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Review Article

Next-Generation Strategies for Developing and Commercializing Rust-Resistant Wheat through High-Throughput Phenotyping and Genomic Innovations

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ABSTRACT

Wheat rusts are the most important diseases leading to substantial yield losses. Early and precise detection of wheat rusts for early mitigation and disease control is imperative. This review summarizes the advances in high-throughput phenotyping (HTP) approaches for rust detection. Additionally, various genomic interventions leading to the development of rust resistance in wheat are discussed in detail. High-throughput phenotyping (HTP) approaches enable early, non-destructive, and repeatable detection of wheat diseases. However, they need initial investment, expertise, and computational resources. RGB imaging achieves ~80% accuracy by capturing infected leaf coloration, while hyperspectral and fluorescence imaging can predict rust with over 90% accuracy, 3–8 days before visible symptoms. LiDAR, UAVs, and robotic platforms automate large-scale field phenotyping, and spectral indices (NDVI, PRI), thermal, and chlorophyll sensors detect early physiological changes. AI and machine learning models, including CNNs and SVMs, enhance diagnostic precision and reduce bias, while mobile apps, lateral flow devices, and IoT-based systems facilitate affordable, real-time rust detection and forecasting. Genomic interventions complement phenotyping, with marker-assisted selection (MAS) enabling precise tracing of rust resistance genes, and genomic selection (GS) allowing early multi-trait prediction. QTL mapping and GWAS identify major and minor resistance loci, while introgression from wild relatives and MAS reduce linkage drag and introduce novel alleles. Transgenic approaches, RNA interference (RNAi), and CRISPR/Cas9 gene editing enhance resistance through targeted gene modification, and gene pyramiding combines multiple loci for durable protection. Wheat pan-genome resources further support precise trait targeting, and speed breeding integrated with MAS, GS, or gene editing accelerates rust-resistant line development. Efficient seed system pathways ensure rapid dissemination, adoption, and resilience. The development and commercialization of rust-resistant wheat varieties under harsh climatic conditions are crucial for mitigating yield losses, reducing fungicide use, safeguarding farmer livelihoods, and ensuring sustainable food security.

Keywords: Artificial intelligence, Computer Vision, Machine Learning, Multi-omics, Plant stress, Precision agriculture, Rust resistance, Smart agriculture

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INTRODUCTION

Wheat (*Triticum aestivum* L.) serves as major source of carbohydrates in addition to protein, fibers and minerals and provides food security globally, but its production and yield is threatened by fungal pathogens. Wheat rusts are most important disease among all fungal diseases of wheat (Jabran *et al.*, 2021). The impact on wheat yield losses depends upon the susceptible variety, phonological stage, environmental condition, rust type, and severity.

However, significant yield losses ranged from 10-90% even up to 100% in epidemic situation (Figueroa *et al.*2018). These fungal pathogens changed their race quickly, that is why conventional breeding techniques cannot cope with wheat yielding potential demand. Therefore, novel systematic approaches for rustproofing wheat varieties development are playing vital role. For instance, genomic interventions including marker-assisted selection (MAS), genomic selection (GS), quantitative trait loci (QTL) mapping, genome-wide association studies (GWAS) and introgression of rust resistant genes from wild relatives have been largely used for the study of sources for rust resistance (Jabran *et al.*, 2023). Additionally, transgenic approaches including CRISPR/Cas9-Based gene editing, RNA interference (RNAi), pyramiding of resistance genes, Pan-genome and pangenomic analysis, speed breeding integrated with genomics, and re-sequencing and genomic diversity panels are widely being used for enhancement of rust resistance in wheat (Jabran *et al.*, 2023).

For the development of rust resistant wheat varieties, the combined intention of all these methods is to push and accelerate conventional breeding with accuracy. However, each approach has its own merits and demerits. Rust-resistant wheat dissemination through efficient seed system pipelines is important for food security. Establishment links between breeding, certification, and distribution safeguards quicker acceptance and resilience against rust waves. Hence, the present study seeks to categorize and smear advanced breeding approaches for rust-proofing wheat varieties, certifying their operative commercialization and adoption in Punjab.

Similarly, genomics, high-throughput phenotyping and machine learning are revolutionizing disease detection. The joint aim of all approaches is to beat the boundaries of conventional breeding by purifying accelerating breeding cycles, accuracy and increasing overall competence in the development of rust resistant wheat cultivars. However, each approach has its own merits and demerits. Rust-resistant wheat dissemination through efficient seed system pipelines is important for food security. Establishment links between breeding, certification, and distribution safeguards quicker acceptance and resilience against rust waves. Hence, the present study seeks to categorize and smear advanced breeding approaches for rust-proofing wheat varieties, certifying their operative commercialization and adoption in Punjab. In the following sections, various HPT and genomic interventions are discussed followed by the systematic scheme for development and commercialization of rust resistance and high yielding wheat varieties.

HIGH THROUGHPUT PHENOTYPING (HTP) FOR RUST DISEASE DETECTION

HTP approaches have revolutionized rust phenotyping by doing rapid and scalable disease assessment which is essential for timely resistance screening and breeding in diverse environments. Several HTP approaches are discussed in the following with an overview provided in Figure 1.

Imaging based Technology

Imaging-based HTP offers non-destructive, scalable trait analysis with improved accuracy, though limited by cost and environmental sensitivity. It remains key to precision breeding and faster genetic gain. RGB imaging has proven effective for detecting wheat rust diseases. RGB is a color model that signifies colors as blends of red, green and blue light. It is extensively used in digital imaging as rust has discrete red-brown-yellow hues in RGB space. Mahesh (2023) from the University of Minnesota introduced and developed a deep-learning model to recognize rust diseases from RGB images displaying the capability to discriminate between diverse intensities of stem rust with 86% accuracy. Sood *et al.* (2022) used standard RGB images for rust detection and achieved high accuracy and robustness in rust detection, which significantly lessens scrutiny time and human error.

Regarding HIS (hyperspectral imaging), Rutkoski *et al*., 2016 used hyperspectral data and validated that hyperspectral data could envisage yellow rust severity in wheat 5-8 days before pictorial symptoms seemed with more than 90% accuracy. Reynolds et al*.* (2016) used UAV-based thermal sensors to phenotype thousands of wheat lines in field trials to breed resistant cultivar against rusts. Kathrin et al. (2009) quantified leaf rust severity using fluorescence imaging, attained 92% precision by detecting infections between healthy and infected genotypes, 3-5 days prior to appearance of visual symptoms. Forero *et al.* (2022) shared LiDAR and 3D imaging with fluorescence data to detect infections seven days before visual symptoms and accomplished 89% classification accuracy**.** Rodríguez-Vázquez *et al.* (2025) evaluated disease severity using LiDAR reflectance intensities, mainly when larger datasets are analyzed, demonstrating the technology's utility in precision agriculture for disease monitoring and management. The CIMMYT, Mexico (2002) also focused on integrating LiDAR & 3D imaging technology into HTP systems and phenotyped more than10, 000 wheat genotypes to boost the breeding of wheat varieties against rusts.

Spectroscopy and Sensors

Spectroscopy and sensor-based tools permit non-invasive detection of rust resistance by capturing early physiological vicissitudes. Indices such as PRI and NDVI, along with sensors for heat, drought and chlorophyll status, help distinguish resistant genotypes, permitting faster selection and rapid field screening. Vagelas *et al.* (2025) used accurately recognized infections at early stages, even before visible symptoms appeared, enabling timely involvement by using portable spectroscopy that sensed tan spot disease in response to fungicide in wheat with accuracy**.** Portable spectroscopy offers rapid on-site disease detection, whereas hyperspectral systems provide richer spectral information but require complex calibration and data processing. Research on spectroscopy and sensors in wheat has produced substantial outcomes not only in the detection of physiological changes but also quality and composition analysis in wheat (Zhang *et al.,* 2025).

Automated robotic system

Automated robotic systems and unmanned aerial vehicles (UAVs) are transforming HTP by enabling non-destructive, efficient, and large-scale detection of rust resistance in wheat (Zulkiffal *et al*., 2025). These platforms separately steer greenhouse benches and field plots, capturing high-resolution spatial and temporal data to categorize early disease symptoms and track disease development. UAVs enhance these abilities by using multispectral and thermal cameras to detect stress signals, map disease incidence and assess canopy-level traits which provide rapid aerial imaging over wide-ranging breeding plots. Ground-based data collection is labor-intensive or impractical where aerial phenotyping is particularly advantageous for large-scale field trials. By accelerating the identification and development of resistant wheat genotype, collectively, robotic and UAV platforms expressively improve the throughput, precision and spatial exposure of rust resistance screening. Supporting this, Su *et al*. (2018) verified the fruitful application of an automated robotic system for monitoring yellow rust in winter wheat, providing a consistent, quick and non-destructive method for early disease detection-crucial for timely intervention and operative crop protection.

AI and Machine Learning

For automatic disease revealing, AI and machine learning have been successfully used for phenotyping rust resistance across cultivars as these are based on complex data sets and able to screen large populations speedily and enhanced success rate of breeding program. The employed advanced machine learning algorithms, mainly convolutional neural networks (CNNs), which were able to process compound image features and precisely differentiate between infected and non-infected varieties, thus signifying their potential as consistent tools for early disease detection. For example, Atanasov *et al.* (2025) found more than 95% accuracy from UAV-captured imagery for detecting of wheat stripe rust by using a deep learning-based CNNs program. Likewise, for the detection of early rust under field conditions, Terentev *et al. (*2023) applied support vector machine models. Therefore, AI and ML phenotyping are found to be an effective method as it forecasts with reliability and reduce the systematic error based on visual assessment.

Field based HTP Platforms

By using field based, data quality is adversely affected due to fluctuation in temperature, light, wind and humidity including difficulty in management, scaling, maintaining and manual interventions. Besides, this approach is unable to detect the internal physiological traits due to limited focus on whole canopy level. Even with these restrictions, this approach is useful for large scale phenotypic traits insight precisely. For field-based wheat phenotyping, a study was developed for measuring strip rust incidence by Atanasov *et al.* (2025) in which he used a mobile platform connected with the app (Plantix or PlantVillage). This study provided real-time disease severity evidence effectively. For rapid on-site detection and enabling rust pathogens or their linked protein, field-based lateral flow devices were used. These are low-cost, portable and are designed for comfort of use without necessitating specialized laboratory facilities and training (Wang *et al*., 2025). Different IoT designed for rust detection are used in different potential. Some of these HTP platforms do not directly detect rust but only monitor environmental conditions that may warn of inclination or tendency toward disease development such as CropX (2020).

GENOMIC INTERVENTIONS FOR ENHANCEMENT OF RUST RESISTANCE IN WHEAT

Given the constrained effectiveness of conventional breeding in addressing the swiftly evolving rust pathogen populations, genomic interventions have arisen as robust strategies to expedite and improve rust resistance breeding, presenting an innovative avenue for developing resilient and broad-spectrum rust-resistant wheat cultivars. Figure 2 outlines the step-by-step procedure for genomic intervention in detail.

Marker-Assisted Selection (MAS)

Marker-assisted selection (MAS) has significantly enhanced the precision and speed of breeding for rust resistance in wheat by utilizing molecular markers linked to rust resistance genes. The integration of MAS into conventional breeding pipelines has thus played a central role in developing climate-resilient and disease-resistant wheat cultivars. Among various wheat cultivars, numerous molecular markers were established and confirmed which were closely linked to known rust resistance genes. For instance, Lagudah *et al.* (2006), used functional marker (csLV34) conferring durable adult plant resistance to leaf, stripe, and stem rusts for the gene *Lr34/Yr18/Sr57* in wheat. Godoy *et al.* (2018) and Klymiuk *et al.* (2018) revealed that *Lr46/Yr29/Sr58 and Yr15* genes were linked with *Xwmc44 and Xgwm413* markers, respectively.

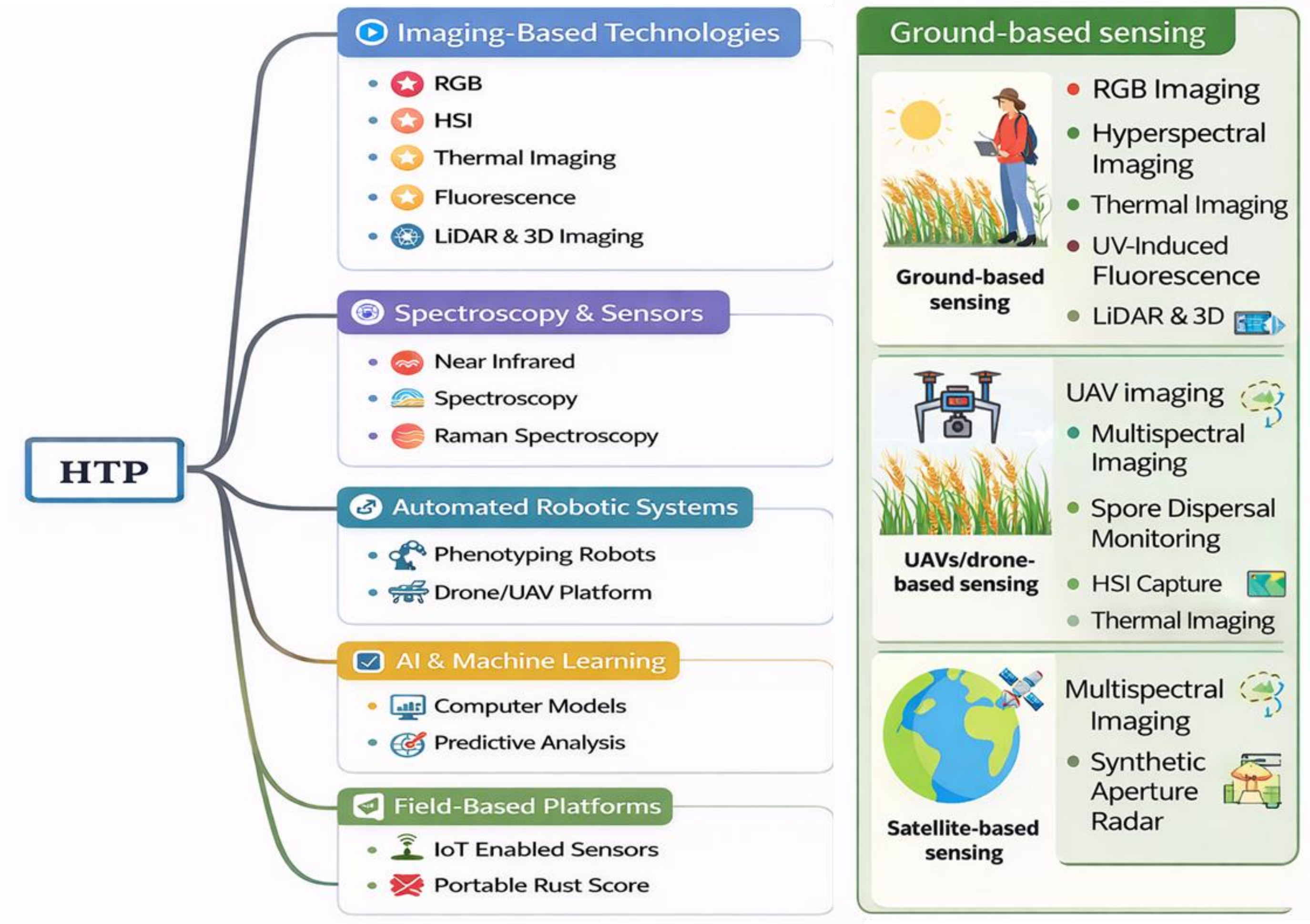

Figure 1. Overview of HTP technologies for the detection, monitoring, and assessment of wheat rust diseases under field conditions. The figure shows integration of various HTP approaches i.e., imaging-based technologies (RGB, hyperspectral, thermal, fluorescence, and LiDAR/3D imaging), spectroscopy and sensor-based approaches (near-infrared and Raman spectroscopy), automated robotic and UAV platforms, and AI- and machine learning–driven analytical models. These components are used across multiple sensing scales, including ground-based, UAV/drone-based, and satellite-based platforms, enabling continuous disease surveillance.

MAS has empowered successful pyramiding of several genes, i.e., *Sr2, Lr24, Yr36,* and *Lr68*, into single genotypes which helped in the release of durable rust resistance wheat varieties. MAS has been used to intercross *Lr34/Yr18* genes into elite cultivars with confirmed field resistance. For instance, MAS has been used to introgress the durable *Lr34/Yr18* gene into elite wheat cultivars, including Pakistani Inqilab-91 derivatives, Indian PBW343 and HD-series lines, Chinese Ning 7840 and Super152 lines, Australian Janz and Avocet lines, CIMMYT germplasm such as Pavon 76, Parula, Attila and Kauz, European winter wheat lines like Renan and Victo, and Argentinian Baguette-series cultivars, resulting in enhanced and field-validated resistance to Yr and Lr rusts (Omar *et al.,* 2021). Recently, MAS has greatly improved the accuracy and speed of breeding rust-resistant wheat, allowing gene pyramiding and the development of durable, climate-resilient cultivars across diverse germplasm (Kumar *et al.*, 2017).

Genomic Selection (GS)

Targeting few major genes is the limitation of MAS which was overcome by GS which is very effective for multiple traits having polygenic inheritance. GS facilitate the breeder to select the desirable plants at seedling stage which reduces the breeding cycle and cost. Moreover, throughout the genome GS exhibit the effect of both major gene (e.g. *Lr43, Sr2*) and minor quantitative resistance loci.

By applying GS for the prediction of multi-rust resistance in CIMMYT's international spring wheat nurseries, Juliana *et al.* (2017) successfully captured additive effects from *Lr46*, *Sr2*, and *Yr18* genes along with minor QTLs. Therefore, GS surpasses MAS by directing both major and minor genes, permitting early selection, reducing breeding time and cost, and effectively predicting multi-rust resistance in wheat.

Quantitative Trait Loci (QTL) Mapping

Using genetic markers in bi-parental populations, either it is recombinant inbred lines or double haploid, QTLs mapping provides a facility to relate phenotypic disparity to precise chromosomal regions. This method is particularly powerful for detecting major resistance genes (*Lr34*, *Sr2*, *Yr18*) or stable adult plant resistance QTLs by allowing the estimation of effect size, heritability and epistatic interactions. For example, Rosewarne *et al.* (2008) used multi-environment QTL analysis to identify durable stripe and leaf rust resistance QTLs such as *QYr.sun-1BL* and *QLr.cim-7DS* in wheat germplasm. Another notable application was by Chenggen *et al.* (2009), who mapped multiple leaf rust resistance QTLs (*QLr.cim-1BL*, *QLr.cim-2BS*) in CIMMYT, Mexico populations. Singh *et al. (*2011) in combination with other QTLs such as *Sr55 (Lr67/Yr46)* and *Sr57 (Lr34/Yr18)*, steadily mapped Sr2 complex on chromosome 3BS across varied populations and deliberated partial resistance to *Puccinia graminis*. QTL mapping leads to the association of phenotypic variation precisely to the exact chromosomal regions, allowing identification of major and durable rust-resistance genes. It provides estimates of effect size, heritability, and gene interactions. This makes it a valuable tool for developing resilient wheat varieties.

Genome-Wide Association Studies (GWAS)

Genome-Wide Association Studies (GWAS) are being used largely for association of genomic regions with stress related traits in crop plants and specially for wheat resistance (Jabran *et al.*, 2023). It comprises mainly on two main steps, disease phenotyping of a large number of wheat germplasm followed by genotyping based on different molecular marker systems. The data from both phenotyping and genotyping is then correlated using different statistical tools to associate the rust resistant phenotypes with specific genomic regions. The main benefit of GWAS is that it delivers high resolution and permits the identification of resistance genes, both major and minor, which are discrete across the genome This opportunity is lacking in traditional QTL mapping as GWAS has positively disclosed novel loci for Lr. Yr, and Sr resistance by connecting high-density SNP markers with phenotypic data which were composed from diverse settings. For example, Singh *et al.* (2013) conducted GWAS on a global panel of spring wheat and identified multiple stripe rust resistance loci on chromosomes 1B, 2A, and 6A, including the well-known *Yr18/Lr34/Sr57* locus. Similarly, Juliana *et al.* (2020) detected over 90 significant marker-trait associations for leaf rust resistance across chromosomes 1B, 2B, and 7D in elite CIMMYT lines. Altogether, GWAS enables high-resolution identification of major and minor rust-resistance genes, uncovering novel loci and enhancing wheat breeding for durable resistance.

Introgression from Wild Relatives

The incorporation of wild relatives is very crucial in wheat breeding program because they enhanced disease and pest resistance, improved stress tolerance, increased genetic diversity, improve better end-use quality and finally contribution to higher yields which is the challenges of new production systems, especially in response to climate change. It has proven to be an effective approach for transferring resistance genes from wild wheat relatives (*Thinopyrum ponticum*, *Aegilops speltoides*, and *Agropyron elongatum*) through wide hybridization followed by backcrossing with the help of MAS to minimize linkage drag and lessening detrimental effects to maintained sustainability. Notable examples include *Lr19, Sr24, Sr26, Lr29* which were introgressed from *Agropyron elongatum, Thinopyrum spp.* and Agropyron *elongatum, respectively.* (Mago *et al.,* 2005). In conclusion, incorporating wild relatives into wheat breeding enhances disease resistance, stress tolerance, genetic diversity, and yield. Through wide hybridization and MAS, resistance genes like *Lr19, Sr24, Sr26,* and *Lr29* can be effectively introgressed while minimizing linkage drag, supporting sustainable crop improvement

Transgenic Approaches

Unlike traditional breeding or introgression from wild relatives, transgenic methods offer a precise and powerful strategy for enhancing rust resistance in wheat by allowing direct transfer of well-characterized genes via transformation into elite cultivars. Several transgenic lines have been developed with enhanced resistance to leaf, stem and stripe rusts by overexpressing either native resistance genes (*Lr34*, *Sr22*, *Sr35*) or pathogen-targeted genes (chitinases, glucanases, and defensins). For example, the Sr35 gene, originally cloned from *Triticum monococcum*, was successfully transferred into bread wheat using Agrobacterium-mediated transformation, conferring complete resistance against Ug99 (Saintenac *et al.,* 2013). For instance, *Sr22*, *Sr33*, *Sr35*, *Sr45*) were cloned and transformed into modern wheat cultivars with the help of binary vectors and stacking multiple cloned R genes to enhance durability and breadth of rust resistance (Dracatos *et al.,* 2023). Although regulatory and biosafety concerns have limited the commercial deployment of transgenic wheat, these approaches have significantly advanced the understanding of host-pathogen interactions and provided valuable germplasm for pre-breeding and functional studies. Ultimately, transgenic approaches allow precise transfer of resistance genes into wheat, enhancing rust resistance and durability, while also advancing understanding of host-pathogen interactions despite regulatory limitations.

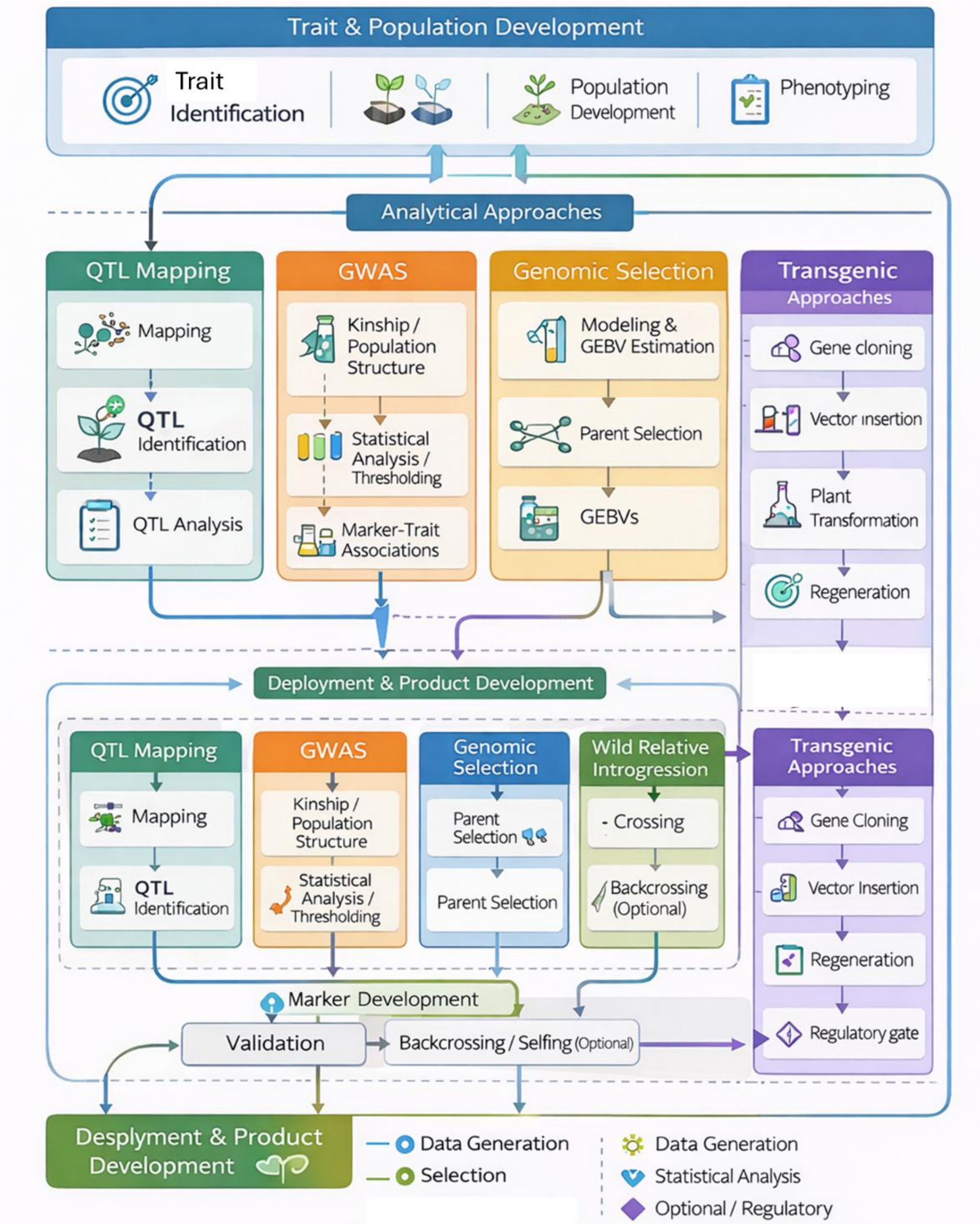

Figure 2. A simplified and integrated framework illustrating how modern molecular and genomic breeding approaches are used for crop improvement. Common early steps like trait identification, population development, phenotyping, and genotyping develop the foundation for multiple analytical strategies, including QTL mapping, genome-wide association studies (GWAS), and genomic selection. These approaches generate complementary outputs such as QTLs, marker–trait associations, and genomic estimated breeding values (GEBVs), which guide parent selection and marker development. Simultaneously, the genes coding for beneficial traits from wild relatives can be introgressed into high yielding genotypes through targeted crossing and backcrossing. Transgenic approaches involving gene cloning, transformation, regeneration, and biosafety evaluation converge at validation and deployment stages, ultimately supporting well-informed selection decisions and efficient cultivar development aimed at rust resistance.

CRISPR/Cas9-Based Gene Editing

CRISPR/Cas9-based gene editing enables represents a breakthrough in wheat improvement, offering unprecedented precision, efficiency and flexibility for modifying genes associated with traits in wheat. It has also ability to target and edit specific genomic loci without introducing foreign DNA. This factor may circumvent some GMO regulatory hurdles that is why direct editing of major *R* genes (*Lr34* or *Sr35*) is still limited also due to polyploidy and gene redundancy. Risk *et al.* (2012). For instance, the TaMLO genes**,** initially associated with powdery mildew susceptibility, serve as a functional model for editing S-genes to block pathogen entry mechanisms. Wang *et al.* (2014) achieved a major milestone by simultaneously editing all three homeoalleles of *TaMLO*, demonstrating that CRISPR is effective even in polyploid crops like hexaploid wheat. Smedley *et al.* (2021) successfully edited multiple wheat genes using a single CRISPR construct, reducing breeding cycles. These efforts, complemented by the availability of the wheat pangenome and rust-responsive transcriptome datasets are accelerating the identification and editing of novel gene targets (*TaNPR1*, *EDS1*, *PAD4)* against leaf, stem, and stripe rusts for broad-spectrum resistance**.** CRISPR/Cas9 is rapidly being adopted in pre-breeding pipelines for precise, next-generation wheat improvement but no commercial rust-resistant wheat variety developed through CRISPR has been released yet, functional validations are underway in pre-breeding pipelines. All in all, CRISPR/Cas9 enables precise, efficient editing of resistance and susceptibility genes in wheat, accelerating pre-breeding for broad-spectrum rust resistance, though no commercial varieties have been released yet.

RNA Interference (RNAi)

It is a powerful post-transcriptional gene silencing tool that has been employed to target both wheat susceptibility genes and essential rust pathogen genes to enhance resistance against leaf, stem and stripe rusts. In RNAi wheat is genetically engineered to express double-stranded RNAs (dsRNAs) that target vital genes in the invading pathogen. For example, Eissa *et al.* (2017) used RNAi to silence the *PtMAPK1* gene in *P. triticina*, which resulted in significantly reduced fungal biomass and disease symptoms. Likewise, Huai *et al.* (2020) applied RNAi to silence wheat susceptibility genes (*TaSTP13)* which led to enhanced resistance against rust. Thus, RNAi contributes in the development of wheat varieties that are durable due to its specificity, flexibility and has capability and can target genes that are problematic to alter with the help of conventional breeding skills. RNAi enhances wheat rust resistance by specifically silencing pathogen and susceptibility genes, enabling durable resistance beyond the reach of conventional breeding.

Pyramiding of Resistance Genes

PRG provide durable and broad-spectrum resistance by stacking multiple rust resistance genes with the help of markers or editing tools. For sustainable rust management gene pyramiding is a vital approach in global wheat breeding programs as the rust pathogens undergo continuous genetic evolution. Pyramiding delivers durable resistance which naturally comprises combinations for their partial and durable APR effects (*Lr34*, *Sr2*, and *Yr18)* in addition to their race-specific genes **(***Sr24*, *Lr19*, or *Yr15*). For instance, Singh *et al.* (2013) stated the effective deployment of multiple APR genes (*Lr34*, *Lr46*, *Sr2*, *Yr29*) through conventional breeding and MAS, resulted in wheat lines with improved resistance to leaf, stem, and stripe rusts across South Asia. For instantaneously tracking of multiple genes, gene pyramiding has also been boosted through marker-assisted backcrossing and genomic selection. A notable example is the development of wheat line by CIMMYT carrying Sr*57 and* Sr2 complex *(Sr2*, *Lr34*, *Yr18*), which shows high resistance to all three rusts under field conditions. Overall, gene pyramiding effectively stacks multiple resistance genes, combining APR and race-specific loci to provide durable, broad-spectrum rust protection, thereby enhancing wheat resilience against evolving pathogens.

Pan-Genome and Pangenomic Analysis

Unlike single-reference genome approaches, the wheat pan-genome explores gene presence/absence variation across wheat genotypes to discover novel R genes including core, dispensable, and accessory genes across multiple cultivars and wild relatives. Zhang *et al.* (2020) identified over 3,500 R genes across the wheat pan-genome, many of which are linked to regions previously associated with leaf rust, stem rust, and stripe rust resistance through GWAS and QTL mapping. Additionally Using the pan-genome, researchers discovered that certain rust resistance genes like *Sr21*, *Yr15*, and *Lr22a* are present only in specific germplasm and would have been missed by single-reference studies. Pan-genomic approaches contribute to the next generation of durable and broad-spectrum rust-resistant wheat varieties by identifying conserved or elite-specific disease resistant alleles that support the arrangement of candidate genes for genome editing or transformation. So, the wheat pan-genome uncovers gene presence/absence variation across diverse genotypes, revealing novel and elite-specific R genes that single-reference genomes miss. This approach enables the discovery and deployment of durable, broad-spectrum rust resistance and guides targeted breeding, genome editing, and transformation efforts.

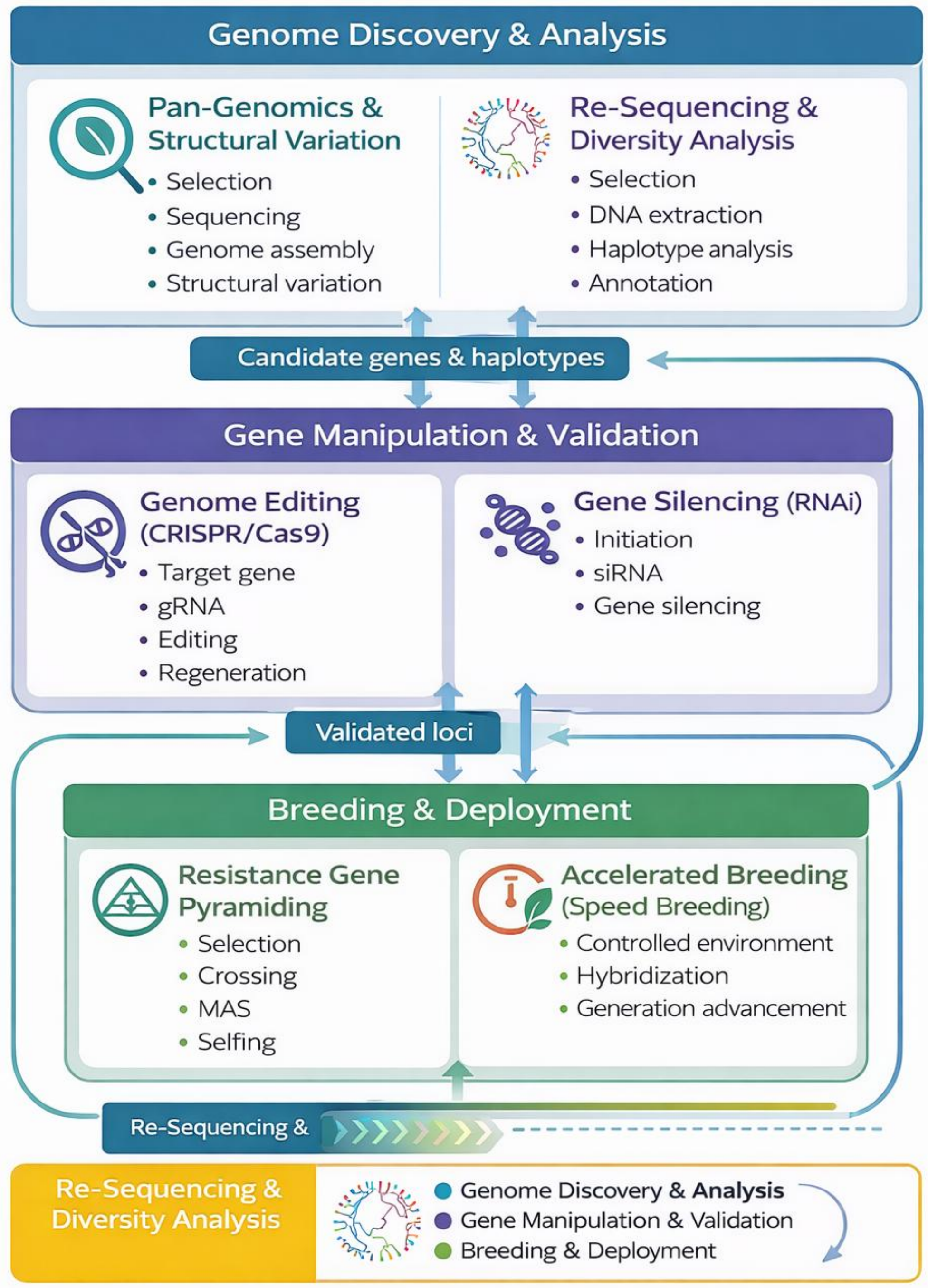

Figure 3. An integrated framework combining genomic discovery, gene functional validation, and advanced breeding strategies for crop improvement. Pan-genomics and re-sequencing approaches enable the identification of candidate genes, haplotypes, and structural variations associated with key agronomic traits. These candidates are functionally validated through genome editing (CRISPR/Cas9) and gene silencing (RNAi) approaches. Validated loci are subsequently incorporated into elite germplasm through resistance gene pyramiding and accelerated deployment using speed breeding under controlled environments. Continuous re-sequencing and diversity analysis provide feedback to refine gene discovery and breeding decisions, creating an iterative pipeline for rapid and durable genetic improvement.

Speed Breeding Integrated with Genomics

Speed breeding shortens generation times and accelerates selection cycles especially when integrated with genomic tools. This approach emerged as a transformation for rapidly developing wheat lines with enhanced rust resistance by involving growing crops under controlled environments, enabling up to 6 generations per year as compared to 2-3 under conventional practice. The rapid fixation and stacking of major rust resistance genes (*Lr34*, *Sr2*, *Yr18*, and *Sr26)* and minor QTLs governing adult plant resistance (APR) can be done by this approach when joint with MAS and GS**.**

Table 1. Merits, demerits and solutions of phenotyping and genomic interventions for rust resistance in wheat.

Items	Merits	Demerits	Solution	Ref
High-throughput phenotyping	• Non-destructive and repeatable • Access multiple pathogens • Speedy, accurate, efficient, scalable • Early and timely disease detection • Provide spatial resolution • Applicable for surveillance • Integrated with genomic selection	• High initial setup cost • Requires expertise • Sensor limitations and data noise • Difficulty in rust types identification • Computational bottlenecks • Phenotype-genotype mismatch • Limited field applicability in smallholder farms	• Cost-sharing models • Capacity building • Multi-sensor trait integration • Cloud computing • HTP-GWAS pipelines • Low-cost mobile apps	Yang *et al.* (2020) Araus *et al.* (2014) Singh *et al.* (2022) Ogidi *et al.* (2023) Yang *et al.* (2020)
Genomic interventions	• Precise gene identification and deployment • Accelerated breeding cycles • Accuracy in disease prediction Precise genome editing • Effective pyramiding of multiple resistance genes for durability • Efficiently integrated with HTP	• High cost of genotyping • Required technical expertise • Resistance breakdown due to pathogen evolution • Environment-specific QTL effects • Complexity of large genomic datasets • Less effective for polygenic traits • Regulatory hurdles for transgenic wheat • Need for field validation • Infrastructure gaps	• Use of low-cost platforms, shared genomic resources • Capacity building Gene pyramiding and adult plant resistance • Multi-environment trials • Use of machine learning • Integrate with GS • Harmonize global policies • Integrated phenotyping approach • Private public partnership	Raffo *et al.*(2023), Gao (2021) Araus *et al.* (2014) Wulff *et al.* (2022), Babu *et al.* (2020).

For example, Hickey *et al.* (2017) developed wheat lines pyramided with *Lr67*, *Sr2*, and *Yr36* which significantly reduced time by speed breeding protocols combined with gene-based markers. Before field testing a breeder can discard susceptible lines by the integration of markers (SNP arrays or KASP) which additionally allows early-generation selection for rust resistance. Furthermore, speed breeding accelerates the validation of edited rust resistance genes (*TaMLO*, *NPR1*) and their deployment into elite backgrounds when it is paired with CRISPR/Cas9 gene editing or host-induced gene silencing (HIGS). Speed breeding platforms have also been applied in pre-breeding pipelines to incorporate wild-relative resistance genes (*Sr22*, *Lr19*, and *Yr15)* with the aid of successive backcrosses. By summing up, speed breeding accelerates improvement of wheat genotypes by enabling rapid stacking and validation of rust resistance genes, and, when combined with genomic tools or gene editing, shortens breeding cycles for durable, resistant lines.

Re-sequencing and Genomic Diversity Panels

Re-sequencing provides nucleotide-level information across the entire genome by capturing single nucleotide polymorphisms (SNPs), insertions/deletions (indels), copy number variations (CNVs) and presence/absence variations (PAVs) which are not captured by traditional genotyping methods that rely on pre-defined markers. For identification and characterization of rust resistance genes in wheat, whole-genome re-sequencing and the use of genomic diversity panels have become powerful tools because these enabled high-resolution analysis of sequence dissimilarity, haplotypes and allelic diversity across a wide range of germplasm. The major advantage of re-sequencing is its direct comparison between resistant and susceptible lines at the nucleotide level which helps to disclose novel resistance loci, gene variants, and structural rearrangements that underlie quantitative and race-specific resistance to Lr, Sr, and Yr**.** For instance, Hao *et al.* (2020) used whole-genome re-sequencing of diverse Chinese wheat accessions to identify regions associated with *Yr9* and *Lr34* resistance. Similarly, Sahin *et al.* (2025) scrutinized a global diversity panel of over 400 wheat accessions and revealed high allelic variation in key APR genes such as *Lr67* and *Sr2*, along with novel haplotypes linked to stripe rust resistance on chromosomes 1B and 6A. These findings provide valuable markers for breeding and also suggest insights into the evolution and distribution of resistance alleles in varied agro-climatic environments. These insights, in turn, support the development of pan-genomic selection models and precision breeding strategies to pyramid diverse and durable rust resistance genes into elite wheat backgrounds. Conclusively, whole-genome re-sequencing enables high-resolution detection of SNPs, indels, CNVs, and PAVs, revealing novel rust resistance loci and allelic diversity. This approach supports precision breeding and the pyramiding of durable Lr, Sr, and Yr resistance genes into elite wheat lines. Table 1 summarizes the merits, demerits, and proposed solutions associated with phenotyping and genomic interventions for rust resistance in wheat.

INTEGRATION OF GENOMIC INTERVENTIONS FOR RUST RESISTANCE IN WHEAT

The advancement of modern crop improvement is increasingly driven by the integration of multiple genomic, molecular, and breeding technologies into a unified framework, instead of utilization of disparate methodologies (Tyagi *et al.*, 2024; Zhang *et al.*, 2022). Advancements in genome sequencing, functional genomics, high-throughput phenotyping, and predictive breeding have resulted in a paradigm shift in the way traits are discovered, validated, and incorporated in elite cultivars (Charan *et al.*, 2025). When employed in a strategic manner, these approaches facilitate a more comprehensive exploration of genetic diversity, a more precise confirmation of gene function, and the ability to make breeding decisions with greater pace and accuracy (Zhang *et al.*, 2022; Charan *et al.*, 2025). This integrated paradigm represents a shift from a linear process to a dynamic, feedback-driven system in which discovery, validation, selection, and field performance continuously inform one another, ultimately accelerating genetic gain and improving breeding efficiency (Tyagi *et al.*, 2024).

As illustrated in Figures 2 and 3, a holistic crop improvement ecosystem is depicted in which genomic discovery, molecular tools, phenotyping and breeding strategies are interconnected, rather than functioning as isolated approaches. The system is predicated on the continuous flow of information between genetic diversity, trait expression and breeding decisions. Genome-level approaches, including pan-genomics, re-sequencing, GWAS, and QTL mapping, collectively capture and interpret natural and induced genetic variation, thereby forming a shared knowledge base for trait discovery (Tyagi *et al.*, 2024). The utilization of functional genomics tools, such as CRISPR/Cas9 and RNAi, does not function as a definitive endpoint. Rather, these tools are employed as validation and refinement instruments, thereby enhancing the reliability of genomic signals that have been identified through population-based analyses (Charan *et al.*, 2025). Similarly, the process of wild relative introgression leads to the broadening of the genetic base, thereby facilitating the availability of novel alleles to the same genomic and phenotypic framework.

The operational engine of this system is constituted by breeding strategies such as genomic selection, marker-assisted selection, gene pyramiding, and speed breeding aimed at the development of rust resistance genotypes (Tyagi *et al.*, 2024). Transgenic approaches, while following a regulated pathway, remain part of the same continuum by contributing functionally proven genes that ultimately enter the shared selection and evaluation pipeline. It is imperative to note that phenotyping, evaluation, and field performance serve to close the loop by generating feedback that continuously informs genomic analyses, model recalibration, and selection strategies (Charan *et al.*, 2025). Rather than following a linear progression, the figures emphasize a dynamic, circular, and data-driven system in which discovery, validation, prediction, and deployment evolve together (Zhang *et al.*, 2022). This integrated framework is indicative of contemporary crop improvement as a unified process designed to deliver faster, more precise, and more durable genetic gains.

PATHWAYS FOR RUST-RESISTANT WHEAT VARIETY DISSEMINATION WITHIN SEED SYSTEM PIPELINES

Genomic innovations speed seed dissemination by rapidly identifying or creating useful alleles, integrating them into elite lines with genomic selection and supporting faster testing and certification. This shortens breeding cycles and accelerates multiplication, allowing improved varieties to reach farmers far more quickly.

Regulatory testing, approval and certification compliance

DUS testing ensures variety distinct**,** uniformity, and stability and is cornerstone and prerequisite for varietal approval and certification. It safeguards both breeders' innovations and farmers' investments. DUS trials are conducted over two consecutive seasons**,** during which candidate wheat varieties are compared against checked registered varieties for trait expression at different growth stages according to standardized states of expression by The Federal Seed Certification and Registration Department (FSC&RD**),** Islamabad, Pakistan. The outcomes of DUS testing inform variety registration, release, and seed certification processes in Pakistan.

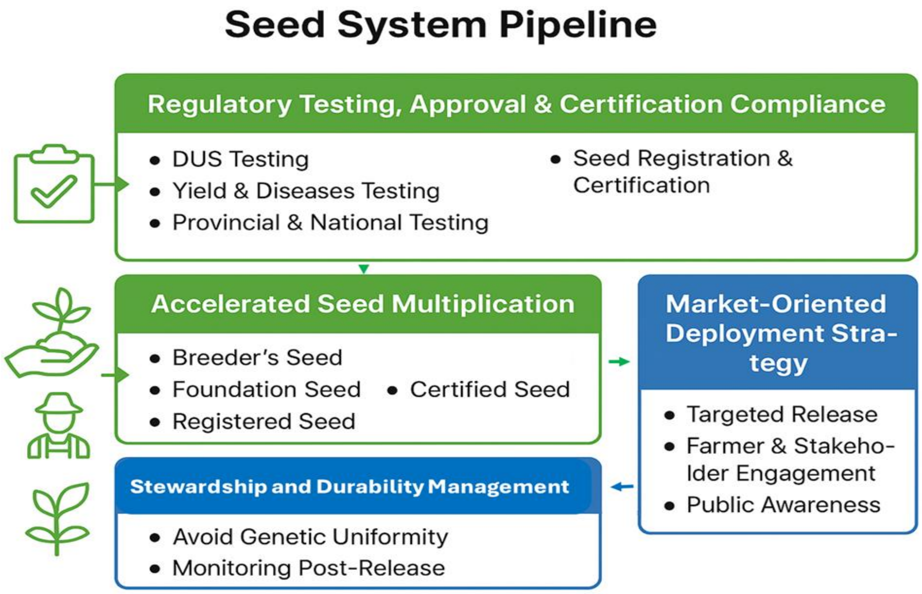

Figure 4. Seed system pathway for dissemination of rust resistant wheat variety.

For yield and disease testing, station yield trials are conducted for one year each as preliminary and regular yield trials in which best performing lines are promoted for one year Punjab uniform wheat yield trials and then out yield lines are further tested in national uniform wheat yield trials for two consistent years. A variety that meets all the requirements is accepted for registration by the FSC&RD. The department submits all its reports to expert sub-committee and all the decisions are made based on consensus of opinion by the experts. The committee evaluates the report and National/Punjab seed council recommends its registration. Notification is issued by Federal Government (Year book 2021-22). The seed certification procedure begins with the submission of an application (form-1), followed by crop inspection (form-2) and preparation of a seed inspection report. Seed sampling is then conducted (form-3), and a lot number is issued. Preliminary seed testing is performed before processing, while final testing is carried out after processing to ensure quality standards. Upon successful evaluation, certification-colored tags are issued as pre-basic (white with purple crossed lines), basic (white), certified (blue) and approved (pink) by FSC&RD.

Safeguarding varietal purity

Maintaining the genetic purity of wheat is vital to ensure varietal stable performance, identity and seed certification compliance. Seed multiplication should be carried out under strict agronomic and isolation protocols to prevent cross-pollination. During harvesting and post-harvest operations, threshers, harvesters, and storage containers must be thoroughly cleaned to prevent mechanical mixtures, and seed lots should always be handled separately. Processing and storage also require careful labeling, sealing, and environmental control to preserve varietal identity.

Market oriented deployment strategy

The release is often targeted to specific regions or mega-environments where the variety's specific traits (high yield potential and stability, climate-resilient, short-duration and zone-specific with improved quality and bio-fortified seed) will provide a clear benefit to farmers. Strengthen wheat seed multiplication and distribution networks to safeguard farmer access to improved varieties and enforce seed certification laws to ensure the availability of high-quality wheat seeds. In this case, public-private partnerships comprise alliance between government agencies and national, multinational companies, industries, scientists and experts that provide gains to all parties should be encouraged. For effective communication and capacity building farmers, scientists, extension workers and all other stakeholders, farmer field days, demonstration plots, education material, national and international seminars will also be organized. Continue to monitor the performance of the deployed variety. If virulence emerges, this early warning system allows breeders to go back to their pipeline and deploy the next solution. Figure 4 provides an overview of whole seed system pipeline for the development and commercialization of high yielding and rust resistant cultivars.

CONCLUSION AND WAY FORWARD

To accelerate the development of disease resistant wheat varieties under harsh climatic conditions, the future lies in an integrated approach that combines advanced HTP and genomic techniques with traditional breeding methods on sustainable basis. Challenges include high costs, technical and computational demands, field variability, polyploidy-related genomic complexity, regulatory hurdles, and slow or uneven seed delivery, all limiting the impact of innovations. Under core priorities and actionable recommendation, understanding pathogen mechanisms, identifying resistance sources, combining defense mechanisms, surveillance and early warning systems are vital. Similarly, for operative commercialization and deployment of disease-resistant wheat varieties, it is indispensable to develop market-oriented varieties, reinforce the seed sector, safeguard rationalized regulatory support pathways and foster public-private partnerships, coupled with dynamic farmer involvement and training to support extensive varietal acceptance to accomplish widespread, well-timed and equitable seed dissemination.

AUTHOR CONTRIBUTIONS

MZ, AA and JA conceived the concept and prepared the initial draft of the manuscript. SM, SA, FH, MIK, MUF, MN and MA performed the literature review and contributed to the manuscript’s refinement and editing.

CONFLICTS OF INTEREST

The authors affirm that there are no competing interests to disclose.

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