverol, J. Bai, C. Bandla, D. García-Seisdedos, S. Hewapathirana, S. Kamatchinathan, D. J. Kundu, A. Prakash, A. Frericks-Zipper, M. Eisenacher, M. Walzer, S. Wang, A. Brazma, J. A. Vizcaíno, The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences, *Nucleic Acids Research*, 2022, **50**, D543–D552, doi: 10.1093/nar/gkab1038,
- [46] D. Szklarczyk, A. L. Gable, D. Lyon, A. Junge, S. Wyder, J. Huerta-Cepas, M. Simonovic, N. T. Doncheva, J. H. Morris, P. Bork, L. J. Jensen, C. von Mering, STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets, *Nucleic Acids Research*, 2019, **47**, D607–D613, doi: 10.1093/nar/gky1131.
- [47] M. Kanehisa, M. Furumichi, Y. Sato, M. Ishiguro-Watanabe, M. Tanabe, KEGG: integrating viruses and cellular organisms, *Nucleic Acids Research*, 2021, **49**, D545–D551, doi: 10.1093/nar/gkaa970.
- [48] P. Zhang, H. Foerster, C. P. Tissier, L. A. Mueller, S. Paley, P. D. Karp, S. Y. Rhee, MetaCyc and AraCyc: metabolic pathway databases for plant research, *Plant Physiology*, 2005, **138**, 27–37, doi: 10.1104/pp.105.060376.
- [49] K. Haug, K. Cochrane, V. C. Nainala, M. Williams, J. Chang, K. V. Jayaseelan, C. O'Donovan, MetaboLights: a resource evolving in response to the needs of its scientific community, *Nucleic Acids Research*, 2020, **48**, D440–D444, doi: 10.1093/nar/gkz1019.
- [50] M. Wang, J. J. Carver, V. V. Phelan, L. M. Sanchez, N. Garg, Y. Peng, D. D. Nguyen, J. Watrous, C. A. Kapon, T. Luzzatto-Knaan, C. Porto, A. Bouslimani, A. V. Melnik, M. J. Meehan, W.-T. Liu, M. Crusemann, P. D. Boudreau, E. Esquenazi, M. Sandoval-Calderón, R. D. Kersten, L. A. Pace, R. A. Quinn, K. R. Duncan, C.-C. Hsu, D. J. Floros, R. G. Gavilan, K. Kleigrew, T. Northen, R. J. Dutton, D. Parrot, E. E. Carlson, B. Aigle, C. F. Michelsen, L. Jelsbak, C. Sohlenkamp, P. Pevzner, A. Edlund, J. McLean, J. Piel, B. T. Murphy, L. Gerwick, C.-C. Liaw, Y.-L. Yang, H.-U. Humpf, M. Maansson, R. A. Keyzers, A. C. Sims, A. R. Johnson, A. M. Sidebottom, B. E. Sedio, A. Klitgaard, C. B. Larson, C. A. Boya P, D. Torres-Mendoza, D. J. Gonzalez, D. B. Silva, L. M. Marques, D. P. Demarque, E. Pociute, E. C. O'Neill, E. Briand, E. J. N. Helfrich, E. A. Granatosky, E. Glukhov, F. Ryffel, H. Houson, H. Mohimani, J. J. Kharbush, Y. Zeng, J. A. Vorholt, K. L. Kurita, P. Charusanti, K. L. McPhail, K. F. Nielsen, L. Vuong, M. Elfeki, M. F. Traxler, N. Engene, N. Koyama, O. B. Vining, R. Baric, R. R. Silva, S. J. Mascuch, S. Tomasi, S. Jenkins, V. Macherla, T. Hoffman, V. Agarwal, P. G. Williams, J. Dai, R. Neupane, J. Gurr, A. M. C. Rodríguez, A. Lamsa, C. Zhang, K. Dorrestein, B. M. Duggan, J. Almaliti, P.-M. Allard, P. Phapale, L.-F. Nothias, T. Alexandrov, M. Litaudon, J.-L. Wolfender, J. E. Kyle, T. O. Metz, T. Peryea, D.-T. Nguyen, D. VanLeer, P. Shinn, A. Jadhav, R. Müller, K. M. Waters, W. Shi, X. Liu, L. Zhang, R. Knight, P. R. Jensen, B. Ø. Palsson, K. Poglian, R. G. Linington, M. Gutiérrez, N. P. Lopes, W. H. Gerwick, B. S. Moore, P. C. Dorrestein, N. Bandeira, Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking, *Nature Biotechnology*, 2016, **34**, 828–837, doi: 10.1038/nbt.3597.
- [51] Z. Pang, J. Chong, G. Zhou, D. A. de Lima Morais, L. Chang, M. Barrette, C. Gauthier, P.-É. Jacques, S. Li, J. Xia, MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights, *Nucleic Acids Research*, 2021, **49**,

- W388–W396, doi: 10.1093/nar/gkab382.
- [52] G. Zhou, Z. Pang, Y. Lu, J. Ewald, J. Xia, OmicsNet 2.0: a web-based platform for multi-omics integration and network visual analytics, *Nucleic Acids Research*, 2022, **50**, W527–W533, doi: 10.1093/nar/gkac376.
- [53] Y. Van de Peer, E. Mizrachi, K. Marchal, The evolutionary significance of polyploidy, *Nature Reviews Genetics*, 2017, **18**, 411–424, doi: 10.1038/nrg.2017.26.
- [54] P. S. Soltis, D. E. Soltis, Ancient WGD events as drivers of key innovations in angiosperms, *Current Opinion in Plant Biology*, 2016, **30**, 159–165, doi: 10.1016/j.pbi.2016.03.015.
- [55] N. Panchy, M. Lehti-Shiu, S.-H. Shiu, Evolution of gene duplication in plants, *Plant Physiology*, 2016, **171**, 2294–2316, doi: 10.1104/pp.16.00523.
- [56] X. Qiao, Q. Li, H. Yin, K. Qi, L. Li, R. Wang, S. Zhang, A. H. Paterson, Gene duplication and evolution in recurring polyploidization–diploidization cycles in plants, *Genome Biology*, 2019, **20**, 38, doi: 10.1186/s13059-019-1650-2.
- [57] H. W. Nützmann, C. Scazzocchio, A. Osbourn, Metabolic gene clusters in eukaryotes, *Annual Review of Genetics*, 2018, **52**, 159–183, doi: 10.1146/annurev-genet-120417-031237.
- [58] G. L. Cawood, J. Ton, Decoding resilience: ecology, regulation, and evolution of biosynthetic gene clusters, *Trends in Plant Science*, 2025, **30**, 185–198, doi: 10.1016/j.tplants.2024.09.008.
- [59] M. Rafiq, M. Guo, A. Shoaib, J. Yang, S. Fan, H. Xiao, K. Chen, Z. Xie, C. Cheng, Unraveling the hormonal and molecular mechanisms shaping fruit morphology in plants, *Plants*, 2025, **14**, 974, doi: 10.3390/plants14060974.
- [60] C. Gao, Genome engineering for crop improvement and future agriculture, *Cell*, 2021, **184**, 1621–1635, doi: 10.1016/j.cell.2021.01.005.
- [61] K. A. Molla, S. Sretenovic, K. C. Bansal, Y. Qi, Precise plant genome editing using base editors and prime editors, *Nature Plants*, 2021, **7**, 1166–1187, doi: 10.1038/s41477-021-00991-1.
- [62] Q. Lin, Y. Zong, C. Xue, S. Wang, S. Jin, Z. Zhu, Y. Wang, A. V. Anzalone, A. Raguram, J. L. Doman, D. R. Liu, C. Gao, Prime genome editing in rice and wheat, *Nature Biotechnology*, 2020, **38**, 582–585, doi: 10.1038/s41587-020-0455-x.
- [63] Y. Zong, Y. Wang, C. Li, R. Zhang, K. Chen, Y. Ran, J.-L. Qiu, D. Wang, C. Gao, Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion, *Nature Biotechnology*, 2017, **35**, 438–440, doi: 10.1038/nbt.3811.
- [64] B. Wang, M. Regulski, E. Tseng, A. Olson, S. Goodwin, W. R. McCombie, D. Ware, A comparative transcriptional landscape of maize and sorghum obtained by single-molecule sequencing, *Genome Research*, 2018, **28**, 921–932, doi: 10.1101/gr.227462.117.
- [65] R. Stark, M. Grzelak, J. Hadfield, RNA sequencing: the teenage years, *Nature Reviews Genetics*, 2019, **20**, 631–656, doi: 10.1038/s41576-019-0150-2.
- [66] P. Xu, T. Zhang, Transcriptional Landscape of Arabidopsis Seedling Roots in Response to Simulated Microgravity at Single-Cell Resolution, Research Square Preprint, 2026, doi: 10.21203/rs.3.rs-9938609/v1.
- [67] J. Goossens, P. Fernández-Calvo, F. Schweizer, A. Goossens, Jasmonates: signal transduction components and their roles in environmental stress responses, *Plant Molecular Biology*, 2016, **91**, 673–689, doi: 10.1007/s11103-016-0480-9.
- [68] C. Wasternack, B. Hause, Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany, *Annals of Botany*, 2013, **111**, 1021–1058, doi: 10.1093/aob/mct067.
- [69] S. Vishwakarma, J. H. Nishad, Anjali, S. Sinha, R. Bharti, L. Shekhar, R. N. Kharwar, A metabolomics approach to target the effect of histone protein methyltransferase-specific probe UNC1999 on cryptic metabolites in an endophytic fungus *Xylaria papulis* Lloyd, *Frontiers in Microbiology*, 2026, **17**, 1894461, doi: 10.3389/fmicb.2026.1894461.
- [70] V. Verma, P. Ravindran, P. P. Kumar, Plant hormone-mediated regulation of stress responses, *BMC Plant Biology*, 2016, **16**, 86, doi: 10.1186/s12870-016-0771-y.
- [71] J. V. Jorriin-Novo, Plant proteomics methods and protocols, *Plant Proteomics: Methods and Protocols*, Humana Press, 2014, **1072**, 3–13, doi: 10.1007/978-1-62703-631-3_1.
- [72] K. Kosová, P. Vítámvás, I. T. Prášil, J. Renaut, Plant proteome changes under abiotic stress—contribution of proteomics studies to understanding plant stress response, *Journal of Proteomics*, 2011, **74**, 1301–1322, doi: 10.1016/j.jprot.2011.02.006.
- [73] J. V. Jorriin-Novo, Proteomics and plant biology: contributions to date and a look towards the next decade, *Expert Review of Proteomics*, 2021, **18**, 93–103, doi: 10.1080/14789450.2021.1910028.
- [74] A. H. Millar, J. L. Heazlewood, C. Gligione, M. J. Holdsworth, A. Bachmair, W. X. Schulze, The scope, functions, and dynamics of posttranslational protein modifications, *Annual Review of Plant Biology*, 2019, **70**, 119–151, doi: 10.1146/annurev-arplant-050718-100211.
- [75] L. D. Vu, K. Gevaert, I. De Smet, Protein language: post-translational modifications talking to each other, *Trends in Plant Science*,

- 2018, **23**, 1068–1080, doi: 10.1016/j.tplants.2018.09.004.
- [76] G. Nieto Feliner, J. Casacuberta, J. F. Wendel, Genomics of evolutionary novelty in hybrids and polyploids, *Frontiers in Genetics*, 2020, **11**, 792, doi: 10.3389/fgene.2020.00792.
- [77] M. Y. Annis, P. Routray, N. H. Bhuiyan, B. Yuan, K. J. van Wijk, The chloroplast CLPD chaperone: consequences of under- and overexpression, interaction with the CLP protease core, and candidate substrates, *Plant Physiology*, 2026, doi: 10.1093/plphys/kiag681.
- [78] L. Fürtauer, J. Weiszmänn, W. Weckwerth, T. Nägele, Dynamics of plant metabolism during cold acclimation, *International Journal of Molecular Sciences*, 2019, **20**, 5411, doi: 10.3390/ijms20215411.
- [79] S. Alseekh, F. Scossa, W. Wen, J. Luo, J. Yan, R. Beleggia, H. J. Klee, S. Huang, R. Papa, A. R. Fernie, Domestication of crop metabolomes: desired and unintended consequences, *Trends in Plant Science*, 2021, **26**, 650–661, doi: 10.1016/j.tplants.2021.02.005.
- [80] A. C. Schrimpe-Rutledge, S. G. Codreanu, S. D. Sherrod, J. A. McLean, Untargeted metabolomics strategies—challenges and emerging directions, *Journal of the American Society for Mass Spectrometry*, 2016, **27**, 1897–1905, doi: 10.1007/s13361-016-1469-y.
- [81] R. Katam, C. Lin, K. Grant, C. S. Katam, S. Chen, Advances in plant metabolomics and its applications in stress and single-cell biology, *International Journal of Molecular Sciences*, 2022, **23**, 6985, doi: 10.3390/ijms23136985.
- [82] L. J. Sweetlove, R. G. Ratcliffe, Flux-balance modeling of plant metabolism, *Frontiers in Plant Science*, 2011, **2**, 38, doi: 10.3389/fpls.2011.00038.
- [83] W. D. Lehmann, A timeline of stable isotopes and mass spectrometry in the life sciences, *Mass Spectrometry Reviews*, 2017, **36**, 58–85, doi: 10.1002/mas.21497.
- [84] B. A. Boughton, D. Thinagaran, D. Sarabia, A. Bacic, U. Roessner, Mass spectrometry imaging for plant biology: a review, *Phytochemistry Reviews*, 2016, **15**, 445–488, doi: 10.1007/s11101-015-9440-2.
- [85] M. Kubicki, Imaging the uptake, distribution and metabolism of agrochemicals using MALDI mass spectrometry imaging, INFU, PhD Thesis, Technische Universität Dortmund, 2022, <https://d-nb.info/126520926X/34>.
- [86] B. Kazemi Oskuei, A. Masi, A. Kosmala, N. Mahna, Plant stress and proteomics in medicinal plants, *Frontiers in Plant Science*, 2025, **16**, 1656247, doi: 10.3389/fpls.2025.1656247.
- [87] M. Glinski, W. Weckwerth, The role of mass spectrometry in plant systems biology, *Mass Spectrometry Reviews*, 2006, **25**, 173–214, doi: 10.1002/mas.20063.
- [88] S. Uddin, S. Gull, J. Wang, J. Yin, H. A. Hussain, U. Mahmood, X. Yang, Synergistic inoculation of *Funneliformis mosseae* and *Pseudomonas putida* enhances drought resilience in *Citrus reticulata* through coordinated physiological and transcriptomic responses, *Physiologia Plantarum*, 2026, **178**, e71067, doi: 10.1111/ppl.71067.
- [89] A. H. Emwas, R. Roy, R. T. McKay, L. Tenori, E. Saccenti, G. A. N. Gowda, D. Raftery, F. Alahmari, L. Jaremko, M. Jaremko, D. S. Wishart, NMR spectroscopy for metabolomics research, *Metabolites*, 2019, **9**, 123, doi: 10.3390/metabo9070123.
- [90] C. Gao, Epigenomic Landscape Profiling and Bioinformatics Application in Breast Cancer Model, Doctoral Dissertation, The University of Vermont and State Agricultural College, 2024.
- [91] A. R. Fernie, T. Tohge, The genetics of plant metabolism, *Annual Review of Genetics*, 2017, **51**, 287–310, doi: 10.1146/annurev-genet-120116-024640.
- [92] D. Selmar, M. Kleinwächter, Stress enhances the synthesis of secondary plant products: the impact of stress-related over-reduction on the accumulation of natural products, *Plant and Cell Physiology*, 2013, **54**, 817–826, doi: 10.1093/pcp/pct054.
- [93] A. Piasecka, N. Jedrzejczak-Rey, P. Bednarek, Secondary metabolites in plant innate immunity: conserved function of divergent chemicals, *New Phytologist*, 2015, **206**, 948–964, doi: 10.1111/nph.13325.
- [94] T. Pusztahelyi, I. J. Holb, I. Pócsi, Secondary metabolites in fungus-plant interactions, *Frontiers in Plant Science*, 2015, **6**, 573, doi: 10.3389/fpls.2015.00573.
- [95] R. L. Berendsen, C. M. J. Pieterse, P. A. H. M. Bakker, The rhizosphere microbiome and plant health, *Trends in Plant Science*, 2012, **17**, 478–486, doi: 10.1016/j.tplants.2012.04.001.
- [96] P. Trivedi, J. E. Leach, S. G. Tringe, T. Sa, B. K. Singh, Plant-microbiome interactions: from community assembly to plant health, *Nature Reviews Microbiology*, 2020, **18**, 607–621, doi: 10.1038/s41579-020-0412-1.
- [97] P. Vandenkoornhuyse, A. Quaiser, M. Duhamel, A. Le Van, A. Dufresne, The importance of the microbiome of the plant holobiont, *New Phytologist*, 2015, **206**, 1196–1206, doi: 10.1111/nph.13312.
- [98] R. C. Lynch, L. K. Padgitt-Cobb, A. R. Garfinkel, B. J. Knaus, N. T. Hartwick, N. Allsing, A. Aylward, P. C. Bentz, S. B. Carey, A. Mamerto, J. K. Kitony, K. Colt, E. R. Murray, T. Duong, H. I. Chen, A. Trippe, A. Harkess, S. Crawford, K. Vining, T. P. Michael, Domesticated cannabinoid synthases amid a wild mosaic *Cannabis* pangenome, *Nature*, 2025, **643**, 1001–1010, doi: 10.1038/s41586-025-09065

- 0.
- [99] M. Hesami, M. Alizadeh, A. M. P. Jones, D. Torkamaneh, Machine learning: its challenges and opportunities in plant system biology, *Applied Microbiology and Biotechnology*, 2022, **106**, 3507–3530, doi: 10.1007/s00253-022-11963-6.
- [100] J. H. Nishad, S. Vishwakarma, A. Singh, M. Yadav, R. Bharti, R. Kharwar, Metabolites profiling, bioactivity assessment and extracellular enzymes estimation of *Pestalotiopsis* sp. L12, an endophytic fungus recovered from *Saraca asoca* (Roxb.) Willd. leaves, *Microbiology*, 2025, **94**, 1075–1086, doi: 10.1134/S0026261725600223.
- [101] S. Sinha, S. Navathe, Anjali, S. Vishwakarma, P. Prajapati, R. Chand, R. N. Kharwar, Whole genome sequencing and annotation of *Pseudocercospora abelmoschi*, a causal agent of black leaf mould of okra, *World Journal of Microbiology and Biotechnology*, 2025, **41**, 174, doi: 10.1007/s11274-025-04398-4.
- [102] W. Peng, Y. Zhang, H. Xie, Y. Yu, M. Zhu, Research progress on the synergistic regulation of MYB transcription factor-mediated developmental plasticity and stress responses in rice, *Frontiers in Plant Science*, 2025, **16**, 1668800, doi: 10.3389/fpls.2025.1668800.
- [103] S. P. Mohanty, D. P. Hughes, M. Salathé, Using deep learning for image-based plant disease detection, *Frontiers in Plant Science*, 2016, **7**, 1419, doi: 10.3389/fpls.2016.01419.
- [104] A. K. Singh, B. Ganapathysubramanian, S. Sarkar, A. Singh, Deep learning for plant stress phenotyping: trends and future perspectives, *Trends in Plant Science*, 2018, **23**, 883–898, doi: 10.1016/j.tplants.2018.07.004.
- [105] M. W. Libbrecht, W. S. Noble, Machine learning applications in genetics and genomics, *Nature Reviews Genetics*, 2015, **16**, 321–332, doi: 10.1038/nrg3920.
- [106] G. Eraslan, Ž. Avsec, J. Gagneur, F. J. Theis, Deep learning: new computational modelling techniques for genomics, *Nature Reviews Genetics*, 2019, **20**, 389–403, doi: 10.1038/s41576-019-0122-6.
- [107] C. Angermueller, T. Pärnamaa, L. Parts, O. Stegle, Deep learning for computational biology, *Molecular Systems Biology*, 2016, **12**, MSB156651, doi: 10.15252/msb.20156651.
- [108] S. Min, B. Lee, S. Yoon, Deep learning in bioinformatics, *Briefings in Bioinformatics*, 2017, **18**, 851–869, doi: 10.1093/bib/bbw068.
- [109] P. Shannon, A. Markiel, O. Ozier, N. S. Baliga, J. T. Wang, D. Ramage, N. Amin, B. Schwikowski, T. Ideker, Cytoscape: a software environment for integrated models of biomolecular interaction networks, *Genome Research*, 2003, **13**, 2498–2504, doi: 10.1101/gr.1239303.
- [110] J. von Steimker, E. L. Rey, C. Stanschewski, R. Wendenburg, A. Klemmer, M. Macho, V. P. Thirumalaikumar, N. O. Saber, A. Skirycz, A. R. Fernie, M. Tester, S. Alseekh, Multi-tissue metabolic GWAS and drought-responsive multi-omics reveal the genetic basis of the quinoa metabolome, *Advanced Science*, 2026, **13**, e76426, doi: 10.1002/advs.76426.
- [111] L. Gerlin, S. Genin, C. Baroukh, Metabolic modeling of a plant-pathogen interaction quantifies the metabolic bottlenecks underlying bacterial wilt, *bioRxiv*, 2024, doi: 10.1101/2024.12.12.628148.
- [112] J. D. Orth, I. Thiele, B. Ø. Palsson, What is flux balance analysis?, *Nature Biotechnology*, 2010, **28**, 245–248, doi: 10.1038/nbt.1614.
- [113] C. Zhao, Y. Zhang, J. Du, X. Guo, W. Wen, S. Gu, J. Wang, J. Fan, Crop phenomics: current status and perspectives, *Frontiers in Plant Science*, 2019, **10**, 714, doi: 10.3389/fpls.2019.00714.
- [114] R. T. Furbank, M. Tester, Phenomics—technologies to relieve the phenotyping bottleneck, *Trends in Plant Science*, 2011, **16**, 635–644, doi: 10.1016/j.tplants.2011.09.005.
- [115] F. Tardieu, L. Cabrera-Bosquet, T. Pridmore, M. J. Bennett, Plant phenomics, from sensors to knowledge, *Current Biology*, 2017, **27**, R770–R783, doi: 10.1016/j.cub.2017.05.055.
- [116] D. Zhang, F. Xu, F. Wang, L. Le, L. Pu, Synthetic biology and artificial intelligence in crop improvement, *Plant Communications*, 2025, **6**, 101220, doi: 10.1016/j.xplc.2024.101220.
- [117] R. Fu, S. Jiang, T. Wu, C. Yin, J. Yan, X. Wang, AI-driven protein engineering: a new paradigm for plant trait design, *Molecular Plant*, 2026, **19**, 1172–1190, doi: 10.1016/j.molp.2026.03.011.
- [118] W. Liu, C. N. Stewart Jr, Plant synthetic biology, *Trends in Plant Science*, 2015, **20**, 309–317, doi: 10.1016/j.tplants.2015.02.004.
- [119] K. K. Rai, M. Aamir, A. Zehra, A. C. Rai, Research trends in genetically modified (GM) plants, *Modified (GM) Plants, Policy Issues in Genetically Modified Crops*, 2021, 453–480, doi: 10.1016/B978-0-12-820780-2.00020-0.
- [120] J. D. Keasling, Synthetic biology and the development of tools for metabolic engineering, *Metabolic Engineering*, 2012, **14**, 189–195, doi: 10.1016/j.ymben.2012.01.004.
- [121] J. Nielsen, J. D. Keasling, Engineering cellular metabolism, *Cell*, 2016, **164**, 1185–1197, doi: 10.1016/j.cell.2016.02.004.
- [122] Q. Que, M.-D. M. Chilton, C. M. de Fontes, C. He, M. Nuccio, T. Zhu, Y. Wu, J. S. Chen, L. Shi, Trait stacking in transgenic crops: challenges and opportunities, *GM Crops*, 2010, **1**, 220–229, doi: 10.4161/gmcr.1.4.13439.
- [123] C. Halpin, Gene stacking in transgenic plants—the challenge for 21st century plant biotechnology, *Plant Biotechnology Journal*, 2005, **3**, 141–155, doi: 10.1111/j.1467-7652.2004.00113.x.

- [124] D. Jaganathan, K. Ramasamy, G. Sellamuthu, S. Jayabalan, G. Venkataraman, CRISPR for crop improvement: an update review, *Frontiers in Plant Science*, 2018, **9**, 985, doi: 10.3389/fpls.2018.00985.
- [125] F. Danila, T. Schreiber, M. Ermakova, L. Hua, D. Vlad, S.-F. Lo, Y.-S. Chen, J. Lambret-Frotte, A. S. Hermanns, B. Athmer, S. von Caemmerer, S.-M. Yu, J. M. Hibberd, A. Tissier, R. T. Furbank, S. Kelly, J. A. Langdale, A single promoter-TALE system for tissue-specific and tuneable expression of multiple genes in rice, *Plant Biotechnology Journal*, 2022, **20**, 1786–1806, doi: 10.1111/pbi.13864.
- [126] C. Zhang, Y. Tang, S. Tang, L. Chen, T. Li, H. Yuan, Y. Xu, Y. Zhou, S. Zhang, J. Wang, H. Wen, W. Jiang, Y. Pang, X. Deng, X. Cao, J. Zhou, X. Song, Q. Liu, An inducible CRISPR activation tool for accelerating plant regeneration, *Plant Communications*, 2024, **5**, 100823, doi: 10.1016/j.xplc.2024.100823.
- [127] B. K. Sarmah, B. K. Borah, Genome Engineering for Crop Improvement, Springer, 2021, doi: 10.1007/978-3-030-63372-1.
- [128] A. Raza, S. Charagh, K. H. M. Siddique, C. S. Prakash, P. V. Vara Prasad, R. J. Harper, V. Fotopoulos, Z. Hu, R. K. Varshney, Breeding drought-tolerant crops for sustainable agriculture, *Agriculture & Food Security*, 2026, **15**, 6, doi: 10.1186/s40066-025-00587-4.
- [129] K. Chen, Y. Wang, R. Zhang, H. Zhang, C. Gao, CRISPR/Cas genome editing and precision plant breeding in agriculture, *Annual Review of Plant Biology*, 2019, **70**, 667–697, doi: 10.1146/annurev-arplant-050718-100049.
- [130] D. Jangpangi, B. Patni, V. Chandola, S. Chandra, Medicinal plants in a changing climate: understanding the links between environmental stress and secondary metabolite synthesis, *Frontiers in Plant Science*, 2025, **16**, 1587337, doi: 10.3389/fpls.2025.1587337.
- [131] Sana, T. Aftab, M. Naeem, P. K. Jha, P. V. Vara Prasad, Production of secondary metabolites under challenging environments: understanding functions and mechanisms of signalling molecules, *Frontiers in Plant Science*, 2025, **16**, 1569014, doi: 10.3389/fpls.2025.1569014.
- [132] A. M. Tessema, F. M. Abebe, Y. G. Kidane, D. Hailegiorgis, N. S. Tehulie, A. Hassen, S. Abebe, S. Ibrahim, Crop biofortification for enhanced nutritional density: breeding, agronomical, and biotechnological strategies, *Crop Science*, 2026, **66**, e70246, doi: 10.1002/csc.2.70246.
- [133] A. G. Atanasov, S. B. Zotchev, V. M. Dirsch, I. E. Orhan, M. Banach, J. M. Rollinger, D. Barreca, W. Weckwerth, R. Bauer, E. A. Bayer, M. Majeed, A. Bishayee, V. Bochkov, G. K. Bonn, N. Braidy, F. Bucar, A. Cifuentes, G. D'Onofrio, M. Bodkin, M. Diederich, A. T. Dinkova-Kostova, T. Efferth, K. El Bairi, N. Arkells, T.-P. Fan, B. L. Fiebich, M. Freissmuth, M. I. Georgiev, S. Gibbons, K. M. Godfrey, C. W. Gruber, J. Heer, L. A. Huber, E. Ibanez, A. Kijjoo, A. K. Kiss, A. Lu, F. A. Macias, M. J. S. Miller, A. Mocan, R. Müller, F. Nicoletti, G. Perry, V. Pittalà, L. Rastrelli, M. Ristow, G. L. Russo, A. Sanches Silva, D. Schuster, H. Sheridan, K. Skalicka-Woźniak, L. Skaltsounis, E. Sobarzo-Sánchez, D. S. Bredt, H. Stuppner, A. Sureda, N. T. Tzvetkov, R. A. Vacca, B. B. Aggarwal, M. Battino, F. Giampieri, M. Wink, J.-L. Wolfender, J. Xiao, A. W. K. Yeung, G. Lizard, M. A. Popp, M. Heinrich, I. Berindan-Neagoe, M. Stadler, M. Daglia, R. Verpoorte, C. T. Supuran, Natural products in drug discovery: advances and opportunities, *Nature Reviews Drug Discovery*, 2021, **20**, 200–216, doi: 10.1038/s41573-020-00114-z.
- [134] M. D. Wilkinson, M. Dumontier, I. J. Aalbersberg, G. Appleton, M. Axton, A. Baak, N. Blomberg, J.-W. Boiten, L. Bonino da Silva Santos, P. E. Bourne, J. Bouwman, A. J. Brookes, T. Clark, M. Crosas, I. Dillo, O. Dumon, S. Edmunds, C. T. Evelo, R. Finkers, A. Gonzalez-Beltran, A. J. G. Gray, P. Groth, C. Goble, J. S. Grethe, J. Heringa, P. A. C. 't Hoen, R. Hooft, T. Kuhn, R. Kok, J. Kok, S. J. Lusher, M. E. Martone, A. Mons, A. L. Packer, B. Persson, P. Rocca-Serra, M. Roos, R. van Schaik, S.-A. Sansone, E. Schultes, T. Sengstag, T. Slater, G. Strawn, M. A. Swertz, M. Thompson, J. van der Lei, E. van Mulligen, J. Velterop, A. Waagmeester, P. Wittenburg, K. Wolstencroft, J. Zhao, B. Mons, The FAIR Guiding Principles for scientific data management and stewardship, *Scientific Data*, 2016, **3**, 160018, doi: 10.1038/sdata.2016.18.
- [135] N. Coca-Lopez, V. Alcolea-Rodriguez, M. A. Bañares, S. Brockhauser, J. Gorenflot, A. Henderson, R. Hildebrandt, N. Jeliazkova, N. Kochev, E. Lozano-Diz, Z. Pilat, D. Polli, P. Strömert, C. Sturm, R. Vanna, R. Portela, Artificial intelligence-powered Raman spectroscopy through open science and FAIR principles, *ACS Nano*, 2025, **19**, 38189–38203, doi: 10.1021/acsnano.5c09165.
- [136] R. De La Peña, E. S. Sattely, Rerouting plant terpene biosynthesis enables momilactone pathway elucidation, *Nature Chemical Biology*, 2021, **17**, 205–212, doi: 10.1038/s41589-020-00669-3.
- [137] X.-Y. Sun, W. Li, H.-X. Zhao, J.-L. Cui, The molecular regulatory network for the accumulation of metabolites in the stems and roots of Ephedra sinica by the fungus Didymella keratinophila, *Physiological and Molecular Plant Pathology*, 2026, **144**, 103261, doi: 10.1016/j.pmpp.2026.103261.
- [138] J. L. Arais, S. C. Kefauver, Breeding to adapt agriculture to climate change: affordable phenotyping solutions, *Current Opinion in Plant Biology*, 2018, **45**, 237–247, doi:

10.1016/j.pbi.2018.05.003.

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