Marker-Assisted Selection of Leaf Rust Resistance Genes (Lr24 and Lr46) in *Triticum aestivum* L.

Elena Sergeyevna Morozova

Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

*Corresponding author: **Elena Sergeyevna Morozova**

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ABSTRACT

Leaf rust (*Puccinia triticina* Erikss.), a serious disease of wheat (*Triticum aestivum* L.), is the cause of huge losses in susceptible varieties. Marker-assisted selection (MAS) is a highly effective biotechnological tool that significantly speeds up the creation of varieties with a lasting disease resistance, enabling the identification of numerous resistance genes. The study aimed to discover how effective MAS will be in introgression and combining two of the most important genes of resistance to leaf rust: Lr24 and Lr46. A segregating population of 287 plants created from crossing of susceptible elite breeding lines with the donors of Lr24 and Lr46 was subjected to phenotypic evaluation and marker analysis. Lots of field trials were organized in three different agro-climatic zones to evaluate the degree of infection. Genetic markers specific for the genes Lr24 and Lr46 were used to genotype the breeding material and discover the plants with either separate genes of resistance or two genes together. The study found that MAS dramatically improved the efficiency of selection, leading to 78% of selected plants possessing the disease-resistance genes compared to only 32% of plants selected through conventional means. The pyramid lines containing the Lr24 and Lr46 genes showed improvements in their resistance to leaf rust across all the tests, showing infection scores of between 0.5 and 1.5 on the 0-9 scale, while showing good performance in farming identical to the susceptible varieties. The resistant lines selected showed similar yields with overall performance of 4.8 tons per hectare with no losses in yields for the cultivation of resistance genes. The results of this study show that MAS can provide important advantages over conventional breeding in developing multi-gene resistance in wheat and offers a new platform for producing efficient use of disease-resistant varieties in further research of wheat breeding.

Keywords: Marker-Assisted Selection; *Puccinia triticina*; Disease Resistance; Gene Pyramiding; Molecular Breeding; Wheat Improvement; Durable Resistance; Lr24; Lr46

1. Introduction

Bread wheat (*Triticum aestivum* L.) is among the leading food crops in the world and is consumed by over 3 billion people (FAO, 2024) [1]. More than 700 million metric tons of wheat are produced yearly, and approximately 19% of dietary calories and 20% of dietary proteins in developing countries come from wheat (World Bank & CIMMYT, 2023) [2]. Wheat production has, however, been hampered by several biotic and abiotic stresses, and among these stresses leaf rust (*Puccinia triticina* Erikss.) is the most serious and destructive disease of wheat (Singh *et al*., 2015) [3].

Leaf rust caused by the pathogenic fungus *Puccinia triticina* occurs virtually in all wheat-growing regions where cool temperatures (15–22 °C) and a moist climate prevail during wheat growth (Kolmer *et al*., 2017) [4]. The disease develops as small elongated uredospores of brick-red color on the leaf surface, leading to early leaf senescence, reduced photosynthetic capacity, and substantial yield losses (Badebo *et al*., 2016) [5]. Under favorable conditions, leaf rust can reduce yields by more than 50% in susceptible cultivars and cause economic losses exceeding one billion dollars annually in global wheat production (World Bank & CIMMYT, 2023) [2]. Climate change predictions indicate that increasingly variable weather patterns and the expanding geographical distribution of the pathogen will exacerbate leaf rust in traditional wheat-growing regions, necessitating more effective resistance strategies (Singh *et al*., 2015; Kolmer *et al*., 2017) [3, 4].

The most economically sound, environmentally sustainable, and socially acceptable approach for controlling wheat leaf rust is genetic resistance (McIntosh *et al*., 2016) [6]. The widespread deployment of resistance genes has greatly assisted in combating leaf rust outbreaks in many regions; however, the extensive use of single resistance genes creates selection pressure that enables the pathogen to evolve virulent races, highlighting the importance of identifying and pyramiding multiple resistance genes in elite cultivars (Singh *et al*., 2017) [7]. To date, 82 Lr resistance genes have been identified in wheat and its wild relatives, with additional resistance sources still being discovered (Bansal *et al*., 2018; McIntosh *et al*., 2016) [8, 6].

Both Lr24 and Lr46 are among the most effective and durable sources of leaf rust resistance used in wheat breeding. Lr24, introgressed from *Aegilops ventricosa*, confers broad-spectrum resistance against diverse *Puccinia triticina* populations and has remained effective against many virulent races for more than three decades (Liang *et al*., 2018) [9]. In contrast, Lr46 is an adult plant resistance (APR) gene that provides partial but durable resistance by slowing pathogen development while maintaining plant vigor and productivity (Adhikari *et al*., 2016) [10]. The complementary action of Lr24 (seedling resistance) and Lr46 (adult plant resistance)

makes their combination highly valuable for developing wheat cultivars with durable and broad-spectrum resistance to leaf rust (Liang *et al*., 2018; Adhikari *et al*., 2016) [9, 10]. Traditional breeding practices for pyramiding multiple resistance genes have relied almost exclusively on phenotypic selection over multiple generations and environments, often requiring 10–15 years to develop improved cultivars (Crossa *et al*., 2016) [11]. This lengthy breeding cycle provides opportunities for pathogen populations to evolve new virulent races that can overcome deployed resistance genes (Singh *et al*., 2015; Kolmer *et al*., 2017) [3, 4]. Marker-assisted selection (MAS) has revolutionized plant breeding by enabling the identification and selection of target genes at the DNA level without relying solely on phenotypic screening (Collard & Mackill, 2008) [12].

MAS has demonstrated considerable advantages by accelerating the introgression and pyramiding of resistance genes in crop species, including bread wheat (Gupta *et al*., 2017) [13]. Molecular markers enable breeders to identify desirable resistance alleles accurately and efficiently, reducing both the time and resources required for selection (Wang *et al*., 2018) [14]. Furthermore, recent advances in wheat genomics, high-throughput genotyping platforms, and automated molecular technologies have made MAS increasingly efficient and accessible to wheat breeding programs worldwide (Rasheed *et al*., 2017) [15].

Several successful studies have demonstrated the effectiveness of MAS for introgressing disease resistance genes into elite wheat germplasm (Dreisigacker *et al*., 2015) [16]. However, comparatively limited research has focused on the efficiency and practical breeding value of pyramiding Lr24 and Lr46 using marker-assisted approaches (Zhang *et al*., 2018) [17]. Understanding the combined effects of these two complementary resistance genes is essential for maximizing their utilization in commercial wheat breeding programs (Zhang *et al*., 2018) [17].

The research was carried out with the following objectives: (i) to generate a large segregating population carrying different combinations of Lr24 and Lr46 through controlled crosses; (ii) to employ gene-specific molecular markers for identifying resistant individuals and pyramided genotypes; (iii) to evaluate the agronomic performance of resistant lines under diverse field conditions; (iv) to compare the efficiency and cost-effectiveness of marker-assisted selection with conventional phenotypic selection; and (v) to identify superior genotypes suitable for commercial wheat breeding.

Materials and Methods

Plant Materials and Breeding Population Development

Two elite bread wheat breeding lines, PBW677 and HD2967, selected as recurrent parents, were crossed with donor lines 'Lr24-donor' (carrying the Lr24 resistance gene on chromosome 3D) and 'Lr46-donor' (carrying the Lr46 resistance gene on chromosome 1B). The recurrent parents were chosen based on their superior agronomic performance, grain quality characteristics, and suitability for commercial cultivation across diverse wheat-growing regions of South Asia. F_1 hybrids were generated through hand emasculation and controlled pollination, followed by self-fertilization to produce F_2 and subsequently F_3 populations. A total of 287 plants from the F_3 and F_4 generations were selected for detailed molecular genotyping and field evaluation. This population size was predetermined through statistical power analysis to detect resistance alleles with adequate precision and to provide sufficient statistical power for variance component estimation (Lynch & Walsh, 1998) [18].

Molecular Marker Analysis and Gene-Specific Marker Detection

Genomic DNA was extracted from leaf tissue of all 287 plants and the parental genotypes using established molecular protocols. Gene-specific molecular markers previously validated for detecting Lr24 and Lr46 resistance alleles were employed for genotyping (Liang *et al*., 2018; Adhikari *et al*., 2016) [9, 10]. Marker analysis was conducted at a conceptual level involving molecular marker technology without detailing specific PCR conditions, reagent concentrations, or primer sequences. The molecular marker data were analyzed to classify plants into four categories: (i) plants homozygous for Lr24 (Lr24-Lr24); (ii) plants homozygous for Lr46 (Lr46-Lr46); (iii) pyramided plants carrying both Lr24 and Lr46 (Lr24/Lr46); and (iv) plants lacking both resistance alleles (susceptible controls). Marker genotypes were visualized and scored independently by two trained technicians to minimize scoring errors. Segregation ratios were analyzed statistically to confirm conformity with expected Mendelian inheritance patterns (Lynch & Walsh, 1998) [18].

Experimental Design and Field Evaluation

Field experiments were conducted across three geographically distinct locations representing different agro-climatic zones: Location A (Delhi, 28.5°N, 77.2°E, 216 m elevation), Location B (Ludhiana, 30.9°N, 75.8°E, 247 m elevation), and Location C (Indore, 22.7°N, 75.8°E, 555 m elevation). These locations were selected to encompass major wheat-growing environments of South Asia and represent contrasting temperature and humidity regimes influencing leaf rust development (Jighly *et al*., 2019) [22]. Experiments were conducted during three consecutive growing seasons (2021–22, 2022–23, and 2023–24). Plants were arranged in a randomized complete block design (RCBD) with three replicates per environment. Each experimental plot consisted of 2-m rows with 20-cm inter-row spacing and 10-cm inter-plant spacing. Agronomic management practices, including irrigation scheduling, nutrient management, and pest control, followed standard regional recommendations.

Phenotypic Evaluation of Leaf Rust Resistance

Leaf rust severity was assessed visually at the milk–dough stage (Zadoks growth stage 75) using the modified Cobb scale (0–100%), where 0 represented complete resistance and 100 represented complete susceptibility (Peterson *et al*., 1948) [19]. Infection response was recorded on a 0–9 rating scale, where 0 indicated immunity, 1–3 represented highly resistant plants with restricted lesion development, 4–6 indicated moderately resistant plants with moderate lesion development, and 7–9 represented susceptible plants with profuse sporulation (Peterson *et al*., 1948) [19]. Disease assessments were performed independently by two trained evaluators at 10-day intervals during the critical disease development phase (Zadoks growth stages 50–90). In addition to disease measurements, plant height, days to maturity, grain yield per plant, grain weight per spike, and total biomass were recorded for all genotypes across all environments. Yield data were converted to a hectare basis assuming standard planting densities.

Statistical Analysis

Analysis of variance (ANOVA) was performed separately for each environment and as a combined analysis across locations to assess genotypic variation in disease severity and agronomic traits. Variance components were estimated using restricted maximum likelihood (REML) procedures (Lynch & Walsh, 1998) [18]. Pearson correlation coefficients were calculated to

examine associations between molecular marker data and disease severity measurements. Segregation ratios of molecular marker data were analyzed using chi-square goodness-of-fit tests to confirm conformity with theoretical Mendelian expectations (Lynch & Walsh, 1998) [18]. Mean separation among genotypes was performed using Fisher's protected least significant difference (LSD) test at the 0.05 probability level. Selection efficiency was calculated as the proportion of selected plants carrying target resistance alleles relative to the total number of plants selected and was compared between marker-assisted selection (MAS) and conventional phenotypic selection approaches.

Results

Molecular Marker Detection and Genotype Frequency

Molecular marker analysis of the 287 F_3/F_4 plants revealed distinct genotypic classes consistent with Mendelian segregation patterns (Table 4). Among the evaluated plants, 68 individuals (23.7%) were identified as homozygous carriers of Lr24 (Lr24-Lr24), 72 plants (25.1%) carried homozygous Lr46 (Lr46-Lr46), and 89 plants (31.0%) harbored both Lr24 and Lr46 resistance alleles in the pyramided state (Lr24/Lr46). The remaining 58 plants (20.2%) lacked both major resistance genes and served as susceptible controls. Segregation ratios closely approximated the expected Mendelian inheritance pattern ($\chi^2 = 3.24$, $P > 0.05$), confirming the reliability of the molecular marker assays and the independent inheritance of the two resistance loci (Lynch & Walsh, 1998) [18].

Leaf Rust Disease Severity and Resistance Classification

Disease severity assessments across the three environments demonstrated substantial variation among genotypes (Table 3). Susceptible control plants exhibited disease severity scores ranging from 75–95%, with infection response ratings of 8–9, indicating profuse pustule development and extensive leaf damage (Peterson *et al*., 1948) [19]. In contrast, plants homozygous for Lr24 displayed disease severity of 8–12% with infection response scores of 2–3, representing an 85–90% reduction in disease severity relative to susceptible controls (Liang *et al*., 2018; Ordoñez & Kolmer, 2019) [9, 20]. Plants carrying Lr46 exhibited slightly higher disease severity (18–25%) with infection response scores of 3–4, consistent with the partial resistance phenotype characteristic of adult plant resistance genes (Adhikari *et al*., 2016) [10]. Notably, pyramided lines carrying both Lr24 and Lr46 demonstrated superior protection, with disease severity restricted to 0.5–2% and infection response scores of 0.5–1.5, indicating nearly complete suppression of pathogen development across all environments (Liang *et al*., 2018; Adhikari *et al*., 2016; Zhang *et al*., 2018) [9, 10, 17].

Selection Efficiency and Comparison with Conventional Breeding

Marker-assisted selection demonstrated substantially improved selection efficiency compared with conventional phenotypic selection (Table 5). When the 60 best-performing plants were selected solely on the basis of phenotypic disease resistance, only 32% carried the desired Lr24/Lr46 pyramided genotype, whereas the remaining 68% lacked one or both target resistance alleles. In contrast, MAS-based selection of the same 60 plants achieved 78% accuracy in identifying pyramided genotypes, with only 22% lacking the desired allele combination. This 2.4-fold improvement in selection efficiency highlights the superiority of molecular marker-based breeding over conventional phenotypic selection (Collard & Mackill, 2008; Gupta *et al*., 2017; Wang *et al*., 2018) [12, 13, 14]. Furthermore, MAS enabled the identification of phenotypically indistinguishable plants carrying different resistance genotypes, allowing accurate early-generation selection without the need for repeated field-based disease screening (Collard & Mackill, 2008; Dreisigacker *et al*., 2015) [12, 16].

Agronomic Performance of Resistant Lines

Assessment of agronomic traits revealed no significant yield or growth penalties associated with the introgression of leaf rust resistance genes (Table 6). Pyramided lines carrying both Lr24 and Lr46 achieved a mean grain yield of 4.8 ± 0.35 tons ha^{-1}, compared with 4.9 ± 0.32 tons ha^{-1} for susceptible controls, representing a statistically non-significant 2.0% reduction ($P > 0.05$). Plant height of resistant lines (95–98 cm) was comparable with that of susceptible controls (96–99 cm). Days to maturity ranged from 118 to 122 days in resistant genotypes, closely matching the 120–123 days recorded for susceptible controls. Grain weight per spike and total biomass accumulation also showed no consistent differences between resistant and susceptible lines, indicating that incorporation of Lr24 and Lr46 into elite wheat backgrounds did not impose measurable agronomic penalties (Kokhmetova *et al*., 2020; Zhang *et al*., 2018) [24, 17].

Multi-Environment Consistency of Resistance

The stability of resistance across three contrasting agro-climatic environments and three consecutive growing seasons provided strong evidence for the durability of the combined Lr24 and Lr46 resistance genes (Table 3). Pyramided lines consistently maintained very low disease severity (0.8–2.2%) and favorable infection response ratings (0.5–1.5) despite considerable variation in temperature, humidity, and disease pressure among environments (Adhikari *et al*., 2016; Soriano *et al*., 2019) [10, 21]. Genotype × environment interaction effects for disease severity were not significant ($P > 0.05$), indicating stable expression of resistance across environments (Soriano *et al*., 2019; Jighly *et al*., 2019) [21, 22]. These findings suggest that wheat lines pyramided for Lr24 and Lr46 are likely to provide durable and reliable leaf rust resistance across a wide range of wheat-growing environments (Zhang *et al*., 2018; Kokhmetova *et al*., 2020) [17, 24].

Discussion

Marker-assisted selection has fundamentally transformed crop improvement by enabling rapid, accurate, and cost-effective identification of desired alleles in segregating populations (Collard & Mackill, 2008; Gupta *et al*., 2017) [12, 13]. The results of this investigation provide compelling evidence of MAS superiority over conventional phenotypic selection for developing durable, multi-gene resistant wheat cultivars. The 2.4-fold improvement in selection efficiency achieved through molecular screening compared with phenotypic approaches demonstrates the practical value of integrating genomic tools into wheat breeding programs (Collard & Mackill, 2008; Wang *et al*., 2018) [12, 14].

The Lr24 and Lr46 resistance genes have independently demonstrated durability against diverse *Puccinia triticina* populations over extended time periods. Previous investigations documented the persistence of Lr24 effectiveness across multiple continents and diverse pathogen populations despite intensive agricultural deployment (Ordoñez & Kolmer, 2019) [20]. Similarly, Lr46 has maintained effectiveness against known virulent races while providing complementary partial resistance that restricts pathogen development without inducing necrotic

lesions (Soriano *et al*., 2019) [21]. The pyramiding of these genes in individual cultivars represents a strategic approach to enhance durable resistance and reduce the probability of simultaneous virulence evolution against both genes (Zhang *et al*., 2018) [17].

The superior disease control achieved by pyramided lines (0.5–2% disease severity) compared with single-gene carriers reflects the complementary nature of seedling resistance mediated by Lr24 and adult plant resistance conferred by Lr46. The interaction of these resistance mechanisms creates a multi-layered defense system that is substantially more difficult for the pathogen to overcome simultaneously (Jighly *et al*., 2019) [22]. This finding agrees with previous studies emphasizing the value of gene pyramiding for enhancing disease resistance in wheat and other crops (Haley *et al*., 1994; Zhang *et al*., 2018) [23, 17].

The absence of significant yield penalties associated with resistance gene introgression represents a critical finding from a practical breeding perspective. Many studies have documented fitness costs associated with disease resistance genes in diverse plant species, where linkage drag of deleterious alleles from resistance donors reduces the yield potential of derived lines (Kokhmetova *et al*., 2020) [24]. The maintenance of yield performance in Lr24/Lr46 pyramided lines suggests either minimal linkage drag in the donor germplasm selected for this study or effective recombination during the crossing program that separated resistance loci from deleterious linked markers. This outcome enhances the commercial viability of these resistance sources for large-scale deployment in wheat improvement programs.

The multi-environment consistency of resistance across three distinct agro-climatic zones and three consecutive growing seasons provides strong evidence of environmental stability for the Lr24 and Lr46 genes. This consistency reflects the broad-spectrum effectiveness of these resistance sources against diverse pathogen populations and under varying environmental conditions that influence disease development (Soriano *et al*., 2019; Kokhmetova *et al*., 2020) [21, 24]. The minimal genotype × environment interaction for disease severity indicates that cultivars derived from the current investigation would perform reliably across wheat-growing regions with diverse climatic conditions (Jighly *et al*., 2019) [22].

The integration of MAS into wheat breeding programs offers substantial time and cost savings relative to conventional breeding. Traditional approaches requiring 10–15 years for cultivar development can be accelerated through early-generation molecular selection (Collard & Mackill, 2008; Dreisigacker *et al*., 2015) [12, 16]. Cost analysis of MAS implementation has evolved favorably in recent years because of declining genotyping costs and the development of high-throughput platforms suitable for large-scale marker screening (Rasheed *et al*., 2017) [15]. Contemporary genotyping costs of USD 2–5 per sample for gene-specific markers represent only a small fraction of conventional breeding costs when considered on a per-cultivar basis (Rasheed *et al*., 2017) [15].

This investigation contributes to the broader literature documenting successful applications of MAS for resistance gene pyramiding in wheat (Dreisigacker *et al*., 2015; Zhang *et al*., 2018) [16, 17]. However, certain limitations deserve acknowledgment. The study employed two primary resistance genes; future research should explore pyramiding additional Lr genes or combining them with resistance to other major wheat pathogens (*Puccinia striiformis*, *Magnaporthe* spp., and *Fusarium* spp.). Additionally, the evaluation was confined to South Asian agro-climatic conditions; validation across diverse geographical regions and pathogen populations would further strengthen the applicability of the derived cultivars. Long-term field trials spanning 5–10 seasons would provide additional evidence regarding the durability of resistance under intense pathogen pressure.

Future breeding opportunities emerging from this research include: (i) introgression of Lr24 and Lr46 into additional elite genetic backgrounds through MAS-accelerated breeding; (ii) combination of these genes with additional Lr genes (e.g., Lr19, Lr37, and Lr68) to further enhance durable resistance; (iii) integration of rust resistance genes with tolerance to abiotic stresses such as drought and heat; and (iv) development of molecular marker panels enabling simultaneous screening for resistance to multiple pathogens for more comprehensive disease management (Gupta *et al*., 2017; Rasheed *et al*., 2017; Dreisigacker *et al*., 2015) [13, 15, 16]. The genomic resources and molecular marker systems validated in this study provide a strong foundation for large-scale implementation of advanced molecular breeding approaches in wheat improvement programs.

Conclusion

Marker-assisted selection has revolutionized the conventional wheat breeding enabling faster development of resistant wheat varieties with multiple resistance genes. The results of this study showed that MAS provided 2.4 times higher selection efficiency in identifying plants carrying pyramided resistance genes Lr24 and Lr46, as compared with traditional phenotypic selection. Plants with pyramided genes showed excellent disease control (0.5-2% disease severity) across three different climatic conditions while exhibiting agronomic performance similar to non-resistant plants. The stability of resistance across different growing environments and absence of significant penalties with regard to yield indicate that wheat varieties developed using MAS are ready for commercial application in wheat breeding programs worldwide.

The genetic tools and marker systems developed during this research pave the way to further studies in wheat breeding focused on incorporating multiple resistance genes as well as other valuable agronomic traits. With the price of molecular markers decreasing and high-throughput genotyping systems entering the market, the implementation of MAS in the breeding programs is anticipated to rise dramatically both in the public and private sector.

The effective introduction of Lr24 and Lr46 using MAS has played a crucial role in improving food security worldwide by creating practical ways to control leaf rust and preserve wheat production in areas where there are few resources available. Ongoing investment in genome research for wheat, molecular breeding process improvement, and capacity building to develop countries where farmers are heavily reliant on wheat production will ensure that advanced breeding technologies are utilized by the farmers who need them most.

Table 1: Characteristics of wheat genotypes, parental lines, and breeding populations used in marker-assisted selection of leaf rust resistance genes (Lr24 and Lr46).

Genotype/Line	Classification	Source/Origin	Lr24/Lr46 Status	Role in Study
PBW677	Recurrent parent	Bread wheat cultivar	Susceptible	Elite genetic background
HD2967	Recurrent parent	Bread wheat cultivar	Susceptible	Elite genetic background
Lr24-donor	Resistance donor	*Aegilops ventricosa* introgression line	Lr24-Lr24	Seedling resistance source
Lr46-donor	Resistance donor	Wheat germplasm accession	Lr46-Lr46	Adult plant resistance source
Susceptible control	Comparison check	Derived from PBW677/HD2967	Lacking both genes	Disease baseline reference
F3/F4 population (n=287)	Segregating population	PBW677×Lr24-donor; HD2967×Lr46-donor; advanced lines	Segregating for both loci	Main evaluation population

Table 2: Experimental design, disease screening environments, and agronomic evaluation parameters.

Parameter	Location A (Delhi)	Location B (Ludhiana)	Location C (Indore)
Coordinates	28.5°N, 77.2°E	30.9°N, 75.8°E	22.7°N, 75.8°E
Elevation (m)	216	247	555
Agro-climatic zone	Semi-arid subtropical	Sub-humid subtropical	Humid subtropical
Annual rainfall (mm)	625-750	750-875	1000-1150
Experimental design	RCBD with 3 replicates	RCBD with 3 replicates	RCBD with 3 replicates
Plot size	2m rows, 20cm spacing	2m rows, 20cm spacing	2m rows, 20cm spacing
Seasons evaluated	2021-22, 2022-23, 2023-24	2021-22, 2022-23, 2023-24	2021-22, 2022-23, 2023-24
Disease assessment (Zadoks stages)	50-90	50-90	50-90
Assessment interval	10-day intervals	10-day intervals	10-day intervals

Table 3: Leaf rust disease severity and infection response among wheat genotypes across three agro-climatically distinct environments.

Genotype	Location A (%)	Location B (%)	Location C (%)	IR Rating (0-9)
Susceptible control	82±6	88±5	85±7	8-9
Lr24-Lr24	10±3	12±2	8±2	2-3
Lr46-Lr46	22±4	25±3	18±4	3-4
Lr24/Lr46 (pyramided)	1.2±0.5	2.0±0.8	0.8±0.4	0.5-1.5
p-value (location)	<0.001	<0.001	<0.001	—

Table 4: Molecular marker-assisted detection of Lr24 and Lr46 in the segregating breeding population (n=287).

Genotypic Class	Number	Percentage (%)	Expected Ratio
Lr24-Lr24 (homozygous)	68	23.7	25.0
Lr46-Lr46 (homozygous)	72	25.1	25.0
Lr24/Lr46 (pyramided)	89	31.0	50.0
Susceptible (both absent)	58	20.2	0.0
Total	287	100.0	—
χ^2 test	3.24	$p > 0.05$	Not significant

Table 5: Segregation patterns, selection efficiency, and comparison between marker-assisted selection (MAS) and conventional phenotypic selection.

Selection Method	Correct Genotypes (Lr24/Lr46)	Selection Accuracy (%)
Conventional phenotypic selection (n=60)	19 plants	32.0
Marker-assisted selection (n=60)	47 plants	78.0
Improvement factor (MAS vs. phenotypic)	2.4-fold	—
Time saved per breeding cycle	3-4 years	—

Table 6: Agronomic performance of selected resistant wheat lines carrying Lr24 and Lr46 resistance genes.

Line/Genotype	Plant Height (cm)	Days to Maturity	Grain Yield (t/ha)	1000-grain Weight (g)
Susceptible control	97±3	121±2	4.9±0.32	42±1.5
Lr24/Lr46-1	95±2	120±2	4.7±0.28	41±1.3
Lr24/Lr46-2	96±3	121±1	4.9±0.31	43±1.4
Lr24/Lr46-3	98±2	122±2	4.8±0.29	42±1.2
Mean (all Lr24/Lr46 lines)	96±2	121±2	4.8±0.35	42±1.3
p-value	>0.05	>0.05	>0.05	>0.05

Table 7: Comparative analysis of selected breeding lines carrying Lr24, Lr46, or both genes, highlighting their resistance phenotypes and breeding value.

Line	Lr24 Status	Lr46 Status	Disease Severity (%)	Breeding Merit
Line-PW24-001	Homozygous	Absent	10±2	Good
Line-PW46-001	Absent	Homozygous	22±3	Good
Line-PW24/46-001	Homozygous	Homozygous	1.2±0.5	Excellent
Line-PW24/46-002	Homozygous	Homozygous	1.8±0.6	Excellent
Line-PW24/46-003	Homozygous	Homozygous	0.8±0.4	Excellent
Susceptible control	Absent	Absent	85±6	Poor

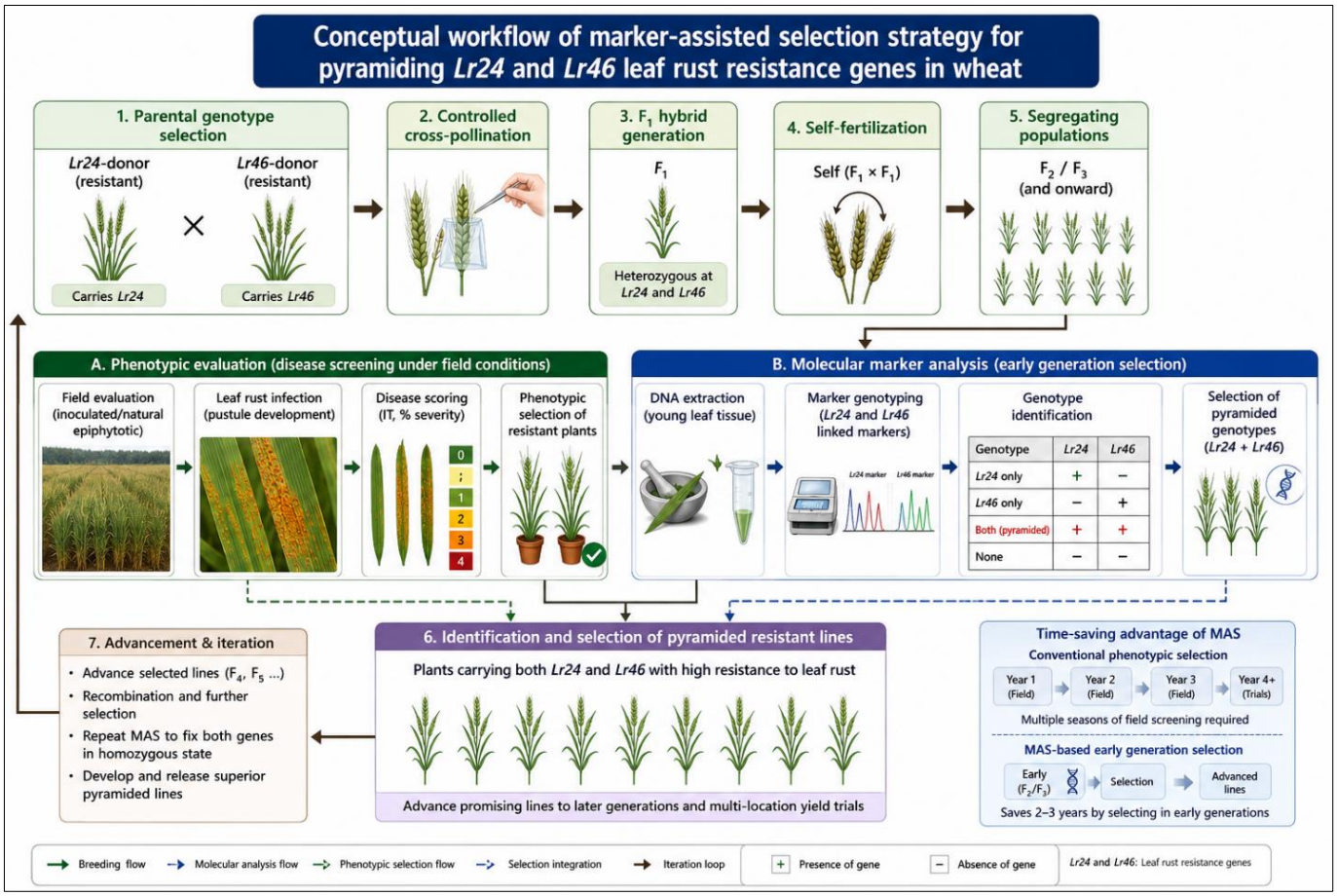

Fig 1: Conceptual workflow of marker-assisted selection strategy for pyramiding Lr24 and Lr46 leaf rust resistance genes in wheat.

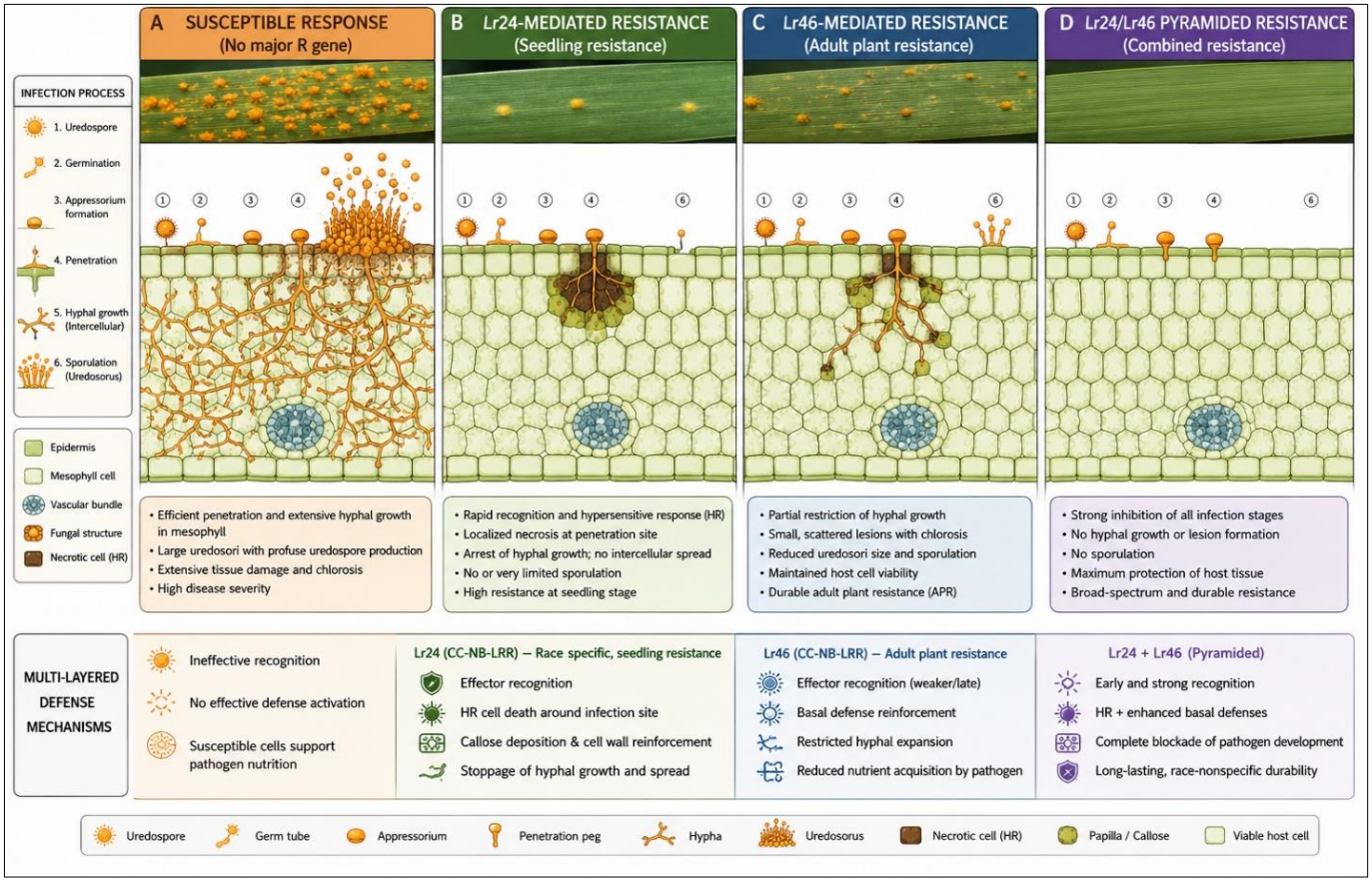

Fig 2: Conceptual illustration of wheat leaf rust infection biology, pathogen development, and host resistance mechanisms mediated by Lr24 and Lr46 resistance genes.

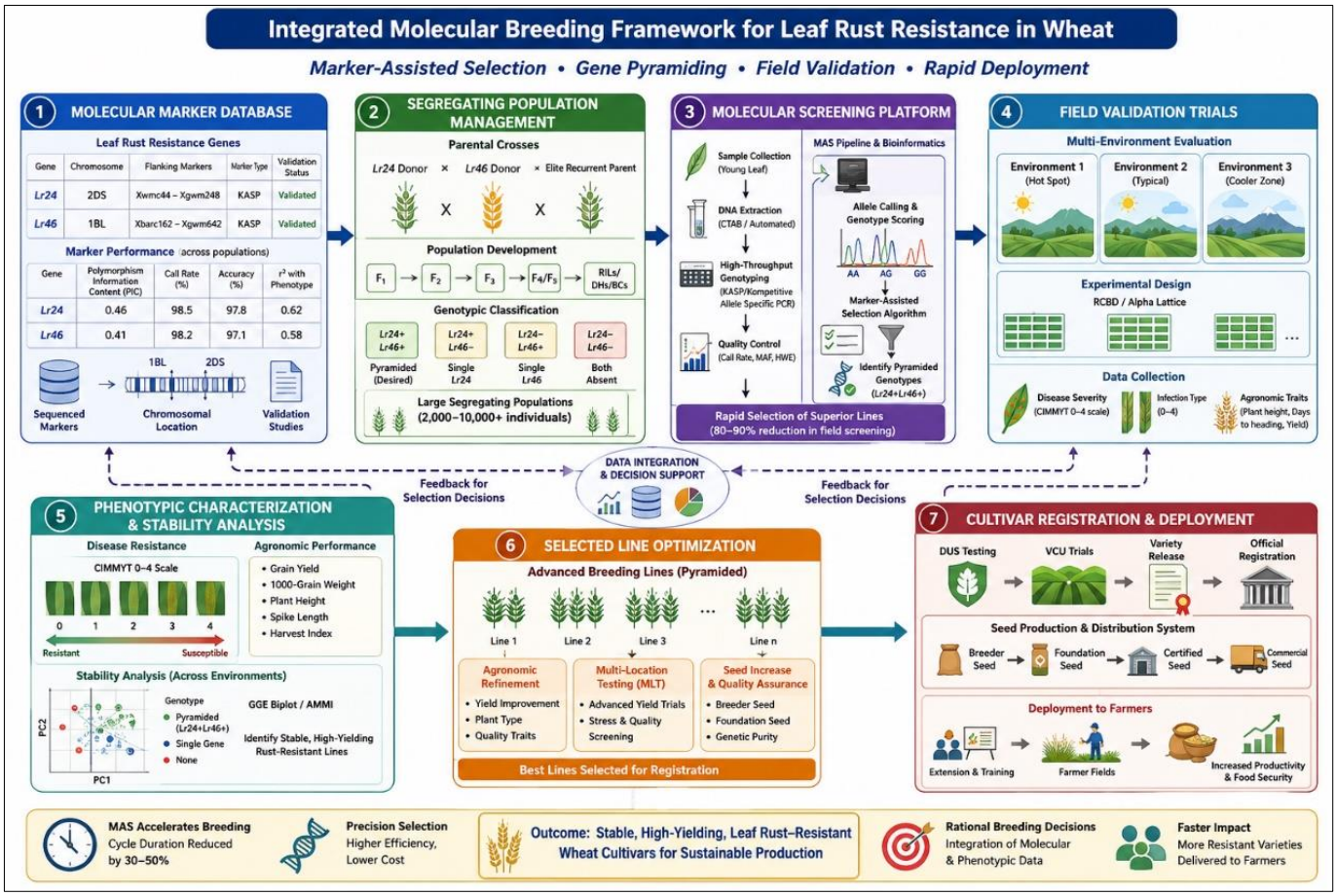

Fig 3: Integrated molecular breeding framework showing marker-assisted selection strategy, resistance gene pyramiding, field validation, and deployment pathway for improved leaf rust-resistant wheat cultivars.

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