



Multi-Omics Discovery of Yield and Disease-Resistance Traits in Rapeseed Improvement

Soo-Jin Yim

Department of Bio-Environmental Chemistry, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 34134, Korea

Abstract

*Rapeseed is a globally significant oilseed crop, providing essential edible oils for human consumption, high-protein meal for livestock, and renewable raw materials for biofuel production. As the global population expands and climate change introduces unprecedented environmental stresses, enhancing the yield and disease resistance of rapeseed has become a paramount objective for agricultural researchers. Traditional breeding programs, which rely predominantly on phenotypic selection and single-marker assisted breeding, have achieved substantial historical gains but are increasingly constrained by the narrow genetic base of modern cultivars and the complex, polygenic nature of key agronomic traits. This paper provides a comprehensive analysis of how the paradigm of crop improvement has shifted toward systems biology, specifically through the integration of multi-omics technologies. By combining genomics, transcriptomics, proteomics, and metabolomics, researchers can now delineate the complex regulatory networks that govern plant architecture, seed development, lipid biosynthesis, and basal immunity. This review critically examines the methodologies employed in multi-omics integration, highlighting both computational frameworks and empirical validation strategies. Furthermore, the discussion elucidates the molecular mechanisms underpinning yield determination and defense responses against devastating fungal pathogens such as *Sclerotinia sclerotiorum*. The synthesis of multi-omics data not only accelerates the discovery of novel quantitative trait loci but also facilitates the design of next-generation ideotypes via targeted genome editing. Ultimately, this comprehensive approach bridges the genotype-to-phenotype gap, ensuring sustainable rapeseed production in an era of global agricultural volatility.*

Keywords: *Brassica napus, Multi-omics integration, Yield enhancement, Disease resistance, Systems biology*

1. Introduction

1.1 The Global Significance of Rapeseed Production

Rapeseed, scientifically known as *Brassica napus*, occupies a critical position in the global agricultural landscape. It is currently one of the leading sources of vegetable oil worldwide, second only to soybean and palm oil. The oil extracted from its seeds is characterized by a favorable fatty acid profile, including high levels of oleic acid and low levels of saturated fats, making it highly desirable for human nutrition and cardiovascular health. Furthermore, the residual meal left after oil extraction is rich in high-quality protein and is extensively utilized as a premium feed supplement in the poultry, swine, and aquaculture industries. Beyond its food and feed applications, rapeseed serves as a primary feedstock for the burgeoning biodiesel industry, particularly in European and North American markets. Consequently, stabilizing and increasing rapeseed production is intrinsically linked to global food security, economic stability, and the transition toward renewable energy economies. The agricultural demands placed on this crop necessitate continuous genetic improvement to maximize productivity per unit of arable land,

especially as urbanization and soil degradation threaten available farming areas [1]. The economic imperatives driving rapeseed cultivation require a paradigm shift in how researchers approach crop improvement, moving beyond incremental gains to achieve transformative leaps in agronomic performance [2].

1.2 Current Challenges in Rapeseed Improvement

Despite the historical successes of modern breeding programs, which have significantly increased seed yields and modified oil quality profiles, the trajectory of rapeseed improvement is currently facing profound biological and environmental bottlenecks. Yield stagnation is a pressing concern, driven largely by the depletion of accessible genetic variation within elite breeding pools. This narrow genetic diversity restricts the ability of breeders to select for novel traits that could push the physiological limits of the plant. Concurrently, global climate change is exacerbating the frequency and severity of both abiotic and biotic stresses [3]. Pathogenic infections pose a particularly severe threat to sustainable yields. Diseases such as Sclerotinia stem rot caused by *Sclerotinia sclerotiorum*, blackleg caused by *Leptosphaeria maculans*, and clubroot caused by *Plasmodiophora brassicae* inflict massive annual economic losses, often decimating entire fields under conducive weather conditions. The genetic architecture controlling yield and broad-spectrum disease resistance is highly complex, typically governed by numerous quantitative trait loci with minor individual effects. Furthermore, these traits are heavily influenced by environmental fluctuations and genotype-by-environment interactions. Traditional breeding methodologies, relying on phenotypic evaluation and limited molecular markers, are inherently inefficient at capturing the multidimensional regulatory networks that dictate these complex traits. There is a critical need for advanced technological frameworks capable of dissecting the entire biological system of the plant to identify synergistic gene combinations and regulatory hubs [4].

1.3 The Advent of Systems Biology and Multi-Omics

In response to the limitations of traditional genetics, the field of plant biology has embraced the era of systems biology, an interdisciplinary approach that models complex biological systems through the comprehensive analysis of their constituent components and interactions. At the heart of this revolution is the deployment of multi-omics technologies. Genomics provides the foundational blueprint of the organism, identifying structural variations, single nucleotide polymorphisms, and gene presence or absence across diverse populations. Transcriptomics captures the dynamic expression of these genes across different tissues, developmental stages, and environmental conditions. Proteomics bridges the gap between transcription and cellular function by quantifying protein abundance, identifying post-translational modifications, and mapping protein-protein interaction networks. Finally, metabolomics offers a snapshot of the ultimate biochemical phenotype, profiling the vast array of primary and secondary metabolites that dictate nutritional quality, structural integrity, and chemical defense mechanisms. By integrating these disparate, high-dimensional datasets, researchers can construct holistic, multi-layered networks that accurately represent the flow of biological information from the DNA sequence to the observable agronomic trait. This paper systematically explores the application of multi-omics approaches in rapeseed improvement, detailing the historical context, the methodologies of data integration, and the specific molecular discoveries that are currently reshaping our understanding of yield potential and disease resistance in this vital crop.

2. Literature Review and Biological Background

2.1 Traditional Breeding and Single-Omics Approaches

The trajectory of rapeseed breeding over the past century has been marked by significant milestones, beginning with the mass selection of locally adapted landraces and progressing to pedigree breeding and the development of hybrid varieties utilizing cytoplasmic male sterility systems. A monumental achievement in the history of rapeseed improvement was the development of double-low varieties, which exhibit minimal levels of erucic acid in the oil and low glucosinolate content in the meal, dramatically improving the nutritional and economic value of the crop [5]. Following these early successes, the advent of molecular biology introduced marker-assisted selection, allowing breeders to track specific genomic regions associated with desirable traits without relying solely on time-consuming phenotypic evaluations. Early genetic studies in rapeseed predominantly utilized mapping populations, such as recombinant inbred

lines or doubled haploids, to identify quantitative trait loci linked to yield components and disease resistance [6]. The transition to single-omics approaches, primarily genomics and transcriptomics, significantly accelerated trait discovery. The publication of the first reference genomes for *Brassica napus* provided a foundational framework for understanding its complex genetic architecture. *Brassica napus* is a recent allotetraploid, formed by the natural hybridization of the diploid species *Brassica rapa* and *Brassica oleracea*. This polyploid nature results in extensive gene duplication, homologous recombination, and structural variations, complicating genetic analyses but also providing a rich reservoir of functional diversity [7]. Genome-wide association studies leveraging single nucleotide polymorphism arrays began to identify hundreds of loci associated with agronomic traits across diverse accession panels [8]. Concurrently, microarray and early RNA-sequencing studies characterized the transcriptional reprogramming that occurs during seed development and pathogen infection. However, these single-omics studies, while foundational, often failed to provide a complete mechanistic understanding of the traits under investigation, as they could not account for post-transcriptional regulation, protein dynamics, or metabolic flux.

2.2 The Complexity of Yield and Disease Traits

Yield in rapeseed is a classic polygenic trait, determined by the multiplicative interaction of several distinct yield components, including the number of plants per unit area, the number of branches per plant, the number of siliques or pods per branch, the number of seeds per silique, and the individual seed weight. Each of these components is controlled by independent yet interacting genetic networks that respond dynamically to resource availability, hormonal signals, and environmental cues [9]. For instance, source-sink dynamics play a crucial role in yield determination; the efficiency of carbon fixation in the leaves and silique walls must be perfectly coordinated with the transport of photoassimilates to the developing seeds. Perturbations at any level of this biological hierarchy, from gene transcription to sugar metabolism, can significantly impact the final yield, illustrating the necessity of a systems-level approach to yield improvement [10]. Similarly, the interaction between rapeseed and its pathogens is governed by an intricate and dynamic evolutionary arms race. Pathogens utilize diverse infection strategies, ranging from biotrophic pathogens that feed on living tissue to necrotrophic pathogens, such as *Sclerotinia sclerotiorum*, which actively kill host cells to extract nutrients [11]. The plant immune system comprises two primary tiers: pathogen-associated molecular pattern-triggered immunity, which provides broad-spectrum basal resistance, and effector-triggered immunity, which provides race-specific resistance mediated by intracellular resistance proteins. The defense response involves a massive reallocation of cellular resources, shifting from growth and development to the production of reactive oxygen species, reinforcement of cell walls, and the synthesis of antimicrobial secondary metabolites [12].

2.3 The Limitations of Isolated Data Streams

The inherent complexity of yield and disease resistance highlights the limitations of isolated omics data streams. A transcriptomic analysis might identify a gene that is highly upregulated during pathogen infection; however, without proteomic data, it remains unknown whether the corresponding protein is successfully translated, appropriately localized, or activated via phosphorylation. Likewise, genomic data might reveal a single nucleotide polymorphism within a promoter region, but metabolomic data is required to determine if this mutation actually alters the biochemical composition of the plant [13]. Furthermore, biological systems are characterized by extensive epistasis, where the effect of one gene is dependent on the genetic background, and pleiotropy, where a single gene influences multiple seemingly unrelated traits. Single-omics approaches are mathematically and biologically ill-equipped to unravel these non-linear interactions. Therefore, the transition to multi-omics integration is not merely a technological upgrade but a fundamental necessity for comprehensively deciphering the molecular logic of rapeseed performance [14].

3. Multi-Omics Methodologies in Rapeseed Research

3.1 Genomic and Transcriptomic Sequencing Technologies

The foundation of any multi-omics study in rapeseed begins with high-resolution genomic and transcriptomic profiling. Recent advancements in third-generation long-read sequencing technologies, such as those developed by Pacific Biosciences and Oxford Nanopore, have revolutionized rapeseed

genomics. Unlike short-read sequencing, which struggles to resolve the highly repetitive regions and duplicated homeologous chromosomes inherent in the allotetraploid genome, long-read technologies enable the assembly of contiguous, high-quality reference genomes. This has facilitated the transition from single reference genomes to pangenomics, which captures the entire repertoire of genetic diversity within the species, including large-scale structural variants, presence-absence variations, and copy number variations that are often the true causal drivers of complex traits [15]. Transcriptomic methodologies have similarly evolved. Standard bulk RNA-sequencing is now routinely deployed across high-resolution spatiotemporal gradients, mapping gene expression profiles across dozens of tissue types and developmental stages. Furthermore, the emergence of single-cell RNA-sequencing and spatial transcriptomics is allowing researchers to resolve transcriptional heterogeneity at the cellular level, providing unprecedented insights into the localized cellular interactions that occur in the meristem during branch initiation or at the infection interface during pathogen penetration [16]. These transcriptomic datasets are often integrated with epigenetic profiling, such as chromatin immunoprecipitation sequencing and whole-genome bisulfite sequencing, to understand how chromatin accessibility and DNA methylation regulate gene expression in response to environmental stimuli [17].

3.2 Proteomics and Metabolomics Advancements

While genomics and transcriptomics identify the potential of the biological system, proteomics and metabolomics define its actualized state. Modern plant proteomics relies heavily on advanced liquid chromatography coupled with high-resolution tandem mass spectrometry. Techniques such as tandem mass tags and data-independent acquisition have significantly increased the throughput, sensitivity, and reproducibility of protein quantification [18]. In rapeseed research, proteomics is crucial for identifying enzymes involved in lipid biosynthesis and regulatory proteins that mediate hormonal signaling pathways. Moreover, specialized sub-disciplines like phosphoproteomics and ubiquitinomics are employed to track the rapid post-translational modifications that serve as the primary on-off switches in signal transduction cascades, particularly during the rapid onset of innate immune responses [19]. Metabolomics represents the ultimate downstream readout of cellular activity. In rapeseed, metabolomic analyses are generally divided into targeted and untargeted approaches. Targeted metabolomics utilizes triple quadrupole mass spectrometry to precisely quantify specific, pre-defined classes of compounds, such as fatty acids, amino acids, or specific defense-related secondary metabolites like glucosinolates and phytoalexins. Untargeted metabolomics, utilizing high-resolution mass spectrometers such as quadrupole time-of-flight instruments, aims to detect as many diverse chemical entities as possible within a biological sample, providing a comprehensive metabolic fingerprint [20]. The integration of these chemical profiles with proteomic and transcriptomic data is essential for mapping biochemical pathways and identifying the specific enzymes responsible for the synthesis of key agronomic compounds.

Table 1: Comparison of multi-omics integration methodologies in crop research

Integration Strategy	Primary Data Types Utilized	Analytical Goal	Computational Complexity
Correlation-based Networks	Transcriptomics, Metabolomics	Identify co-expressed gene-metabolite modules	Moderate
Pathway Mapping	Genomics, Proteomics, Metabolomics	Map functional enzymes to biochemical pathways	High
Machine Learning	All Omics Layers	Predict phenotypic outcomes from complex datasets	Very High
Matrix Factorization	Genomics, Transcriptomics	Reduce dimensionality and find latent variables	High

Multi-layer Perceptrons	Transcriptomics, Phenomics	Model non-linear interactions across tissue types	Very High
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3.3 Computational Integration Strategies

The sheer volume and heterogeneous nature of multi-omics data present formidable computational challenges. The integration of these datasets requires sophisticated bioinformatic pipelines and statistical frameworks. One widely adopted approach is correlation-based network analysis, such as Weighted Gene Co-expression Network Analysis. This method constructs interaction networks based on the correlated abundance patterns of transcripts, proteins, and metabolites across multiple samples, identifying densely connected modules or hubs that represent functional biological pathways [21]. Another powerful strategy involves the use of multi-block partial least squares discriminant analysis, which projects different omics datasets into a common latent space to identify the subset of variables from each layer that most strongly associate with a specific trait or phenotypic outcome [22]. In recent years, artificial intelligence and machine learning algorithms have become indispensable for multi-omics integration. Deep learning architectures, such as multi-modal autoencoders, can ingest disparate data types and extract high-level, non-linear representations of the biological system. These computational models are capable of identifying non-obvious, higher-order interactions between genomic structural variants, alternative splicing events, and metabolic fluxes that traditional statistical methods often overlook. Furthermore, these predictive models can simulate the effects of virtual gene knockouts or overexpressions, prioritizing candidate genes for empirical validation and significantly accelerating the breeding cycle [23]. The continued refinement of these computational algorithms is critical for maximizing the utility of the massive datasets generated by modern sequencing and spectrometry platforms.

4. Discovery of Yield-Associated Traits

4.1 Genetic Regulation of Plant Architecture

Plant architecture, encompassing traits such as plant height, branch number, branch angle, and pod distribution, fundamentally dictates the yield potential of rapeseed by determining light interception efficiency and the physical space available for reproductive organs. Multi-omics analyses have deeply illuminated the regulatory networks controlling these morphological traits. By integrating genome-wide association data with tissue-specific transcriptomes from the shoot apical and axillary meristems, researchers have identified key regulatory hubs, particularly those related to phytohormone biosynthesis and signaling. Auxin, cytokinin, and strigolactones operate in a complex antagonistic and synergistic network to control apical dominance and the outgrowth of axillary buds. Integrated omics approaches have successfully mapped the specific transcription factors and receptor kinases that modulate the local concentrations and sensitivities of these hormones [24]. For example, the combined analysis of the transcriptome and the metabolome in varied branching mutants has revealed how the precise spatiotemporal accumulation of distinct cytokinin derivatives directly correlates with the proliferation of lateral meristems, thereby increasing the total number of siliques per plant. Furthermore, proteomic studies have identified crucial ubiquitin ligases that target specific branching repressors for degradation, highlighting the importance of post-translational regulation in morphological development [25].

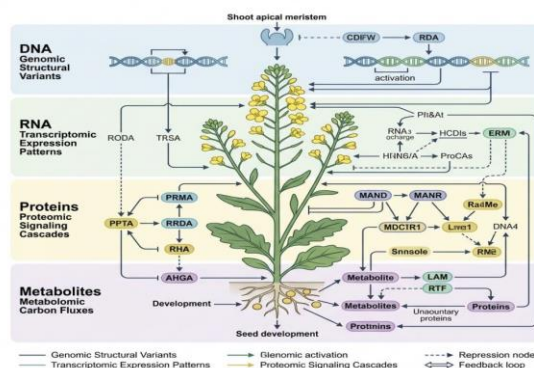


Figure 1: Comprehensive Schematic of Multi-Omics Integration in Plant Architecture

4.2 Seed Development and Lipid Biosynthesis

The ultimate determinant of economic yield in rapeseed is the quantity and quality of the oil stored within the seeds. Seed development is a highly orchestrated process involving embryogenesis, seed expansion, and a prolonged maturation phase characterized by the massive accumulation of storage lipids, primarily triacylglycerols, and storage proteins. Multi-omics integration has proven exceptionally powerful in dissecting the carbon partitioning networks that fuel this process. Comparative genomics and transcriptomics across high-oil and low-oil rapeseed accessions have highlighted the transcriptional upregulation of key enzymes in the de novo fatty acid synthesis pathway located in the plastids, such as acetyl-CoA carboxylase and the fatty acid synthase complex [26]. However, transcript abundance alone does not perfectly correlate with final oil content. By incorporating proteomic and lipidomic profiling during the seed filling stages, researchers have uncovered critical rate-limiting steps at the level of protein complex assembly and substrate specificity during the assembly of triacylglycerols in the endoplasmic reticulum [27]. These multi-level analyses have also elucidated the intricate balance between carbon and nitrogen metabolism. The competition for carbon skeletons between lipid biosynthesis and protein synthesis is a major physiological bottleneck. Multi-omics network analyses have identified specific transcription factors, such as WRINKLED1 and LEAFY COTYLEDON1, which act as master regulators coordinating this balance. By mapping the downstream targets of these transcription factors across the transcriptome and validating the resulting metabolic shifts via untargeted metabolomics, scientists have established comprehensive gene-to-metabolite interaction maps. These maps reveal how manipulating a single master regulator can cascade through the biological network to fundamentally alter the biochemical composition of the seed, providing highly precise targets for genetic engineering aimed at enhancing both total seed weight and overall oil yield [28].

Table 2: Key molecular modules identified through multi-omics associated with yield components.

Yield Component	Master Gene	Regulatory Pathway	Dominant Biological Pathway	Validating Omics Layer
Branch Number	BnaMAX1		Strigolactone Biosynthesis	Metabolomics (Hormone profiling)
Pod Density	BnaPIN1		Auxin Efflux Transport	Proteomics (Membrane localization)
Seed Weight	BnaARF18		Auxin Response Regulation	Transcriptomics (Co-expression)
Oil Content	BnaWRI1		Glycolysis and Fatty Acid Synthesis	Lipidomics (Triacylglycerol profiling)
Carbon Partitioning	BnaSnRK1		Sucrose Sensing and Energy Balance	Phosphoproteomics (Kinase activity)

5. Unraveling Disease-Resistance Mechanisms

5.1 Pathogen Recognition and Early Signaling Events

The successful defense against devastating fungal pathogens in rapeseed requires rapid recognition and the subsequent mobilization of massive cellular resources. Multi-omics technologies have been instrumental in characterizing the earliest events in the plant-pathogen interaction. Upon perception of pathogen-associated molecular patterns, such as chitin from fungal cell walls, plant cells initiate a rapid signaling cascade. Phosphoproteomics has been particularly vital in this domain, as the initial signal transduction relies heavily on the rapid phosphorylation of receptor-like kinases and cytoplasmic signaling proteins, rather than de novo gene expression. Quantitative phosphoproteomic time-course studies, combined with transcriptomic data, have mapped the immediate activation of mitogen-activated protein

kinase cascades and calcium-dependent protein kinases within minutes of pathogen exposure [29]. These kinase cascades subsequently activate specific transcription factors, primarily from the WRKY and MYB families, which then drive the massive transcriptional reprogramming required for defense. By comparing the integrated genomic-transcriptomic-proteomic profiles of highly resistant versus highly susceptible rapeseed accessions upon challenge with *Sclerotinia sclerotiorum*, researchers have identified specific receptor variants and hyper-responsive kinase networks that define the resistant phenotype. This multi-layered approach has clarified how physical variations in receptor structure, mapped via genomics, translate into faster and more robust signal transduction, mapped via phosphoproteomics, ultimately leading to enhanced survival [30].

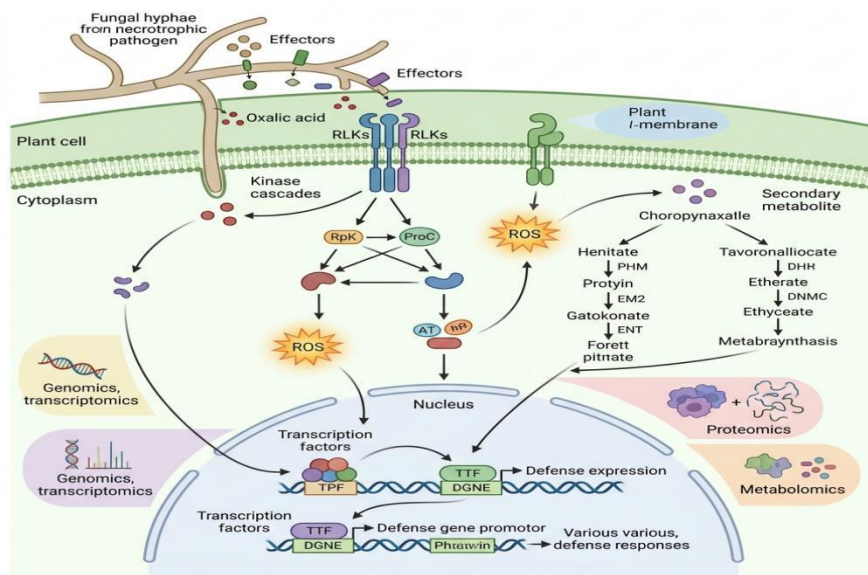


Figure 2: Multi

5.2 Secondary Metabolism and Late Defense Responses

Following the initial recognition and signaling phases, the execution of the defense response relies heavily on the production of antimicrobial compounds and the reinforcement of physical barriers. In rapeseed, the late defense response is characterized by profound metabolic shifts. *Sclerotinia sclerotiorum*, a necrotrophic pathogen, secretes substantial amounts of oxalic acid and cell wall degrading enzymes to induce host cell death and facilitate tissue maceration. Multi-omics integration has revealed the multifaceted strategies employed by resistant rapeseed varieties to counteract these virulence factors. Transcriptomic and proteomic datasets consistently show the upregulation of enzymes involved in the detoxification of reactive oxygen species and the degradation of oxalic acid, such as oxalate oxidase [31]. However, it is the integration with untargeted metabolomics that provides the most comprehensive view of the chemical warfare occurring at the infection site. Brassica species are renowned for their glucosinolate-myrosinase defense system. Upon tissue damage, glucosinolates are hydrolyzed by myrosinase enzymes to produce toxic isothiocyanates. Through combined transcriptomic and metabolic quantitative trait loci mapping, researchers have identified the specific genetic loci controlling the biosynthesis of indolic glucosinolates, which are highly inducible upon fungal infection and play a crucial role in restricting pathogen proliferation [32]. Furthermore, multi-omics network analyses have uncovered the biosynthesis pathways of novel phytoalexins, complex secondary metabolites synthesized de novo in response to infection. By tracking carbon flux using stable isotope labeling coupled with transcriptomics and high-resolution mass spectrometry, researchers have traced the redirection of primary metabolites away from growth pathways and into the shikimate and phenylpropanoid pathways, culminating in the production of lignin for cell wall reinforcement and highly specific fungitoxic flavonoids. The correlation of these distinct metabolic profiles with global gene expression patterns has provided an unprecedented, holistic understanding of the metabolic cost and the biochemical architecture of broad-spectrum disease

resistance in rapeseed [33].

Table 3: Multi-omics markers associated with defense responses against Sclerotinia sclerotiorum.

Defense Phase	Key Biological Indicator	Primary Omics Detection Method	Functional Outcome
Early Recognition	Receptor-Like Kinase Phosphorylation	Phosphoproteomics	Signal Transduction Initiation
Oxidative Burst	Reactive Oxygen Species Accumulation	Targeted Metabolomics	Direct Antimicrobial Action
Transcriptional Shift	WRKY33 Factor Upregulation	RNA-Sequencing (Transcriptomics)	Activation of Defense Genes
Detoxification	Oxalate Oxidase Accumulation	Quantitative Proteomics	Neutralization of Fungal Toxins
Chemical Defense	Indolic Glucosinolate Biosynthesis	Untargeted Metabolomics	Fungal Growth Inhibition

6. Conclusion and Future Directions

6.1 Synthesis of Discoveries and Current Limitations

The integration of genomic, transcriptomic, proteomic, and metabolomic data has fundamentally transformed the landscape of rapeseed research. The transition from reductionist, single-gene analyses to holistic systems biology has provided unprecedented clarity regarding the complex regulatory architectures governing yield potential and disease resistance. As discussed throughout this review, multi-omics methodologies have successfully identified the core master regulators of plant branching, pinpointed the precise biochemical bottlenecks in lipid biosynthesis, and mapped the highly dynamic and multi-layered defense signaling networks activated during fungal pathogenesis. These comprehensive datasets bridge the critical gap between genetic variation and phenotypic realization, offering a robust foundation for next-generation breeding programs. However, despite these profound advancements, significant limitations persist. The biological interpretation of massive, multi-dimensional networks remains computationally challenging and conceptually difficult. Many of the correlations identified through network analyses are merely associative and lack definitive causal proof. Furthermore, the high cost and technical complexity of large-scale proteomic and untargeted metabolomic profiling continue to limit their application to relatively small sample sizes compared to genomic sequencing, thereby reducing statistical power in diverse population studies.

6.2 Future Perspectives and Application

Looking forward, the future of rapeseed improvement relies on the seamless transition of multi-omics discoveries from theoretical models to practical agricultural applications. The integration of advanced artificial intelligence and machine learning algorithms will be paramount in distilling complex omics data into actionable breeding targets. Moreover, the true validation of these computational models will be realized through the expanded use of precision genome editing technologies, such as CRISPR/Cas9. Multi-omics networks can provide highly specific candidate genes for targeted knockout, promoter editing, or base editing, allowing researchers to artificially reconstruct the ideal combinations of alleles necessary for maximum yield and robust disease resistance without the hindrance of linkage drag associated with traditional crossbreeding [34]. Additionally, the continued development of spatial omics and single-cell sequencing technologies will provide even greater resolution, unraveling the precise cellular microenvironments where crucial developmental decisions and pathogen interactions occur. By maintaining this trajectory of technological integration and biological discovery, the agricultural scientific community can successfully develop highly resilient, high-yielding rapeseed varieties tailored to withstand the escalating pressures of global climate change and ensure future agricultural sustainability.

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