Computational Approaches to Fight Against Wheat Rust

Kiran Kushwaha¹, Amaan Arif², Ruby Singh³, Prachi Bhargava^{1*}, Prachi Srivastava^{2*}

Abstract

Wheat rust diseases, caused by the fungal pathogens *Puccinia graminis*, *Puccinia triticina*, and Puccinia striiformis, pose significant threats to global wheat production, leading to substantial economic losses and food insecurity. Stem rust, leaf rust, and stripe rust, though distinct in their symptoms and environmental adaptations, collectively affect crop yields, with severe outbreaks historically devastating wheat fields. Traditional control methods, such as breeding resistant varieties and applying fungicides, face limitations due to pathogen evolution and sustainability concerns. This review explores the role of computational approaches, including genomics, bioinformatics, machine learning, and systems biology, in advancing wheat rust management. Genomic prediction models, systems biology analyses, and machine learning have proven effective in understanding host-pathogen interactions, predicting disease outbreaks, and developing resilient wheat varieties. The integration of computational tools with conventional agricultural practices offers a promising pathway to mitigate wheat rust's impact, enhancing global food security. Continued innovation and interdisciplinary collaboration are crucial for addressing challenges such as data heterogeneity and computational limitations, ensuring the sustainability of wheat production in the face of evolving threats.

Keyword

Wheat rust diseases, Host-pathogen interactions, Computational approaches, Genomic prediction, Food security

¹ Institute of Agricultural Sciences and Technology at SRMU, Lucknow, UP, India

² Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus 226028

³ Gevanam Genomics, Gevanam Genomics, Govardhan enclave, Lucknow Rd, Lucknow, Neemteekar, Uttar Pradesh 226301, Lucknow, Uttar Pradesh, India, 226301

Introduction

Wheat rust diseases, which include stem rust, leaf rust, and stripe rust, represent significant challenges to global wheat production [1]. These fungal diseases, caused by pathogens *Puccinia graminis*, *Puccinia triticina*, and *Puccinia striiformis* respectively, have historically destroyed crops, leading to substantial economic losses and food insecurity [2], [3], [4], [5], [6]. The lifecycle of these rust fungi involves complex interactions with their host plants and environmental conditions, making their control particularly challenging [7].

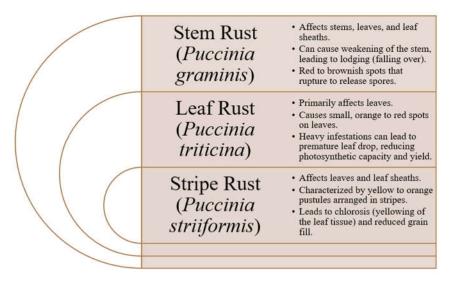


Figure 1 Types of Wheat Rust and Their Symptoms

Stem rust, often considered most severe, has caused widespread epidemics, notably severe outbreaks in early 20th century that devastated wheat fields across North America. Stem rust, which is characterized by reddish-brown pustules on stems, leaves, and leaf sheaths, can cause significant yield losses, sometimes up to 100% in susceptible cultivars. Leaf rust, on other hand, presents as orange-brown lesions primarily on leaves, leading to reduced photosynthetic capability and premature leaf senescence. While generally less damaging than stem rust, leaf rust can still cause significant yield reductions. Stripe rust, identifiable by its yellow stripe-like pustules, is particularly adapted to cooler climates and can be equally destructive, particularly in regions/areas with favourable environmental conditions. Wheat rust has a major economic and agricultural impact. Globally, wheat is a staple/ basic food crop, essential for sustenance of millions of people. Recurrent outbreaks of rust diseases/ infection can severely disrupt wheat production, resulting in increased costs for fungicides and other control measures, decreased yields, and volatile market prices. In developing countries, where resources for disease management are often limited, the impact can be particularly severe, worsening food security and economic stability concerns [8].

Stripe Rust 25% Stem Rust 40%

PROPORTION OF CROP LOSSES DUE TO WHEAT RUST

Figure 2 Proportion of Crop Losses Due to Wheat Rust

Traditionally, primary methods for combating wheat rust have included breeding for resistant varieties and use of chemical treatments. Breeding for resistance involves identifying and incorporating rust-resistant genes into commercial wheat varieties. This approach has yielded significant successes; however, durability of resistance is often compromised by pathogen's ability to evolve and overcome resistance genes. As a result, breeding efforts must be continuous and adaptable to constantly shifting rust populations. Chemical treatments, primarily fungicides, are another key component of rust management. These treatments can be effective in reducing rust outbreaks and protecting crops, but they have several drawbacks. The continuous use of fungicides can result in development of resistant pathogen strains, reducing long-term effectiveness of these chemicals. Additionally, environmental impact and cost of fungicide application can be high, creating issues for sustainable agriculture operations [9].

Given limitations of traditional methods present, there is a growing recognition of need for innovative and new approaches to managing wheat rust more effectively. Computational approaches provides promising solutions by combining developments and advancements in genomics, bioinformatics, machine learning, and systems biology. These approaches can help better understanding of rust pathogens, improve prediction of disease outbreaks, and support development of more resilient wheat varieties. As such, integrating computational approaches into wheat rust management strategies represents an important step towards ensuring sustainability and security of global wheat production.

2. Importance of Computational Approaches

Traditional methods for controlling wheat rust, while historically significant, have various limitations that restrict their long-term effectiveness and sustainability. One primary limitation is ongoing evolution of rust pathogens. Pathogens like *Puccinia graminis*,

Puccinia triticina, and Puccinia striiformis have high degree of genetic variability and can quickly adapt to resistant wheat varieties, making resistance genes useless over time. This necessitates a continuous cycle of developing new resistant strains, which is both timeconsuming and resource intensive. Additionally, the reliance on chemical treatments, such as fungicides, presents and raises challenges related to environmental effects, cost, and rise of fungicide-resistant pathogen strains. Given these limits, computational approaches offer significant advantages in management of wheat rust diseases. Computational approaches use power of advanced/modern technologies in genomics, bioinformatics, machine learning, and systems biology to give more precise, efficient, and sustainable solutions. One important advantage is ability to analyze large amounts of genetic or genomic data quickly and accurately. Through bioinformatics tools and genomic databases, researchers can identify resistance genes, better understand pathogen genetics, and predict how pathogens may evolve. This enables more informed decisions in breeding programs, leading to development of wheat varieties with durable or long-term resistance. Furthermore, computational approaches make it easier to integrate multi-omics data, including as genomics, transcriptomics, proteomics, and metabolomics etc. By combining data from these different layers, scientists can gain a comprehensive understanding of host-pathogen interaction, identifying critical regulatory networks and metabolic pathways involved in disease resistance and susceptibility. This comprehensive understanding can inform the development of targeted interventions or therapies that disrupt critical processes in the pathogen's lifecycle or strengthen plant's defence mechanisms [10].

Machine learning (ML) and artificial intelligence (AI) further improve disease management by providing tools for predictive modelling and disease forecasting. These technologies or tools can analyze complex datasets to predict disease outbreaks based on environmental conditions or factors, historical data, and pathogen behaviour. Early warning systems can be developed to alert or notify farmers and agricultural stakeholders about potential rust epidemics, allowing for timely and proactive measures to mitigate impact [11]. Additionally, AI-driven image analysis can improve accuracy and speed of rust detection in the field, allowing for more effective monitoring and control efforts. The integration of computational approaches also supports and promotes precision agriculture practices. Farmers may improve fungicide application by integrating data analytics and machine learning, which can ensure that treatments are applied only when necessary and in most

effective manner. This reduces the overall use of chemicals, minimizing environmental impact/ effects and lowering costs [12], [13].

3. Types of Computational Approaches

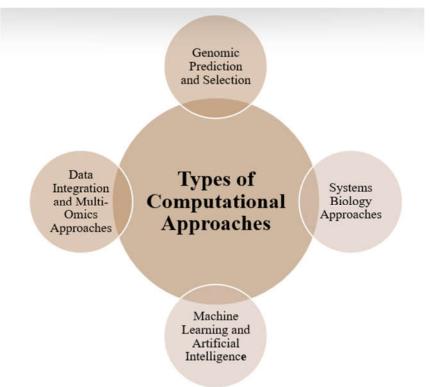


Figure 3 Types of Computational Approaches

Computational approaches in fight against wheat rust use various bioinformatics tools and databases to improve understanding of pathogen genomics/ genomes and development of resistant wheat varieties. These tools provide invaluable resources for researchers to analyze and evaluate genetic data, identify resistance genes, and better understand pathogen variability and evolution.

3.1. Genomic and Proteomic Databases

Genomic and proteomic databases are essential repositories that store large amounts of genetic data or information, making it easier to study both wheat and rust pathogens. Key databases include:

• Ensembl Plants: This comprehensive database provides access to genome sequences and functional annotations of a diverse range of plant species, including wheat varieties. It allows researchers to explore genetic variation, gene structure, and related function, as well as identify candidate or potential resistance genes. The integrated tools and technologies enable comparative genomics, which helps in understanding evolutionary relationships and mechanisms of resistance across different wheat varieties and related species [14], [15].

• NCBI (National Center for Biotechnology Information): NCBI offers a multitude of databases, such as GenBank, which stores nucleotide sequences and their protein translations etc. We can access genome sequences of various rust pathogens, allowing them to examine their genetic makeup and identify target genes for resistance. The NCBI's Sequence Read Archive (SRA) also provides raw sequencing data, allowing for in-depth genomic analyses and interpretation [16].

Software for Sequence Alignment and Gene Prediction

Accurate sequence alignment and gene prediction are important for identifying resistance genes and understanding genetic structure of rust pathogens. Key software tools include:

- BLAST (Basic Local Alignment Search Tool): BLAST is a widely used popular algorithm for comparing nucleotide or protein sequences to sequence databases and identifying regions of similarity. This tool helps in finding homologous genes, understanding and analyses gene function, and identifying potential resistance genes by comparing wheat genomic sequences with known resistance genes from other species or varieties [17].
- HMMER: HMMER uses hidden Markov models for sequence alignment and gene prediction, particularly focusing on identifying protein families and domains. This method is highly effective in detecting conserved motifs and functional domains within proteins, which are important for understanding molecular mechanisms of disease resistance. By analyzing protein sequences of wheat and rust pathogens, researchers can predict functional impact of genetic variations and identify important resistance-related proteins [18].

The combination of these databases and software tools allows a comprehensive analysis of genetic landscape of wheat and its pathogens. Using these resources, researchers can identify critical and important genes involved in resistance, understand molecular mechanisms behind rust infections, and develop strategies for breeding resistant wheat varieties. Moreover, integration of genomic and proteomic data provides a comprehensive perspective of host-pathogen interaction, allowing for development of targeted therapies to disrupt pathogen lifecycle and strengthen plant defences.

3.2. Systems Biology Approaches

Systems biology approaches provide comprehensive understanding of complex interactions between host plants and pathogens. By integrating various types of biological data and applying sophisticated computational methods, systems biology aims to understand intricate networks and pathways that drive host-pathogen interactions. This comprehensive understanding facilitates the identification of key regulatory genes and pathways, as well as

modelling of metabolic and signalling pathways that are critical for disease resistance. Network analysis is a powerful tool used for understanding complex web of interactions between host plants and pathogens. In the case of wheat rust, network analysis involves mapping interactions between wheat genes, proteins, and rust pathogen molecules to identify important locations of vulnerability and defence [19].

One of the important areas of research in plant-pathogen interactions, is development of protein-protein interaction (PPI) networks. By delving into complex web of interactions between wheat and rust pathogen proteins, researchers gain valuable insights into the mechanisms at work. These networks shed light on how pathogen effectors modify host proteins to avoid plant defences, as well as how host proteins coordinate defence responses. Another important aspect of this approach involves development of gene co-expression networks. This is achieved through a comprehensive analysis of gene expression patterns across diverse conditions. Genes with comparable or similar expression patterns are thought to be functionally related. In the context of wheat and rust infection, this technique holds the potential to identify clusters of genes that undergo co-regulation in response to presence of a pathogen. Network analysis can help identify hub proteins and essential nodes that perform important functions in the network. These elements are often critical for maintaining integrity of network and can be targeted for improving disease resistance. By analyzing the network, researchers can identify pathways that are enriched with interactions, providing understanding into biological processes most affected by rust infection [20].

3.2.1 Identification of Key Regulatory Genes and Pathways

Understanding which genes and pathways play key roles in host's response to rust infection is critical for developing resistant wheat varieties. Systems biology approaches make this easier by integrating data from genomics, transcriptomics, and proteomics to identify critical regulatory elements.

Differential Gene Expression Analysis

Comparing gene expression patterns of resistant and susceptible wheat varieties under rust infection shows significant differences in expression levels, identifying candidates involved in resistance or susceptibility, identifying differentially expressed genes (DEGs) encoding transcription factors, signalling molecules, and other regulatory proteins that are involved in the host defence response.

Pathway Analysis:

- KEGG Pathway Analysis: Utilizing databases such as KEGG (Kyoto Encyclopedia of Genes and Genomes), differentially expressed genes were mapped to known biological pathways and other related pathways. This mapping helps to identify pathways that are either activated or suppressed/inhibited during rust infection. Significant pathways involved in immune responses, such as MAPK signaling pathway and plant-pathogen interaction pathway, were markedly upregulated in resistant varieties [21].
- Novel Pathway Discovery: Systems biology approaches also led to discovery of previously
 unknown pathways associated with disease resistance. By combining multiple omics data
 types, new interactions and regulatory mechanisms were discovered, suggesting
 involvement of secondary metabolites and stress-response pathways that had not been
 previously associated to rust resistance [22].

3.2.2 Modeling of Metabolic and Signaling Pathways

Mathematical and computational modelling of metabolic and signalling pathways provides more information into dynamic processes that govern disease resistance. These models can simulate the behaviour of complex biological systems, predict outcomes of genetic modifications or changes, and identify potential targets for intervention.

Metabolic Pathway Modeling:

- Flux Balance Analysis (FBA): FBA is a computational method used for predicting flow of metabolites through metabolic networks. By modelling metabolic pathways of wheat under rust infection, researchers can identify important metabolites and reactions that are required for resistance [23].
- Impact of Pathogen Effectors: Modelling can also reveal how pathogen effectors influence
 host metabolic pathways to facilitate infection. Understanding these changes or
 modifications can help in developing strategies to restore normal metabolic function in host
 organisms/species.

Signalling Pathway Modeling:

Dynamic Simulations: Dynamic signalling pathways model can simulate how signalling
molecules interact over time to trigger or activate defence responses. These models can
combine data from various sources, such as gene expression and protein interaction
networks, to provide comprehensive view of signaling dynamics [24].

 Perturbation Analysis: By simulating effects of perturbations (e.g., gene knockouts or overexpression), researchers can predict impact on signalling network and identify potential possible resistance-enhancing targets.

3.4. Machine Learning and Artificial Intelligence

Machine Learning (ML) and Artificial Intelligence (AI) have emerged as transformative tools in the fight against wheat rust. By using large datasets and advanced algorithms, these technologies offer innovative solutions for predictive modelling, disease detection, and precision agriculture. These applications enhance efficiency and effectiveness of rust management strategies, ensuring sustainable wheat production.



Figure 4 Steps in Creating Predictive Models for Disease Outbreak Forecasting Using Machine Learning 3.4.1 Predictive Modeling for Disease Outbreak Forecasting

Predictive modelling using ML and AI enables the accurate forecasting of wheat rust outbreaks by analyzing historical and real-time data. These models can integrate various data sources, including weather conditions, historical outbreak data, and environmental factors, to predict the likelihood of rust outbreaks [25].

Data Collection and Integration: ML models use historical data on rust outbreaks, including geographical locations, severity, and environmental factors. This data helps in

understanding patterns or trends and triggers of past outbreaks. Incorporating real-time data from weather stations, satellite imaging, and field sensors improves model's accuracy in predicting imminent outbreaks.

Model Development: Supervised learning algorithms, such as regression models, decision trees, neural networks etc, are trained on historical data to identify patterns and correlations between environmental factors and rust outbreaks. Important features, such as temperature, humidity, rainfall, and wind patterns, are identified and used to train models. These features are critical in determining conditions favourable for rust development and spread [26].

Model Deployment and Application:

- Early Warning Systems: Predictive models can be integrated into early warning systems that alert farmers and agricultural stakeholders about risk of rust outbreaks. These systems enable proactive measures, such as timely fungicide application and crop management practices, to mitigate impact of the disease.
- Decision Support Systems: AI-powered decision support systems can provide recommendations based on predicted risk levels, helping farmers make informed decisions about resource allocation and disease management strategies.

3.4.2 Image-Based Detection and Classification of Rust Symptoms

AI and ML technologies, particularly in the field of computer vision, offer powerful tools for the detection and classification of rust symptoms in wheat. These methods enhance speed and accuracy of disease diagnosis, facilitating timely and effective interventions [27], [28].

Image Acquisition and Preprocessing:

- Data Collection: High-quality images of wheat plants, captured using smartphones, drones, or field cameras, serve as the primary data source. These images should cover a wide range of rust symptoms at different stages [29].
- Image Preprocessing: Preprocessing steps, such as resizing, normalization, and augmentation, are applied to enhance the quality and diversity of the training dataset. This improves the model's robustness and generalization ability [29].

Model Training and Classification:

- Convolutional Neural Networks (CNNs): CNNs are the backbone of image-based detection systems. These deep learning models automatically extract features from images and learn to classify them based on the presence and severity of rust symptoms[30].
- Transfer Learning: Pretrained models, such as VGG16, ResNet, and Inception, can be finetuned on rust-specific datasets. Transfer learning accelerates the training process and

improves classification accuracy by leveraging knowledge from large-scale image datasets [30].

Deployment and Application:

- Mobile Applications: AI-powered mobile applications can provide farmers with on-the-spot diagnosis by simply taking a photo of the wheat plant. These applications can identify rust symptoms, determine their severity, and suggest appropriate management practices.
- Automated Monitoring Systems: Drones and field robots equipped with AI models can autonomously monitor large wheat fields, detecting rust outbreaks early and enabling targeted interventions.

3.5. Data Integration and Multi-Omics Approaches

Data integration and multi-omics approaches provide comprehensive insights into the biological mechanisms underlying wheat rust resistance and susceptibility. By combining data from genomics, transcriptomics, proteomics, and metabolomics, researchers can construct a detailed and holistic view of host-pathogen interactions, uncovering novel targets for intervention and breeding strategies.

3.5.1 Integration of Genomics, Transcriptomics, Proteomics, and Metabolomics Data Integrating data from various omics layers is essential to understanding complex biological processes involved in wheat rust infection and resistance [31], [32]. Each omics layer provides unique and complementary information:

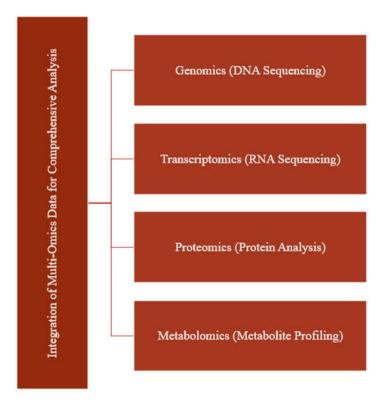


Figure 5 Integration of Multi-Omics Data for Comprehensive Analysis

Genomics: Genomic data include DNA sequences, genetic variants, and structural variations. High-throughput sequencing technologies enable the identification of resistance genes and quantitative trait loci (QTLs) associated with rust resistance. Comparative genomics between resistant and susceptible wheat varieties can reveal genetic markers and regions associated with disease resistance [33].

Transcriptomics: Transcriptomics involves study of RNA transcripts to understand gene expression patterns. RNA sequencing (RNA-seq) allows researchers to compare gene expression levels in infected versus healthy plants, identifying differentially expressed genes (DEGs) that play roles in defense responses. Temporal transcriptomics can track changes in gene expression over time, providing insights into dynamics of the host's response to rust infection [34].

Proteomics: Proteomics analyzes entire set of proteins expressed in a cell, tissue, or organism. Proteomic profiling helps identify proteins involved in rust resistance mechanisms, including defence-related enzymes, signalling molecules, and structural proteins. Post-translational modifications (PTMs) of proteins can be important in regulating their function and interactions during the immune response [35].

Metabolomics: Metabolomics examines complete set of metabolites, small molecules produced during metabolic processes. Metabolomic profiling can identify changes in

metabolic pathways induced by rust infection, highlighting key metabolites that contribute to resistance or susceptibility. Integrating metabolomic data with genomic and transcriptomic information can connect genetic variations and gene expression changes to specific metabolic responses [36].

3.6 Tools for Multi-Omics Data Analysis

Several bioinformatics tools and platforms facilitate integration and analysis of multi-omics data, enabling researchers to uncover complex biological relationships and mechanisms:

Cytoscape: Cytoscape is an open-source software platform for visualizing and analyzing molecular interaction networks. It allows integration of various omics data to construct comprehensive interaction networks, such as protein-protein interaction (PPI) networks and gene regulatory networks. Researchers can use Cytoscape to identify key nodes and hubs in networks, which could represent critical regulatory elements or potential targets for improving rust resistance [37].

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins): STRING is a database and tool that provides information about known and predicted protein-protein interactions. It brings together data from various sources, including experimental data, computational predictions, and public text collections. By using STRING, researchers can explore interaction networks of proteins identified in proteomic studies, showing potential functional interactions and pathways involved in rust resistance [38].

Omics Integrator: Omics Integrator is a tool designed to combine multi-omics datasets and identify essential regulatory elements and pathways. It uses algorithms to combine data from genomics, transcriptomics, proteomics, and metabolomics, providing a unified view of biological processes. This tool helps in identifying causal genes and pathways that contribute to complex traits, such as disease resistance, by utilizing complementary information from different omics layers [39].

4. Benefits of Holistic Approaches in Understanding and Combating Wheat Rust

Adopting holistic approaches through multi-omics data integration offers several benefits in battle against wheat rust [40], [41]:

Comprehensive Understanding of Host-Pathogen Interactions: By combining data from
multiple omics layers, researchers can gain a more complete and nuanced understanding of
interactions between wheat and rust pathogens. This comprehensive view helps in
identifying key regulatory networks and pathways that control resistance and susceptibility.

- Identification of Novel Targets for Breeding and Intervention: Multi-omics approaches can
 identify previously unknown genes, proteins, and metabolites involved in rust resistance.
 These novel targets can be used in breeding programs to develop new resistant varieties or
 in designing targeted interventions to enhance plant's immune response.
- Enhanced Predictive Power: Integrating various omics data improves accuracy and robustness of predictive models. For example, combining genomic and transcriptomic data can improve prediction of resistance traits, while integrating metabolomic data can provide insights into metabolic pathways that support these traits.
- Discovery of Mechanistic Insights: Holistic approaches allow researchers to connect genetic
 variations/ variants and gene expression changes to specific biochemical pathways and
 physiological responses. This mechanistic understanding is crucial for developing strategies
 to manipulate these pathways and improve resistance.
- Data-Driven Decision Making in Breeding Programs: Multi-omics data integration supports
 data-driven decision-making in breeding programs. By providing detailed information on
 genetic and molecular basis of rust resistance, breeders can make more informed selections,
 accelerating development of resistant varieties.

In conclusion, integration of genomics, transcriptomics, proteomics, and metabolomics data through multi-omics approaches provides a powerful framework for understanding and combating wheat rust. These holistic strategies enable the identification of key regulatory elements, enhance predictive models, and support the development of innovative and sustainable solutions for wheat rust management.

5. Case Studies and Applications

5.1 Case Study 1: Enhancing Genomic Prediction in Wheat Breeding through Multimodal Deep Learning

A significant advancement in genomic prediction (GP) of wheat breeding has been achieved through the integration of multimodal deep learning methods. Montesinos-López et al. (2023) explored efficacy of deep learning (DL) neural networks in enhancing accuracy of GP for unobserved phenotypes, taking into account complexities of genotype-environment (GE) interactions. This study stands out as it uniquely combines genomic and phenomic (imaging) data, unlike traditional GP models which often do not incorporate phenomics. The study uses two wheat datasets (DS1 and DS2) to compare a novel DL method with conventional GP models, including Genomic Best Linear Unbiased Prediction (GBLUP), gradient boosting machine (GBM), and support vector regression (SVR). For DS1, which

included both genomic and phenomic data, DL model outperformed other models in terms of GP accuracy over year. However, in other years, GBLUP model exhibited slightly higher accuracy. DS2, comprised solely of genomic data from wheat lines tested over three years across two environments (drought and irrigated) and multiple traits, provided further insights. When predicting performance in irrigated environments using drought environment data, DL model consistently outperformed GBLUP model in accuracy across all traits and years. Conversely, for predictions of drought environments based on irrigated environment data, both models showed similar accuracy levels. The new DL method presented in this study demonstrated a high degree of generalization and adaptability, suggesting its potential for incorporating multiple modules to process diverse data inputs. This capability of multimodal DL methods to improve GP accuracy by integrating complex datasets presents a promising direction for future wheat breeding programs [42].

5.2 Case Study 2: Crop Disease Detection using Machine Learning

In field of agricultural technology, early detection of plant diseases is important for improving crop production efficiency. Abi P Mathew et al. (2023) developed a machine learning-based system for early detection and classification of crop diseases, particularly focusing on leaf-based image classification. This system is important for agricultural sector, where timely and accurate disease diagnosis can significantly enhance yield and reduce losses. The proposed work involved several phases, starting with dataset creation, followed by feature extraction, training classifier, and finally, classification. High-resolution images of crop leaves were used to create robust dataset, capturing various disease symptoms at different stages. Feature extraction techniques were applied to identify and isolate key characteristics of diseases, which were then used to train machine learning classifiers. Various algorithms, such as support vector machines (SVM) and convolutional neural networks (CNN), were employed to classify diseases based on extracted features. In addition to software aspects, study also included design and implementation of hardware components. A microcontroller unit was programmed to interface with sensors that captured data from field. This data was then transmitted to an online database for further analysis and storage. The system demonstrated high accuracy in disease detection and classification, proving its effectiveness as tool for farmers and agricultural professionals. This machine learning-based solution, by allowing for early and precise disease detection, can dramatically improve crop management and output [43].

5.3 Case Study 3: RNA-Seq Profiling for Host-Pathogen Interaction Networks

Advances in next-generation sequencing (NGS) technologies have revolutionized study of host-pathogen interactions, with RNA sequencing (RNA-seq) emerging as powerful tool. Sareshma and Subha (2021) conducted an extensive review of applications of RNA-seq in assessing host-pathogen interaction networks, highlighting its role in understanding the molecular changes during infections. RNA-seq offers a comprehensive approach to transcriptome mapping, providing insights into gene expression profiles under various conditions. This technique surpasses previous methods by offering higher throughput, greater accuracy, and ability to detect new transcripts. Review detailed experimental procedures of RNA-seq, from sample isolation to data analysis, and discussed various NGS platforms, each with its unique advantages and limitations. A significant focus was on dual RNA sequencing technique, which simultaneously profiles transcriptomes of both host and pathogen. This dual approach allows for detailed understanding of interactions and regulatory mechanisms at play during infection. Additionally, review explored other RNAseg techniques such as single-cell RNA sequencing, which provides high-resolution data on gene expression individual cell level, and emerging methods like spatialomics and ribosome profiling, which offer new dimensions of data regarding the spatial organization and translational regulation of gene expression. The study also addressed common challenges in RNA-seq, such as data complexity, variability, and need for modern computational tools for analysis. Strategies to overcome these challenges, including improved experimental designs and advanced bioinformatics approaches, were discussed. By using RNA-seq technologies, researchers can gain a deeper understanding of dynamic host-pathogen interactions, identify critical regulatory genes and pathways, and develop targeted strategies for disease management. This systems biology approach provides a comprehensive framework for studying and combating plant diseases at the molecular level [44].

These case studies collectively demonstrate transformative potential of computational approaches, machine learning, and advanced sequencing technologies in enhancing our understanding and management of wheat rust and other crop diseases. They emphasize importance of integrating diverse data types and employing sophisticated analytical tools to address complex biological challenges in agriculture.

6. Challenges and Future Directions

Challenges in Data Quality and Integration

The integration of heterogeneous datasets from genomics, transcriptomics, proteomics, and metabolomics presents challenges in data quality and standardization. Heterogeneity of data from different/ diverse omics technologies as well as lack of standardized metadata and annotations complicate the integration and analysis of these datasets.

Solutions for Improving Data Quality and Compatibility

To overcome these challenges, several solutions can be implemented:

- Standardization Protocols: Developing and adopting standardized protocols for data collection, processing, and annotation across different omics platforms can significantly enhance data compatibility. International consortia and regulatory bodies can play important role in establishing these standards [45].
- Data Curation and Quality Control: Implementing robust data curation and quality control
 measures can ensure the reliability and accuracy of datasets. This includes use of quality
 metrics, filtering of low-quality data, and validation of results through independent methods
 [45].
- Interoperable Data Formats: Using interoperable data formats and comprehensive metadata standards can help with seamless data integration. Formats such as FASTQ for sequencing data, mzML for mass spectrometry data, and standard nomenclature for gene and protein identifiers should be widely used.
- Integration Platforms: Developing bioinformatics platforms, techniques and tools that are specifically designed for multi-omics data integration can help to streamline process. These platforms should be able to incorporate diverse/ wide range data types and provide comprehensive analytical capabilities.

Computational Limitations

Current computational methods and tools face limitations in scalability, algorithmic complexity, and interpretability when analyzing large-scale biological data and modelling complex systems. To address these challenges, advancements in computational capacity and algorithms, such as high-performance computing, advanced algorithms, and cloud computing, are being explored.

Future Research Directions

As field of computational biology evolves, several emerging technologies and collaborative approaches show promise for advancing wheat rust management. These include

CRISPR/Cas9 genome editing for modifying plant genomes to develop new wheat varieties with increased resistance, and synthetic biology approaches to construct new and novel resistance mechanisms in wheat. Integrating computational models with precision agriculture practices can improve disease management strategies, including early warning systems, variable rate fungicide applications, and real-time monitoring of crop health through remote sensing technologies. Computational predictions and models should be confirmed by extensive field trials in collaborative efforts between computational biologists and agronomists.

7. Conclusion

In conclusion, this review highlight critical role of computational approaches in addressing wheat rust, a chronic challenge to global food security. Wheat rust diseases, including stem rust, leaf rust, and stripe rust, impose significant economic consequences and require appropriate effective management strategies. Traditional methods such as breeding and chemical treatments have limitations, prompting adoption of computational tools and methodologies. Computational approaches provide distinct advantages over conventional methods by using bioinformatics tools and databases like Ensembl Plants and NCBI for genomic and proteomic insights. Sequence alignment techniques (e.g., BLAST, HMMER), genomic prediction models like marker-assisted selection (MAS) and genomic selection (GS), and systems biology analyses have demonstrated efficacy in understanding hostpathogen interactions and identifying resistance mechanisms. Case studies include successful implementations of genomic prediction in wheat breeding, machine learning for disease detection, and systems biology for pathway analysis. These studies demonstrate transformative potential of computational methods in improving agricultural productivity and resilience. However, challenges remain, including data heterogeneity and computational limitations that require continuing attention. Future research directions should focus on improving data quality, advancing computational capacities, and integrating emerging technologies like CRISPR and synthetic biology into wheat rust management strategies.

In conclusion, integration of computational approaches with traditional agricultural practices holds promise for mitigating wheat rust's impact on global food security. Continued interdisciplinary collaboration and innovation are essential to harnessing the full potential of these technologies in safeguarding crop yields and ensuring sustainable agricultural development.

8. Reference

- [1] M. S. Hovmøller, S. Walter, and A. F. Justesen, "Escalating threat of wheat rusts," *Science* (1979), vol. 329, no. 5990, p. 369, Jul. 2010, doi: 10.1126/SCIENCE.1194925/ASSET/C55B12B2-C598-4EF4-AADF-5BDCE4E5DAC2/ASSETS/GRAPHIC/329 369 F1.GIF.
- [2] B. Petre and S. Duplessis, "A decade after the first Pucciniales genomes: A bibliometric snapshot of (post) genomics studies in three model rust fungi," *Front Microbiol*, vol. 13, p. 989580, Sep. 2022, doi: 10.3389/FMICB.2022.989580/BIBTEX.
- [3] H. Dubey *et al.*, "Identification and characterization of Dicer-like genes in leaf rust pathogen (Puccinia triticina) of wheat," *Funct Integr Genomics*, vol. 20, no. 5, pp. 711–721, Sep. 2020, doi: 10.1007/S10142-020-00745-W/METRICS.
- [4] N. Wu *et al.*, "Puccinia striiformis f. sp. tritici effectors in wheat immune responses," *Front Plant Sci*, vol. 13, p. 1012216, Nov. 2022, doi: 10.3389/FPLS.2022.1012216/BIBTEX.
- [5] W. Chen, C. Wellings, X. Chen, Z. Kang, and T. Liu, "Wheat stripe (yellow) rust caused by Puccinia striiformis f. sp. tritici," *Mol Plant Pathol*, vol. 15, no. 5, pp. 433–446, Jun. 2014, doi: 10.1111/MPP.12116.
- [6] A. Wan and X. Chen, "Virulence Characterization of Puccinia striiformis f. sp. tritici Using a New Set of Yr Single-Gene Line Differentials in the United States in 2010," https://doi.org/10.1094/PDIS-01-14-0071-RE, vol. 98, no. 11, pp. 1534–1542, Oct. 2014, doi: 10.1094/PDIS-01-14-0071-RE.
- [7] S. Periyannan, Ed., "Wheat Rust Diseases," vol. 1659, 2017, doi: 10.1007/978-1-4939-7249-4.
- [8] T. Fetch and B. McCallum, "Increased Virulence of Wheat Rusts and the Threat to Global Crop Production," pp. 249–266, 2014, doi: 10.1007/978-1-4939-1188-2 9.
- [9] J. Mapuranga, N. Zhang, L. Zhang, W. Liu, J. Chang, and W. Yang, "Harnessing genetic resistance to rusts in wheat and integrated rust management methods to develop more durable resistant cultivars," *Front Plant Sci*, vol. 13, p. 951095, Oct. 2022, doi: 10.3389/FPLS.2022.951095/BIBTEX.
- [10] A. Oulas, G. Minadakis, M. Zachariou, K. Sokratous, M. M. Bourdakou, and G. M. Spyrou, "Systems Bioinformatics: increasing precision of computational diagnostics and therapeutics through network-based approaches," *Brief Bioinform*, vol. 20, no. 3, pp. 806–824, May 2019, doi: 10.1093/BIB/BBX151.

- [11] K. G. Liakos, P. Busato, D. Moshou, S. Pearson, and D. Bochtis, "Machine Learning in Agriculture: A Review," *Sensors 2018, Vol. 18, Page 2674*, vol. 18, no. 8, p. 2674, Aug. 2018, doi: 10.3390/S18082674.
- [12] N. Zhang, M. Wang, and N. Wang, "Precision agriculture A worldwide overview," *Comput Electron Agric*, vol. 36, no. 2–3, pp. 113–132, Nov. 2002, doi: 10.1016/S0168-1699(02)00096-0.
- [13] A. Sharma, A. Jain, P. Gupta, and V. Chowdary, "Machine Learning Applications for Precision Agriculture: A Comprehensive Review," *IEEE Access*, vol. 9, pp. 4843–4873, 2021, doi: 10.1109/ACCESS.2020.3048415.
- [14] D. Bolser, D. M. Staines, E. Pritchard, and P. Kersey, "Ensembl plants: Integrating tools for visualizing, mining, and analyzing plant genomics data," *Methods in Molecular Biology*, vol. 1374, pp. 115–140, 2016, doi: 10.1007/978-1-4939-3167-5_6.
- [15] B. Contreras-Moreira et al., "Scripting Analyses of Genomes in Ensembl Plants," Methods in Molecular Biology, vol. 2443, pp. 27–55, 2022, doi: 10.1007/978-1-0716-2067-0 2/FIGURES/2.
- [16] G. Stoesser, M. Griffith, and O. L. Griffith, "NCBI (National Center for Biotechnology Information)," *Dictionary of Bioinformatics and Computational Biology*, Oct. 2004, doi: 10.1002/9780471650126.DOB0477.PUB2.
- [17] K. C. Samal, J. P. Sahoo, L. Behera, and T. Dash, "Understanding the BLAST (Basic Local Alignment Search Tool) Program and a Step-by-step Guide for its use in Life Science Research," *Bhartiya Krishi Anusandhan Patrika*, no. Of, Jun. 2021, doi: 10.18805/BKAP283.
- [18] C. Bystroff, V. Thorsson, and D. Baker, "HMMSTR: a hidden Markov model for local sequence-structure correlations in proteins," *J Mol Biol*, vol. 301, no. 1, pp. 173–190, Aug. 2000, doi: 10.1006/JMBI.2000.3837.
- [19] R. Peyraud, U. Dubiella, A. Barbacci, S. Genin, S. Raffaele, and D. Roby, "Advances on plant–pathogen interactions from molecular toward systems biology perspectives," *The Plant Journal*, vol. 90, no. 4, pp. 720–737, May 2017, doi: 10.1111/TPJ.13429.
- [20] P. A. Rodriguez, M. Rothballer, S. P. Chowdhury, T. Nussbaumer, C. Gutjahr, and P. Falter-Braun, "Systems Biology of Plant-Microbiome Interactions," *Mol Plant*, vol. 12, no. 6, pp. 804–821, Jun. 2019, doi: 10.1016/J.MOLP.2019.05.006.
- [21] F. Huang *et al.*, "Analysis and prediction of protein stability based on interaction network, gene ontology, and KEGG pathway enrichment scores," *Biochimica et Biophysica Acta*

- (BBA) Proteins and Proteomics, vol. 1871, no. 3, p. 140889, May 2023, doi: 10.1016/J.BBAPAP.2023.140889.
- [22] Y. Dong *et al.*, "High mass resolution, spatial metabolite mapping enhances the current plant gene and pathway discovery toolbox," *New Phytologist*, vol. 228, no. 6, pp. 1986–2002, Dec. 2020, doi: 10.1111/NPH.16809.
- [23] Y. Liu and H. V. Westerhoff, "Competitive, multi-objective, and compartmented Flux Balance Analysis for addressing tissue-specific inborn errors of metabolism," *J Inherit Metab Dis*, vol. 46, no. 4, pp. 573–585, Jul. 2023, doi: 10.1002/JIMD.12603.
- [24] D. Miljkovic *et al.*, "Signalling Network Construction for Modelling Plant Defence Response," *PLoS One*, vol. 7, no. 12, p. e51822, Dec. 2012, doi: 10.1371/JOURNAL.PONE.0051822.
- [25] A. Syrowatka *et al.*, "Leveraging artificial intelligence for pandemic preparedness and response: a scoping review to identify key use cases," *npj Digital Medicine 2021 4:1*, vol. 4, no. 1, pp. 1–14, Jun. 2021, doi: 10.1038/s41746-021-00459-8.
- [26] M. I. Ahmed and F. Shefaq, "A Study on Machine Learning and Supervised and Deep Learning Algorithms to Predict the Risk of Patients: Ten Year Coronary Heart Disease," https://services.igi-global.com/resolvedoi/resolve.aspx?doi=10.4018/IJPHIMT.305127, vol. 9, no. 1, pp. 1–12, Jan. 1AD, doi: 10.4018/IJPHIMT.305127.
- [27] K. Singh, S. Kumar, and P. Kaur, "Support vector machine classifier based detection of fungal rust disease in Pea Plant (Pisam sativam)," *International Journal of Information Technology (Singapore)*, vol. 11, no. 3, pp. 485–492, Sep. 2019, doi: 10.1007/S41870-018-0134-Z.
- [28] I. Salotti, F. Bove, and V. Rossi, "Development and Validation of a Mechanistic, Weather-Based Model for Predicting Puccinia graminis f. sp. tritici Infections and Stem Rust Progress in Wheat," Front Plant Sci, vol. 13, p. 897680, May 2022, doi: 10.3389/FPLS.2022.897680/BIBTEX.
- [29] D. Ashourloo, M. R. Mobasheri, and A. Huete, "Evaluating the Effect of Different Wheat Rust Disease Symptoms on Vegetation Indices Using Hyperspectral Measurements," *Remote Sensing 2014, Vol. 6, Pages 5107-5123*, vol. 6, no. 6, pp. 5107–5123, Jun. 2014, doi: 10.3390/RS6065107.
- [30] Q. Pan, M. Gao, P. Wu, J. Yan, and M. A. E. AbdelRahman, "Image Classification of Wheat Rust Based on Ensemble Learning," *Sensors 2022, Vol. 22, Page 6047*, vol. 22, no. 16, p. 6047, Aug. 2022, doi: 10.3390/S22166047.

- [31] K. Wanichthanarak, J. F. Fahrmann, and D. Grapov, "Genomic, proteomic, and metabolomic data integration strategies," *Biomark Insights*, vol. 10, pp. 1–6, Jul. 2015, doi: 10.4137/BMI.S29511/ASSET/IMAGES/LARGE/10.4137 BMI.S29511-FIG1.JPEG.
- [32] J. Eichner, L. Rosenbaum, C. Wrzodek, H. U. Häring, A. Zell, and R. Lehmann, "Integrated enrichment analysis and pathway-centered visualization of metabolomics, proteomics, transcriptomics, and genomics data by using the InCroMAP software," *Journal of Chromatography B*, vol. 966, pp. 77–82, Sep. 2014, doi: 10.1016/J.JCHROMB.2014.04.030.
- [33] Y. Yuan, P. E. Bayer, J. Batley, and D. Edwards, "Current status of structural variation studies in plants," *Plant Biotechnol J*, vol. 19, no. 11, pp. 2153–2163, Nov. 2021, doi: 10.1111/PBI.13646.
- [34] C. Cockrum, K. R. Kaneshiro, A. Rechtsteiner, T. M. Tabuchi, and S. Strome, "A primer for generating and using transcriptome data and gene sets," *Development (Cambridge)*, vol. 147, no. 24, Dec. 2020, doi: 10.1242/DEV.193854/226048.
- [35] M. Tyers and M. Mann, "From genomics to proteomics," *Nature 2003 422:6928*, vol. 422, no. 6928, pp. 193–197, Mar. 2003, doi: 10.1038/nature01510.
- [36] J. Fu *et al.*, "Application of metabolomics for revealing the interventional effects of functional foods on metabolic diseases," *Food Chem*, vol. 367, p. 130697, Jan. 2022, doi: 10.1016/J.FOODCHEM.2021.130697.
- [37] P. Shannon *et al.*, "Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks," *Genome Res*, vol. 13, no. 11, pp. 2498–2504, Nov. 2003, doi: 10.1101/GR.1239303.
- [38] D. Szklarczyk *et al.*, "The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets," *Nucleic Acids Res*, vol. 49, no. D1, pp. D605–D612, Jan. 2021, doi: 10.1093/NAR/GKAA1074.
- [39] N. Tuncbag, S. J. C. Gosline, A. Kedaigle, A. R. Soltis, A. Gitter, and E. Fraenkel, "Network-Based Interpretation of Diverse High-Throughput Datasets through the Omics Integrator Software Package," *PLoS Comput Biol*, vol. 12, no. 4, p. e1004879, Apr. 2016, doi: 10.1371/JOURNAL.PCBI.1004879.
- [40] J. Mapuranga, N. Zhang, L. Zhang, W. Liu, J. Chang, and W. Yang, "Harnessing genetic resistance to rusts in wheat and integrated rust management methods to develop more

- durable resistant cultivars," *Front Plant Sci*, vol. 13, Oct. 2022, doi: 10.3389/FPLS.2022.951095.
- [41] J. Mapuranga *et al.*, "The Underexplored Mechanisms of Wheat Resistance to Leaf Rust.," *Plants (Basel)*, vol. 12, no. 23, Nov. 2023, doi: 10.3390/plants12233996.
- [42] A. Montesinos-López *et al.*, "Deep learning methods improve genomic prediction of wheat breeding," *Front Plant Sci*, vol. 15, p. 1324090, Mar. 2024, doi: 10.3389/FPLS.2024.1324090/BIBTEX.
- [43] A. P. Mathew, Sreehari, P. Viswajith, A. Rahman, and V. Murali, "Crop Disease Detection using Machine Learning," *Journal of Applied Science, Engineering, Technology and Management*, vol. 1, no. 01, pp. 28–32, Jun. 2023, doi: 10.61779/JASETM.V1II.6.
- [44] S. D. Sareshma, B. Subha, S. D. Sareshma, and B. Subha, "Assessing Host-Pathogen Interaction Networks via RNA-Seq Profiling: A Systems Biology Approach," *Applications of RNA-Seq in Biology and Medicine*, Oct. 2021, doi: 10.5772/INTECHOPEN.96706.
- [45] T. Luo, J. Huang, S. S. Kanhere, J. Zhang, and S. K. Das, "Improving IoT data quality in mobile crowd sensing: A cross validation approach," *IEEE Internet Things J*, vol. 6, no. 3, pp. 5651–5664, Jun. 2019, doi: 10.1109/JIOT.2019.2904704.