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Identification of Candidate Genes for Late Leaf Spot Resistance in Previous QTL Regions for Late Leaf Spot and Rust Resistance in Groundnut

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Abstract:
Late leaf spot (LLS) and rust are major biotic constraints in *Arachis hypogaea*, governed by complex polygenic resistance largely mapped to QTL-rich regions on chromosomes A02 and A03. This review synthesizes recent advancements in the identification, mapping, and deployment of candidate resistance genes within these loci. Resistance is underpinned by key gene families including NBS-LRRs, RLKs, serine/threonine kinases, and PPR proteins, identified through integrative approaches combining QTL-seq, transcriptomics, metabolomics, and high-density SNP genotyping. Functional validation using RNAi, CRISPR/Cas9, and overexpression has confirmed the regulatory roles of genes such as LLSR1, LLSR2, and LR1 in mediating defense via ROS homeostasis, PTI/ETI signaling, and secondary metabolism. The development of diagnostic KASP markers has enabled marker-assisted selection (MAS) and early-generation pyramiding of non-allelic loci, accelerating the release of resistant cultivars like Girnar 4 and GG 39. Cross-species synteny and comparative QTL analysis further reveal conserved defense networks across legumes and cereals, supporting translational breeding strategies. Collectively, this genomics-driven framework underscores a shift toward precision resistance breeding, integrating functional gene discovery with high-throughput marker deployment for durable, broad-spectrum foliar disease resistance in groundnut.

Keywords: Arachis hypogaea, Late leaf spot (LLS), Quantitative trait loci (QTL), Marker assisted selection (MAS), KASP markers, Functional genomics, Disease resistance breeding.

Introduction:

Groundnut (*Arachis hypogaea* L.) is an economically significant oilseed crop, widely cultivated in tropical and subtropical regions, with India ranking second in global production (Pandey *et al.,* 2024). Despite its nutritional and economic value, groundnut cultivation is severely constrained by foliar fungal diseases, notably late leaf spot (LLS) caused by *Nothopassalora personata* and rust caused by *Puccinia arachidis*. These diseases contribute to premature defoliation, reduced photosynthesis, poor pod filling, and yield losses ranging from 50% to 70% under unmanaged field conditions (Varshney *et al.,* 2014; Khedikar *et al.,* 2010).

Chemical control through fungicide applications has been a traditional strategy; however, it is often cost-prohibitive for smallholder farmers and raises environmental concerns (Pandey *et al.,* 2017). As a result, breeding for host-plant resistance has emerged as a sustainable and economically viable approach. The complex inheritance pattern of resistance, largely governed by multiple quantitative trait loci (QTLs), adds challenges to conventional breeding efforts (Chu *et al.,* 2019).

Significant progress has been made in mapping QTLs for both LLS and rust resistance, particularly on chromosomes A02 and A03, which have been consistently identified as hotspots for foliar disease resistance (Kenta *et al.,* 2018; Sujay *et al.,* 2012). These findings have laid the foundation for identifying candidate genes associated with resistance, many of which encode nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins, serine/threonine kinases, and other defense-related proteins (Phat *et al.,* 2021; Gangurde *et al.,* 2019).

Furthermore, advances in next-generation sequencing, RNA-Seq, and transcriptomic profiling have enabled fine-mapping and differential gene expression studies, enhancing the resolution and accuracy of candidate gene identification (Pandey *et al.*2024). For instance, comparative transcriptomics have revealed several differentially expressed genes in resistant versus susceptible genotypes, including those involved in oxidative stress response and pathogenesis-related pathways (Qin *et al.,* 2012; Gangurde *et al.,* 2019).

Modern marker-assisted selection (MAS) and high-throughput genotyping tools such as KASP assays have further streamlined resistance breeding. These tools not only facilitate early generation selection but also enable the pyramiding of multiple resistance loci, offering the potential for durable disease resistance (Pandey *et al.*2024).

This review aims to consolidate recent advances in the identification of candidate genes associated with LLS and rust resistance within previously mapped QTL regions, especially on A02 and A03. Emphasis is placed on genetic mapping, candidate gene discovery, functional characterization, and the deployment of diagnostic markers in breeding pipelines.

1. Genetic Studies – Identification of Candidate Genes and Trait Dissection

The main goal of breeding approaches around the world has been to comprehend the genetic basis of groundnut resistance to foliar disease like rust and late leaf spot. Integrative methods of combining phenotyping, genotyping and functional genomics are necessary due to the complexity of these traits, which are polygenic and modified by environment. (Mondal *et al.,* 2021; Pandey *et al.,* 2024).

1.1 Trait Complexity and Quantitative Inheritance

LLS and rust resistance are quantitative in nature, controlled by multiple genes across different loci. Recent genetic studies continue to affirm the polygenic nature of LLS resistance. Sadgar *et al.* (2023) conducted a classical inheritance study using Phule Unnati (resistant) and SBXI (susceptible) groundnut parents. They observed a 15:1 susceptible-to-resistant ratio in the F2 population, confirming a duplicate recessive gene interaction. These findings offer important insights into the complex inheritance of LLS resistance and support the hypothesis that multiple minor-effect genes may collectively regulate the trait. Such genetic models complement molecular data and provide useful guidance for marker deployment in early segregating populations. Early studies using segregating populations highlighted the significant role of additive QTLs and epistatic interactions in shaping resistance phenotypes (Mondal *et al.* 2021). The detection of such loci has been facilitated by the use of recombinant inbred lines (RILs) and MAGIC populations, which offer high-resolution mapping power and the ability to capture minor-effect loci (Ankush *et al.* 2021).

1.2 Role of Transcriptomics and Functional Genomics

Advancements in transcriptome analysis have been pivotal in identifying differentially expressed genes during pathogen challenge. For example, Gangurde *et al.,* (2019) and Qin *et al.* (2012) performed comparative transcriptomic profiling in resistant and susceptible groundnut genotypes, revealing a suite of defense-related genes including PR-proteins, oxidative stress enzymes, and WRKY transcription factors. These genes were significantly upregulated in resistant lines, confirming their involvement in plant defense mechanisms.

Moreover, Kumar *et al.* (2021) applied metabolomics alongside transcriptomic data, linking resistance to key biochemical pathways such as flavonoid biosynthesis, cutin and wax production, and linoleic acid metabolism. These findings support the hypothesis that resistance is mediated not only through gene expression but also via metabolic reprogramming of host tissues during infection.

1.3 Molecular Mechanisms and Resistance Gene Families

The NBS-LRR gene family, known for its role in pathogen recognition and signal transduction, is among the most studied in groundnut disease resistance. Several candidate genes identified within QTL intervals on chromosomes A02 and A03 encode NBS-LRR motifs, suggesting their direct role in mediating LLS and rust resistance (Phat *et al.,* 2021; Pandey *et al.,* 2024). In addition, serine/threonine protein kinases and receptor-like kinases (RLKs) have been implicated in signal relay processes that trigger localized and systemic immune responses.

Transcriptome-wide expression analyses have also indicated that the reactive oxygen species (ROS) pathway plays a critical role in LLS resistance, with increased ROS-scavenging activity observed in resistant lines (Jennifer *et al.,* 2019). These findings align with previous work in other crops, such as maize and sorghum, where ROS-related genes have also been linked to foliar disease resistance (Hongbo *et al.,* 2021).

Integration into Breeding Pipelines

The identification of robust candidate genes lays the groundwork for marker-assisted breeding strategies. However, the success of trait dissection and integration depends on accurate phenotyping, high-quality genotypic data, and functional validation. With the increasing availability of high-throughput tools and reference genomes, breeders are now able to move from QTL identification to gene-level precision, enhancing selection efficiency in breeding pipelines (Pandey *et al.,* 2024).

2. Genetic Studies – How to Identify Candidate Genes and Decoding the Traits

2.1. Understanding Trait Complexity and Genetic Architecture

The resistance mechanisms against foliar fungal diseases such as late leaf spot (LLS) and rust in groundnut are controlled by polygenic traits involving multiple quantitative trait loci (QTLs). The expression of these traits is also influenced by environmental factors and genotype-by-environment interactions (Mondal *et al.* 2021). Early studies employing recombinant inbred line (RIL) populations revealed significant additive QTLs and epistatic interactions, particularly involving loci on chromosomes A02 and A03, which are repeatedly highlighted as key genomic regions for resistance (Kenta *et al.,* 2018; Sujay *et al.,* 2012).

2.2. Approaches for Candidate Gene Identification

Identifying candidate genes within QTL regions has become more precise with the availability of high-density genetic maps and next-generation sequencing. For instance, Pandey *et al.* (2024) utilized pooled sequencing and KASP marker development to isolate candidate genes like LLSR1, LLSR2, and LR1, specifically located on LLS-A02 and LLS-A03. These candidate genes were associated with disease

resistance and encoded functions such as NBS-LRR proteins, pentatricopeptide repeat (PPR) proteins, and serine/threonine kinases.

Moreover, Ankush *et al.* (2021) applied a MAGIC population to explore LLS resistance, identifying lines with longer incubation and latent periods. Their approach captured a wide allelic variation, further supporting the use of diverse genetic panels in candidate gene discovery.

Furthermore, combining classical genetics with modern molecular tools enables better prediction of gene-trait relationships. For instance, the use of infected row techniques and a 0–9 disease scoring scale, as utilized by Sadgar *et al.* (2023), not only validates resistance phenotypes but also facilitates the strategic development of segregating populations for downstream QTL mapping. Such integrated approaches ensure that phenotypic screening and genotypic analysis progress hand-in-hand during candidate gene discovery.

2.3. Role of Transcriptomics and Comparative Genomics

Transcriptome profiling has been a powerful method to detect differentially expressed genes under disease pressure. Qin *et al.* (2012) and Gangurde *et al.* (2019) reported that several genes involved in defense signaling, oxidative stress, and cell wall reinforcement are significantly upregulated in resistant lines. This includes genes encoding WRKY transcription factors, PR proteins, and enzymes involved in flavonoid biosynthesis.

Furthermore, Kumar *et al.* (2021) conducted a metabolomic analysis in combination with transcriptomics, revealing the enrichment of defense pathways such as cutin and wax biosynthesis, linoleic acid metabolism, and flavone/flavonol biosynthesis in resistant cultivars, thereby linking gene expression to physiological resistance.

2.4. Molecular Mechanisms Involved in Resistance Expression

The molecular mechanisms underlying resistance are often mediated by classical resistance gene families, particularly the NBS-LRR class, which plays a central role in recognizing pathogen effectors and triggering immune responses (Phat *et al.* 2021). Functional categories of resistance genes also include receptor-like kinases (RLKs) and serine/threonine protein phosphatases, indicating layered defense signaling cascades.

In sorghum, Jennifer *et al.* (2019) observed QTLs for Target Leaf Spot resistance associated with ROS production and PAMP-triggered immunity, offering parallels to groundnut's defense responses. Similarly, Hongbo *et al.* (2021) identified QTLs associated with grey leaf spot resistance in maize, further validating that disease resistance mechanisms often converge on conserved molecular pathways across species.

3. QTL Mapping About Both Diseases

3.1. Progress in QTL Mapping for LLS and Rust Resistance

Quantitative trait loci (QTL) mapping has been a cornerstone in dissecting complex traits like late leaf spot (LLS) and rust resistance in groundnut. Over the past decade, several studies have consistently identified major QTLs on chromosomes A02 and A03, reinforcing their significance in breeding for foliar disease resistance.

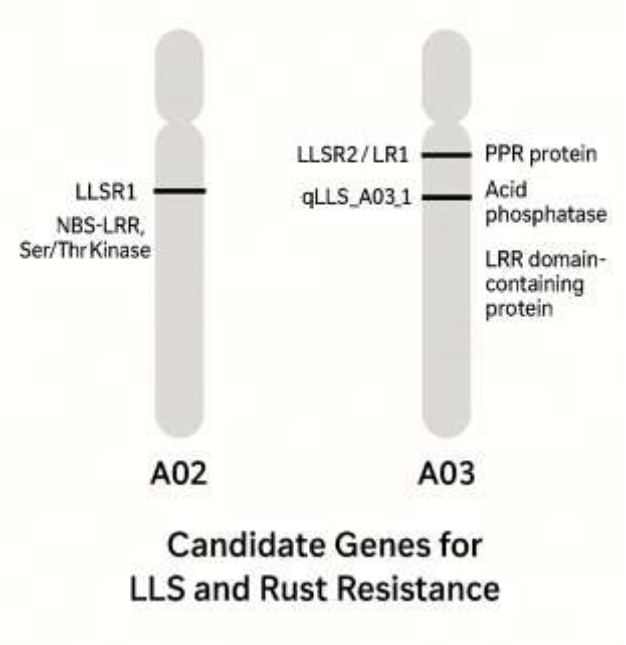

Figure: 1 QTLs and Candidate Genes for LLS and Rust Resistance in Groundnut: Hotspots on A02 and A03.

Khedikar *et al.* (2010) first mapped a major rust resistance QTL on chromosome A03 using a RIL population derived from the cross TAG 24 × GPBD 4. This QTL not only conferred rust resistance but also had a minor effect on LLS. Subsequent refinement by Sujay *et al.* (2012), using additional SSR markers, increased the mapping resolution and confirmed its consistent effect across environments.

Expanding on these findings, Pandey *et al.* (2024) employed QTL-seq and pooled sequencing approaches to identify LLSR1 and LLSR2 on A02 and A03, respectively. Notably, the LLSR2 locus co-localizes with LR1, a gene conferring rust resistance, highlighting the pleiotropic potential of this genomic region. The authors also developed and validated 49 KASP markers, of which 39 were specifically linked to LLS and rust resistance, confirming the functional significance of these loci in multiple genetic backgrounds.

Complementary to these molecular mapping efforts, Sadgar *et al.* (2023) also reinforced the use of classical genetics by showing that the LLS resistance trait in certain crosses follows a digenic inheritance model. Their study supports the notion that conventional inheritance studies remain relevant and can work synergistically with QTL-seq and GBS to validate genomic findings. This dual strategy strengthens the biological confidence in candidate QTLs.

3.2. Fine-Mapping and High-Resolution QTL Discovery

Technological advances have enhanced the precision of QTL mapping. For example, Lu *et al.* (2018) utilized high-density SNP markers to achieve a mapping resolution of 0.7 cM, and further refinement narrowed the LLS resistance locus to 0.38 cM. Such high-resolution mapping is critical for distinguishing tightly linked genes and reducing linkage drag during introgression.

Likewise, Zhao *et al.* (2018), working on spotted wilt virus (SWV) resistance, refined a major QTL on chromosome A01 to a 0.8 Mb interval using amplicon sequencing. Although targeting a different disease, this study illustrates how fine-mapping techniques can sharply delineate genomic regions, thereby accelerating gene cloning and diagnostic marker development.

3.3. Cross-Species and Comparative QTL Insights

Several comparative studies in other crops have revealed similar genetic architectures for foliar disease resistance. For instance, Jennifer *et al.* (2019) mapped QTLs for Target Leaf Spot in sorghum and identified loci controlling reactive oxygen species (ROS) responses and PAMP-triggered immunity, traits also relevant in groundnut. Similarly, Chutintorn *et al.* (2021) identified a major QTL controlling Cercospora leaf spot resistance in mungbean on chromosome 6, containing the candidate gene VrTAF5, involved in transcriptional regulation.

In addition to legumes and cereals, recent mapping in other species like tomato and soybean has revealed similar resistance loci enriched with RLK, PR, and ROS pathway genes, hinting at conserved defense mechanisms. These parallels provide opportunities for candidate gene borrowing across crops through synteny-based approaches.

These insights suggest that core defense pathways are conserved, and groundnut breeding can benefit from functional analogs in related legumes and cereals, especially when genomic resources are limited.

3.4. Consensus QTL Regions and Breeding Implications

The recurring detection of major QTLs on A02 and A03 across studies (Khedikar *et al.,* 2010; Sujay *et al.,* 2012; Pandey *et al.,* 2024) points to the feasibility of using consensus QTL regions for marker-assisted selection (MAS). Varshney *et al.* (2014) demonstrated successful introgression of these QTLs into elite cultivars using marker-assisted backcrossing (MABC), leading to the development of improved lines with enhanced rust resistance and higher pod yields.

Moreover, the integration of diagnostic markers like IPAHM103, GM1536, and KASP assays allows for early-generation selection and gene pyramiding. These strategies enable breeders to combine multiple resistance loci into a single background, offering a more durable and broad-spectrum resistance profile.

4. Management of Diseases

The management of foliar fungal diseases in groundnut, particularly late leaf spot (LLS) and rust, involves an integrated approach combining chemical, cultural, and genetic strategies. However, given the high cost and environmental concerns associated with chemical control, genetic resistance has emerged as the most sustainable and farmer-friendly solution (Varshney *et al.,* 2014; Pandey *et al.,* 2024).

4.1. Chemical Control and Its Limitations

Historically, fungicide applications especially with chlorothalonil and tebuconazole have been the primary method for managing LLS and rust. However, repeated applications increase production costs and pose ecological risks. Moreover, fungicide resistance in pathogen populations is becoming an emerging threat (Clevenger *et al.,* 2018). As a result, chemical control is increasingly seen as a short-term solution best reserved for high-value cultivation or during severe epidemics.

4.2. Cultural Practices

Cultural practices like crop rotation, field sanitation, residue management, and adjusting planting dates can help reduce inoculum pressure. The use of spreader rows has also been adopted in field screening trials to ensure uniform disease pressure for phenotyping (Kumari *et al.,* 2020). However, these approaches alone are insufficient to achieve effective and consistent disease control in large-scale production systems.

4.3. Genetic Resistance – The Most Viable Strategy

Given the limitations of external inputs, the deployment of genetically resistant cultivars has become central to integrated disease management. Resistant cultivars eliminate the need for frequent fungicide applications, reduce input costs, and minimize environmental impact. GPBD 4, for instance, is a well-

known resistant line used as a donor in several breeding programs (Varshney *et al.,* 2014; Sujay *et al.,* 2012). Additionally, newer varieties such as Girnar 4, Girnar 5, and GG 39 have been developed with resistance traits through marker-assisted breeding and have been released for commercial cultivation in India (Pandey *et al.,* 2024).

New insights from Sadgar *et al.* (2023) reinforce the practical value of using highly resistant donor lines such as Phule Unnati in backcross programs. Their findings demonstrated that resistance was transferable across multiple generations without compromising yield, encouraging the use of such donors in elite breeding setups. Incorporating such durable lines ensures that resistance traits remain stable under variable field conditions.

4.4. Durable Resistance Through Gene Pyramiding

To combat the potential breakdown of single-gene resistance, breeders are increasingly employing gene pyramiding strategies. By stacking multiple QTLs or resistance genes, the chances of durable resistance are enhanced. For example, Chu *et al.* (2019) suggested that pyramiding early and late leaf spot resistance loci could stabilize yield performance even under low fungicide regimes.

Moreover, genomic tools like marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS) are being combined with high-throughput genotyping to accelerate the development of multi-disease resistant varieties.

4.5. Future Directions in Disease Management

Advances in functional genomics and genome editing now offer new avenues for disease management. The use of CRISPR/Cas9 for targeted gene editing and RNA interference (RNAi) for silencing susceptibility genes presents exciting opportunities for developing next-generation disease-resistant cultivars (Pandey *et al.,* 2017). Coupled with early generation selection using diagnostic KASP markers, these tools are transforming disease management from field-based screening to precision breeding.

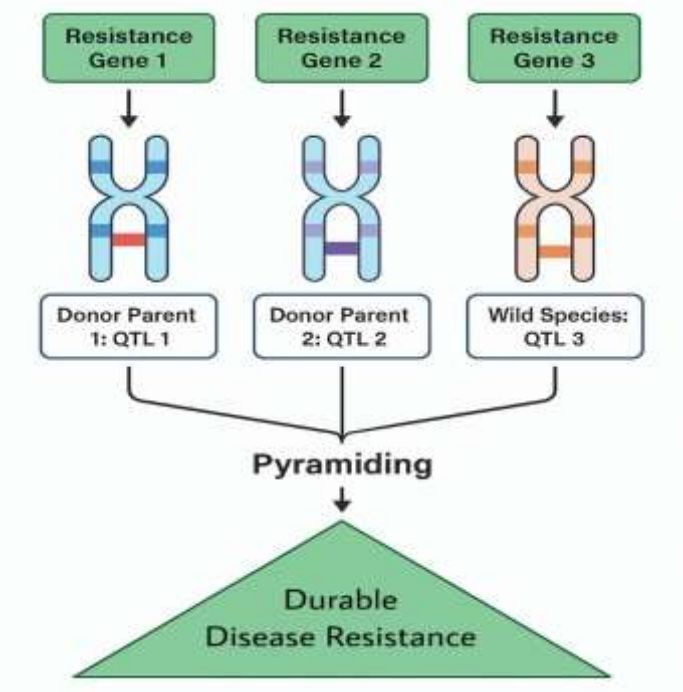

Figure: 2 Pyramiding Non-Allelic Resistance Genes for Durable LLS and Rust Resistance

Additionally, recent studies suggest exploring non-allelic gene pyramiding to delay pathogen evolution. Thakur *et al.* (2008) and Sadgar *et al.* (2023) emphasized the importance of combining resistance genes with different modes of action to achieve pathotype-wide and durable defense. This strategy reduces the selection pressure on pathogens and is a recommended next step for breeding climate-resilient varieties.

5. Marker-Assisted Selection and Genotyping Approaches for LLS and Rust Resistance

The integration of molecular markers into groundnut breeding programs has revolutionized the development of disease-resistant cultivars, especially for complex traits like late leaf spot (LLS) and rust. Marker-assisted selection (MAS) provides a precise and efficient method to introgress resistance loci, greatly reducing the time and cost of breeding compared to traditional methods.

5.1. Evolution of Marker Systems in Groundnut

Early genotyping efforts in groundnut primarily relied on simple sequence repeats (SSRs) and dominant markers such as RAPDs and AFLPs. While effective, these methods had limitations in terms of throughput, reproducibility, and marker density. The advent of high-throughput technologies, especially SNP-based platforms, has significantly improved marker resolution and enabled genome-wide tracking of traits (Pandey *et al.,* 2024; Varshney *et al.,* 2014).

SSR markers such as GM1536, GM2301, and IPAHM103 were among the earliest to be linked with rust resistance QTLs on chromosome A03 (Khedikar *et al.,* 2010). These markers were successfully used in marker-assisted backcrossing (MABC) to introgress resistance into popular but susceptible varieties like TAG 24, JL 24, and ICGV 91114 (Varshney *et al.,* 2014).

5.2. KASP Markers – The Game Changer

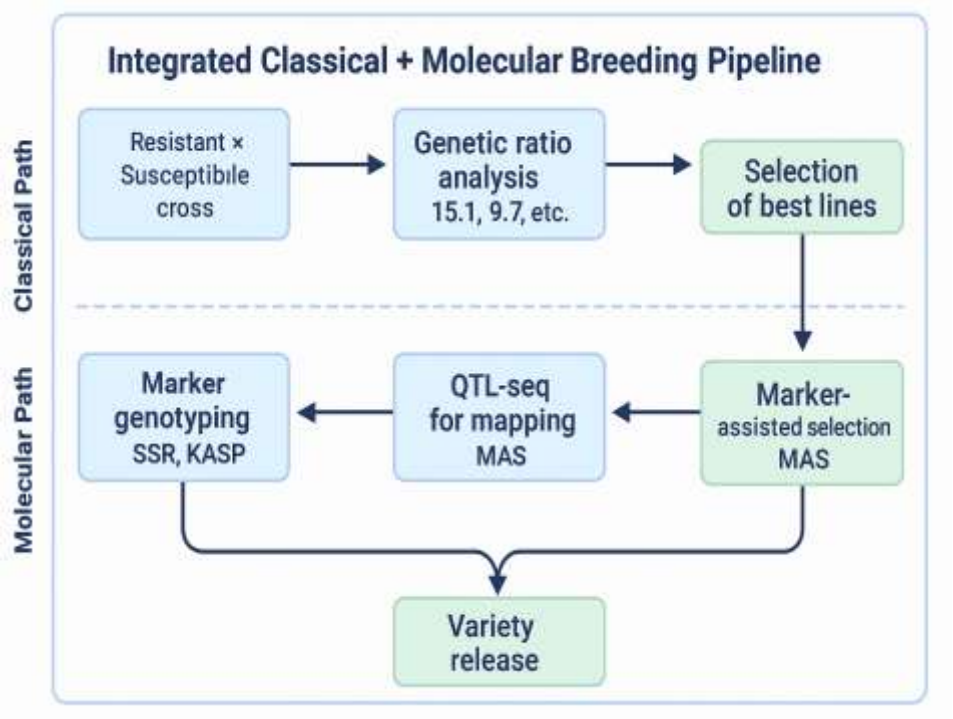

Figure: 3 Integrated Classical and Molecular Breeding Pipeline for LLS and Rust Resistance in Groundnut

One of the most impactful advancements in recent years has been the deployment of Kompetitive Allele-Specific PCR (KASP) markers. These high-throughput, SNP-based markers allow for cost-effective

genotyping and have been validated across diverse germplasm.

Pandey *et al.* (2024) reported the development of 49 KASP markers, 41 of which were specific to LLS and rust resistance. These markers were derived from candidate genes including NBS-LRRs, serine/threonine kinases, and purple acid phosphatases. Of these, a subset has already been deployed in several Indian breeding programs, resulting in the release of improved cultivars such as Improved JL 24 and Super TMV 2, which carry resistance loci derived from GPBD 4. Building upon these advances, Sadgar *et al.* (2023) highlighted the critical importance of early-stage screening using field-based phenotyping. Combining MAS with field-disease scoring during early backcross generations (like BC1 and BC2) helped confirm resistance inheritance patterns and enhanced the accuracy of selection, especially when integrated with diagnostic KASP assays.

5.3. Genotyping-by-Sequencing (GBS) and High-Density SNP Arrays

High-resolution genotyping platforms such as GBS and Axiom_Arachis SNP arrays (~58K SNPs) have enabled fine-mapping of QTLs and identification of candidate genes at the genome level (Pandey *et al.* 2024). These platforms also support genomic selection (GS) and marker-assisted recurrent selection (MARS), which are increasingly being applied in elite × elite crosses to stack favorable alleles across multiple traits. For instance, Lu *et al.* (2018) achieved a mapping resolution as fine as 0.38 cM for LLS resistance by using dense SNP data, significantly enhancing the precision of trait introgression.

5.4. Early Generation Selection and Marker Deployment

The use of marker-assisted early generation selection (MEGS) has become a practical approach in breeding programs. By applying diagnostic markers in F2 or early F3 generations, breeders can discard undesirable genotypes early and focus on lines carrying resistance loci (Pandey *et al.* 2024). This approach significantly saves field space and resources while increasing the genetic gain per cycle. In parallel, low-density SNP panels and functional markers have been developed for routine use, making marker-assisted breeding scalable and accessible to public breeding programs across India and sub-Saharan Africa.

5.5. Towards Genomic Selection (GS)

While MAS focuses on individual loci, genomic selection uses genome-wide marker data to predict breeding values for complex traits. This approach holds promise for accelerating improvement in polygenic traits like LLS and rust resistance. The integration of GS models with field phenotyping data and multi-environment trials is currently underway in groundnut breeding hubs coordinated by ICRISAT and national programs.

6. Candidate Genes (CGs) for LLS and Rust Resistance

6.1. Choice of Candidate Genes

The candidate gene (CG) approach is based on identifying genes with known or predicted roles in a particular biological process—here, plant defense against fungal pathogens. In groundnut, this strategy has been critical for dissecting resistance mechanisms against late leaf spot (LLS) and rust.

Candidate genes can be broadly classified as:

- Functional CGs – identified based on known biological roles (e.g., NBS-LRR genes, PR proteins)
- Positional CGs – located within previously mapped QTL intervals for disease resistance (Pflieger et al., 2001)

Genes involved in signal transduction, oxidative stress management, secondary metabolite production, and cell wall reinforcement have been prioritized. Several of these were identified within resistance QTLs

on chromosomes A02 and A03, reinforcing their functional relevance (Pandey *et al.,* 2024; Gangurde *et al.,* 2019).

6.2. Positioning of Candidate Genes within QTL Regions

Positional cloning has been used to narrow down QTL regions and identify putative resistance genes. Pandey *et al.* (2024) mapped LLSR1 and LLSR2 within the QTL regions LLS-A02 and LLS-A03, respectively. These loci harbored genes encoding:

- NBS-LRR proteins
- Serine/threonine-protein kinases
- Purple acid phosphatases
- PPR (pentatricopeptide repeat) proteins

Notably, LLSR2 and LR1 (a rust resistance gene) were found to co-localize, suggesting a shared regulatory region conferring resistance to both diseases—a critical insight for gene pyramiding efforts.

Table 1. Major candidate genes associated with late leaf spot (LLS) and rust resistance in groundnut, with chromosomal positions, functional roles, and key references.

QTL / Locus	Chromosome	Candidate Gene(s)	Gene Function	Reference(s)
LLSR1	A02	NBS-LRR, Ser/Thr Kinase	Disease resistance, signal transduction	Pandey et al., 2024
LLSR2 / LR1	A03	PPR protein, Acid phosphatase	Defense signaling, oxidative stress response	Pandey et al., 2024
qLLS_A03_1	A03	LRR domain-containing protein	Pathogen recognition	Mondal et al., 2021
-	A03	PR proteins, WRKY TFs	Pathogenesis-related proteins, transcriptional regulation	Qin et al., 2012; Gangurde et al., 2019
qLLS_Lu	-	Unknown gene set	Narrowed LLS QTL region (0.38 cM)	Lu et al., 2018
qGLS8 (Maize)	8 (Maize)	-	Resistance to gray leaf spot (comparative)	Hongbo et al., 2021
qCLS (Mungbean)	6 (Mungbean)	LOC106765332 (VrTAF5)	Transcriptional regulation (TAF protein)	Chutintorn et al., 2021
-	A02	RLKs, ROS-related enzymes	Signal transduction, defense response	Phat et al., 2021
-	A03	Metabolic pathway genes	Resistance-associated biochemical response	Kumar et al., 2021

6.3. Characteristics of Disease Resistance Loci (QTLs)

Resistance-associated QTLs often harbor genes related to pattern recognition receptors (PRRs), transcription factors (e.g., WRKY, MYB), and defense-related enzymes. These genes are responsible for initiating and amplifying defense responses upon pathogen recognition.

For instance,

- Mondal *et al.* (2021) identified LRR domain-containing genes within QTL intervals, indicating classic R-gene signatures.
- Qin *et al.,* (2012) and Gangurde *et al.* (2019) observed that PR proteins and ROS-related enzymes are differentially regulated in resistant genotypes.

Furthermore, some QTLs showed QTL × environment interactions, as reported by Sunitha *et al.* (2021), which suggests that certain candidate genes may be more effective under specific agro-climatic conditions.

6.4. Screening and Identification of Novel Candidate Genes

Recent studies have employed high-throughput comparative transcriptome analysis and metabolomics to screen for novel CGs:

- Phat *et al.* (2021) identified 36 resistance genes (R-genes) differentially expressed in resistant lines most of which were receptor-like kinases (RLKs).
- Kumar *et al.* (2021) highlighted metabolomic markers such as ribonic acid, cinnamic acid, and squalene, linked with pathogen-triggered resistance. These biochemical signatures corresponded to defense pathways like flavonoid biosynthesis and cuticle reinforcement, providing functional validation for several CGs.
- In addition, Zhao *et al.* (2018) used amplicon sequencing to refine QTL intervals and detected SNPs and InDels that helped narrow a major resistance region to 0.8 Mb, paving the way for cloning of high-confidence CGs.
- Incorporating a genetics-first approach, Sadgar *et al.* (2023) demonstrated that segregating populations derived from well-characterized parental lines can still serve as a resource for identifying new CGs. Their study offers strong support for integrating phenotypic ratios with downstream sequencing, ensuring that CG identification remains grounded in both molecular evidence and traditional inheritance patterns.

6.5. Validation and Deployment of Candidate Genes

Candidate genes must undergo functional validation to confirm their role in disease resistance. Techniques like:

RNA interference (RNAi) to silence target genes, CRISPR/Cas9 genome editing for gene knockout studies, and transgenic overexpression in model systems are increasingly being applied (Pandey *et al.,* 2017; Clevenger *et al.,* 2018). Once validated, these genes can be used to develop gene-based markers for breeding. For example, the diagnostic KASP markers developed from LLSR1 and LLSR2 are now routinely used in MAS pipelines across Indian groundnut breeding programs (Pandey *et al.,* 2024).

7. Mapping and Functional Validation of Candidate Genes

7.1. Mapping of Candidate Genes

The precise mapping of candidate genes within known QTL intervals is critical for translating genomic knowledge into breeding outcomes. In groundnut, candidate genes have been mapped using a variety of approaches including linkage mapping, QTL-seq, association studies, and comparative genomics.

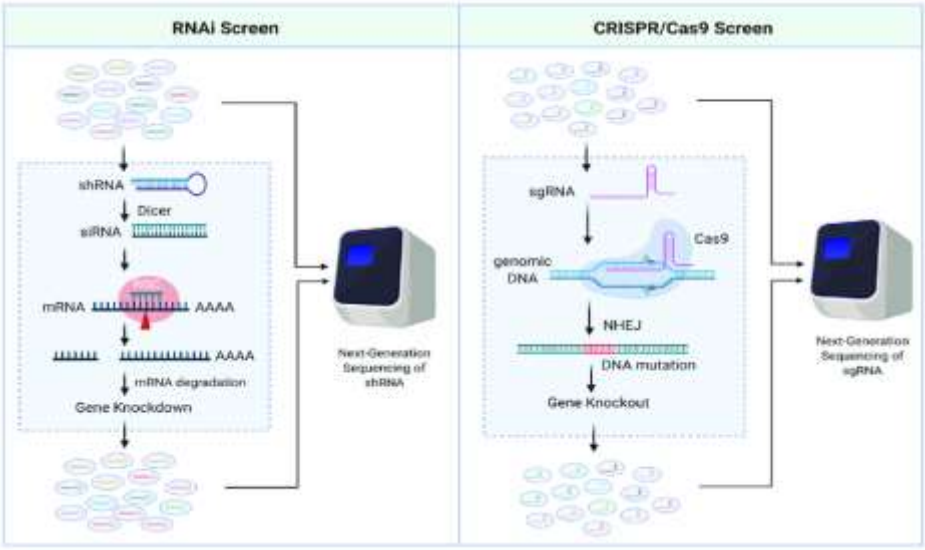

Figure: 4 Schematic overview of RNAi and CRISPR/Cas9 screening approaches for gene function analysis

The functional insights from classical segregation analyses such as those conducted by Sadgar *et al.* (2023) can inform CRISPR/Cas9 target design. By confirming gene action and dominance relationships in a controlled background, breeders can prioritize which CGs to edit or overexpress in follow-up validation experiments.

For example, Pandey *et al.* (2024) successfully mapped LLSR1 and LLSR2 to the LLS-A02 and A03 regions, respectively, using QTL-seq combined with high-throughput genotyping. These candidate loci co-localize with previously reported resistance QTLs and contain functional domains such as NBS-LRR and kinase motifs, which are classical indicators of resistance genes. The mapping accuracy was enhanced using KASP markers, many of which now serve as diagnostic tools in breeding programs.

Similarly, Lu *et al.* (2018) refined a major QTL interval on chromosome A03 to just 0.38 cM, enabling more precise localization of tightly linked candidate genes. This degree of mapping resolution is crucial for reducing linkage drag and allows breeders to focus on fewer genes for validation.

In addition, mapping studies like those by Zhao *et al.* (2018) demonstrated the power of amplicon sequencing in refining large QTL regions. Their approach narrowed a spotted wilt resistance QTL from 15.2 Mb to 0.8 Mb, supporting further candidate gene validation steps.

7.2. Validation of Candidate Genes

Mapping alone does not confirm gene function. Thus, functional validation is essential to establish the causative role of candidate genes in disease resistance.

Key validation strategies include:

RNA interference (RNAi): This technique allows for gene silencing to assess the phenotypic effects of downregulation. In groundnut, RNAi has been used to knock down susceptibility genes, thereby increasing resistance levels (Pandey *et al.,* 2017).

CRISPR/Cas9 genome editing: Though still emerging in peanut, this technology offers the ability to create targeted knockouts or allelic edits. Functional validation using CRISPR can confirm if knocking out a gene eliminates or enhances resistance.

Overexpression studies: Inserting a candidate gene into a model or susceptible groundnut line can demonstrate gain-of-function effects. Genes that improve resistance upon overexpression provide strong

evidence for their involvement.

Differential expression analysis: Transcriptomic studies showing that specific genes are highly expressed in resistant genotypes under disease stress add indirect but compelling evidence of their role. For instance, Gangurde *et al.,* (2019) and Qin *et al.* (2012) observed that several PR genes and oxidative stress enzymes were consistently upregulated in resistant lines, reinforcing their role in LLS resistance. These validations, when supported by genomic, transcriptomic, and phenotypic data, pave the way for developing gene-based markers and transgenic or gene-edited lines with enhanced disease resistance.

8. Case Studies: Deployment of Candidate Genes in Groundnut Breeding

8.1. Development of Rust and LLS Resistant Varieties Using MAS

A notable success story in groundnut breeding is the deployment of marker-assisted backcrossing (MABC) to introgress major QTLs for rust and LLS resistance into elite, farmer-preferred cultivars. Varshney et al. (2014) utilized four molecular markers (including IPAHM103 and GM1536) to introgress a major rust resistance QTL from GPBD 4 into three susceptible but popular Indian cultivars: TAG 24, JL 24, and ICGV 91114. Through two to three backcrosses and careful selection, they developed 200 introgression lines, of which 81 exhibited improved rust resistance and yield gains ranging from 56% to 96% under disease pressure.

These lines retained desirable agronomic traits of the recurrent parent while gaining disease resistance, demonstrating the practical efficiency of MAS in varietal improvement.

8.2. KASP-Based Varietal Development and Marker Deployment

The study by Pandey *et al.* (2024) is another compelling example. They identified and validated 49 KASP markers linked to LLS and rust resistance using pooled sequencing and QTL-seq. These markers were used in multiple breeding programs across India, leading to the release of varieties such as:

Table 2. KASP Markers and Released Groundnut Varieties with LLS and Rust Resistance

Variety	Pedigree/Parentage	Traits Improved	Release Zone
Girnar 4 (ICGV 15083)	SunOleic 95R × GPBD 4	High oleic acid, LLS and rust resistance	Rajasthan, India
Girnar 5 (ICGV 15090)	SunOleic 95R × GPBD 4	High oleic acid, LLS and rust resistance	Rajasthan, India
GG 39 (ICGV 16697)	GPBD 4 derived	LLS and rust resistance	Gujarat, India
GG 40 (ICGV 16668)	ICGV 00350 × ICGV 91114	LLS and rust resistance	Gujarat, India
ICRC-1 (ICGV 16690)	Derived from resistant elite lines	LLS and rust resistance	Central India

These varieties were developed mainly under ICRISAT led programs through marker-assisted breeding, targeting foliar fungal disease resistance (Late Leaf Spot and Rust) and high oleic acid trait.
The markers used were mainly KASP markers linked to major QTLs on chromosomes A02 and A03, derived from donors like GPBD 4 which carry pyramided resistance loci validated through KASP markers and have demonstrated improved pod yields, higher shelling percentages, and elevated oleic acid content (>75%). Their adoption is expected to enhance farmer profitability and reduce reliance on fungicide sprays.

8.3. Spotted Wilt Resistance QTL Refinement

In the U.S., Zhao *et al.* (2018) refined a major spotted wilt resistance QTL on chromosome A01 and showed that the resistance allele was unique to certain lines and absent in the U.S. mini-core germplasm. This refinement helped breeders target novel sources of resistance and guided germplasm enhancement efforts for spotted wilt.

9. Marker-Assisted Selection and Genotyping Approaches for LLS/Rust

Marker-assisted selection (MAS) has emerged as a pivotal tool for improving late leaf spot (LLS) and rust resistance in groundnut. Early efforts using SSR markers identified key QTLs on chromosomes A02 and A03 (Khedikar *et al.* 2010), enabling targeted selection of resistant genotypes.With the advancement of high-throughput genotyping platforms like KASP assays and SNP arrays, selection efficiency has significantly improved. Pandey *et al.* (2024) reported the development of 49 KASP markers for LLS and rust resistance, which are now routinely used across breeding programs in India. These markers have been effectively employed in marker-assisted backcrossing (MABC) to introgress resistance loci from donor genotypes such as GPBD 4 into elite cultivars like JL 24 and TAG 24 (Varshney *et al.,* 2014). The result has been the
development of improved lines with stacked
resistance genes and enhanced agronomic traits. The integration of functional markers, derived from resistance genes like NBS-LRRs and kinases, has further enhanced selection precision. Combined with tools like genotyping-by-sequencing (GBS) and transcriptome-based SNP discovery, MAS now allows for efficient pyramiding of multiple traits in early generations.
In essence, MAS has shifted groundnut breeding from conventional selection to a genomics-driven, trait-focused approach, enabling faster development of disease-resistant varieties.
These molecular deployment strategies are further complemented by classical gene-pyramiding schemes. As discussed by Sadgar *et al.* (2023), combining multiple resistance genes in a single cultivar not only enhances durability but also delays the emergence of virulent pathotypes. Their findings, backed by strong field data, underline the significance of multi-gene resistance stacking as a practical breeding goal for LLS and rust.

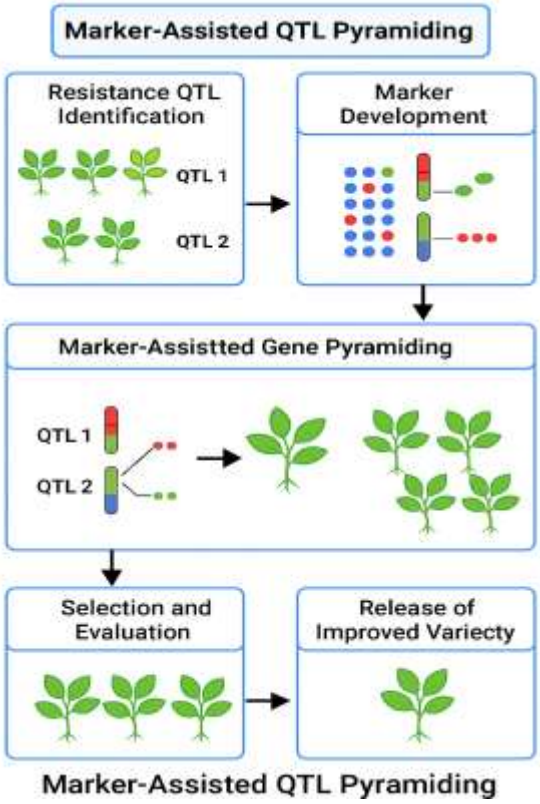

Figure: 5 Workflow of Marker-Assisted Selection (MAS) in Groundnut Breeding

Conclusion:

Breeding for resistance to late leaf spot (LLS) and rust in groundnut has evolved from conventional field-based selection to a precise, gene-targeted approach. The integration of QTL mapping, candidate gene identification, and marker-assisted selection (MAS) has enabled the development of resistant cultivars with minimal trade-offs in yield and quality.

Advances in high-throughput genotyping platforms (like KASP assays), comparative transcriptomics, and metabolomic profiling have greatly accelerated the discovery and validation of resistance-associated genes, particularly those located in QTL regions on chromosomes A02 and A03. The practical deployment of these markers has led to the release of improved varieties with broad-spectrum resistance and enhanced oil quality, reinforcing the effectiveness of genomic tools in breeding. To maximize impact, continued investment in functional validation, breeder-friendly diagnostics, and capacity building in national programs will be essential. The candidate gene approach, when fully embedded within an integrated breeding pipeline, promises to deliver groundnut varieties that are resilient, high-yielding, and climate-smart.

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