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Molecular Diversity of Chickpea Ascochyta Blight Pathogen in Pakistan

Nabeel Ahmad
National Institute for Biotechnology and Genetic Engineering (NIBGE)
Corresponding Author nabeel8700@gmail.com

Shayhaq Sayed
Govt of Balochistan agriculture cooperatives department agriculture extension date farm turbat
shayhaq.sayed@gmail.com

Ameer Jan
Department of Botany University of Makran Panjgur
Corresponding AuthorAmeer.jeehand143@gmail.com
Ameerjan@uomp.edu.pk

Adalat Ali
Dr. A.Q. Khan Institute of Biotechnology and Genetic Engineering, University of Karachi, Karachi, Pakistan,
mehraniadalat786110@gmail.com

Shahbano Ali Kashani
Department of Botany, University of makran panjgur
shahbano.ali@uomp.edu.pk

Shakeela Mohammad
Department of Botany, University of makran panjgur
Shakeela@uomp.edu.pk

Abstract: Ascochyta blight (AB), caused by the necrotrophic fungus *Ascochyta rabiei* (*teleomorph Didymella rabiei*), remains the most devastating disease of chickpea (*Cicer arietinum* L.) in Pakistan, where the crop occupies approximately 2.2 million hectares and serves as a vital source of dietary protein. This comprehensive review synthesizes historical epidemiology, morphological characteristics, pathogenic variability, and molecular diversity of A. rabiei populations in Pakistan. The pathogen exhibits high genetic and pathogenic diversity, driven by factors such as potential historical sexual recombination, high gene flow across regions (particularly in the Thal and Pothwar areas), and strong selection pressure from host resistance genes. Studies using molecular markers including RAPD, SSR, ISSR, and Universal Rice Primers (URP) have revealed extensive polymorphism, distinct genetic clusters, and evidence of long-distance migration, with skewed mating type ratios (predominance of MAT1-2) suggesting limited current sexual reproduction despite

signatures of past panmixia. Pathotype classification identifies dominant groups (I–III), with highly aggressive isolates (comparable to international Pathotype IV) emerging in northern regions. Environmental factors (temperature 15–25°C, high humidity, wind dispersal) and agricultural practices (monoculture, seed-borne transmission) exacerbate epidemics, causing yield losses of 10–100%. Advances in QTL mapping, SNP-based GWAS, and host-pathogen interaction studies highlight key genomic regions (on chromosomes Ca2 and Ca4) for durable resistance breeding. Integrated management strategies combining marker-assisted selection, biocontrol agents (*Trichoderma*), cultural practices, and forecasting models are essential. Continuous molecular surveillance is recommended to anticipate pathogen evolution and safeguard chickpea production in Pakistan.

Keywords: Ascochyta rabiei, Ascochyta blight, chickpea, molecular diversity, genetic diversity, pathotypes, mating types, RAPD, SSR, Pakistan, Thal region, resistance breeding, gene flow

1. Introduction

The cultivation of chickpea (*Cicer arietinum* L.) represents one of the oldest agricultural traditions in human history, tracing its domestication back to the eighth millennium BC in the Fertile Crescent, specifically in regions now occupied by southeastern Turkey and northern Syria (Jamil et al., 2000). As a self-pollinated diploid with a genome size of approximately 740 Mbp, chickpea has evolved from its wild progenitor, *Cicer reticulatum*, to become a foundational pulse crop for global food security (Raman et al., 2022). In contemporary agriculture, it serves as a critical source of vegetable protein (20-30%), carbohydrates (40%), and essential minerals like iron and zinc, particularly in the developing world (Manjunatha et al., 2022). Pakistan stands as a major producer within the global landscape, where the crop is grown on roughly 2.2 million hectares, contributing significantly to the national agricultural GDP and the nutritional requirements of its population (Ali & Malik, 2014).

However, the productivity of this crop is perennially undermined by *Ascochyta rabiei* (Pass.) Labrousse, the causal agent of Ascochyta blight (AB), a devastating necrotrophic fungus that can induce total crop failure under conducive environmental conditions (Jamil et al., 2010). The pathogen was first formally identified in the scientific record in 1911 in the North-West Frontier Province of India, an area that now constitutes part of Pakistan (Gayacharan et al., 2020). Since this initial report, the disease has expanded to more than 40 countries across six continents, becoming the most limiting factor for chickpea production wherever cool and humid climates prevail (Kenis et al., 2022). The molecular diversity of *A. rabiei* in Pakistan is particularly pronounced, driven by a combination of

high selection pressure from host genotypes and potential sexual recombination, which facilitates the rapid evolution of aggressive pathotypes capable of overcoming newly developed resistance genes (Farley et al., 2021).

Table 1. Parameter values for chickpea area and blight yield loss.

Parameter	Value/Statistic	Source
Global Chickpea Cultivation Area	14.84 - 17.8 million hectares	(Ali & Malik, 2014; Manjunatha et al., 2022)
Pakistan Chickpea Cultivation Area	~2.2 million hectares	(Ali & Malik, 2014; et al., 2024)
Typical Yield Loss (AB)	10% - 100%	(Jamil et al., 2010; Manjunatha et al., 2022)
First Report of AB in Pakistan	1911	(Butler, 1918)
Genome Size of *Cicer arietinum*	~740 Mbp	(Jamil et al., 2000)

2. Historical Epidemiology and Epidemic Cycles in Pakistan

The history of Ascochyta blight in Pakistan is defined by cyclical epidemics that have occasionally threatened the very existence of chickpea as a viable crop. Following its discovery in 1911, the disease caused frequent epiphytotics, particularly in the northern regions of the country where the climate is more humid (Vandana et al., 2020). One of the most catastrophic periods occurred between 1979 and 1982, when consecutive epidemics devastated the chickpea industry across Pakistan and North India (Dikshit et al., 2025). During these years, approximately 50% of the growing area was affected, with yield losses reaching 50% or more, leading to a massive "pulses debacle" that necessitated significant national policy shifts and increased reliance on imports (Dikshit et al., 2025).

These historical outbreaks underscore the pathogen's ability to remain dormant in crop residues for several years, waiting for the alignment of cool temperatures (15 to 25 degrees Celsius) and high relative humidity (above 90%) to launch a secondary spread (Jayavelu et al., 2025). The epidemiology in Pakistan is further complicated by the geographic concentration of the crop; nearly 80% of the national production is located in the Thal region, a desert tract where monoculture is the norm (Saqib et al., 2024). This lack of crop diversification creates a perennial reservoir for the fungus, as stubble

retention on the soil surface acts as a primary source of inoculum for subsequent seasons (Chakraborty et al., 2021).

The economic consequences of these epidemics are multifaceted. Beyond the immediate loss of grain yield, the quality of the harvested seeds is severely compromised, with infections causing shriveling, discoloration, and reduced germination rates (Nigam, 2024). In a rainfed agricultural system like Pakistan's, where farmers often save their own seed for planting, the prevalence of seed-borne *A. rabiei* ensures the early establishment of disease foci in the following year, creating a self-perpetuating cycle of infection that is difficult to break without a molecular understanding of the pathogen's diversity and its spread mechanisms (Shahbazi et al., 2025).

3. Morphology and Taxonomy of *Ascochyta rabiei*

The taxonomic classification of the chickpea blight pathogen has undergone several refinements since its early description as *Phyllosticta rabiei*. Today, it is recognized as *Ascochyta rabiei*, a coelomycete fungus within the family Didymellaceae (Abassy, 2024). The pathogen exists in two distinct stages: the asexual anamorph (*A. rabiei*) and the sexual teleomorph (*Didymella rabiei*). Morphological characterization of Pakistani isolates reveals significant diversity in their cultural and structural traits, which reflects their broad genetic base (Iqbal et al., 2004).

3.1 The Anamorph Stage

The asexual stage is characterized by the formation of pycnidiadark brown, spherical to pear-shaped fruiting bodies that develop within host tissues. In Pakistani isolates, the size of these pycnidia can vary significantly, with recorded dimensions ranging from 91 x 85 to 225 x 224 micrometers squared (Ali et al., 2012). These structures produce vast numbers of pycnidiospores (conidia), which are typically hyaline, oval to oblong, and measure approximately 8 x 4 to 13 x 5 micrometers squared (Coram, 2024). While most conidia are unicellular, a small percentage (2-4%) are uniseptate, a characteristic that was historically used to differentiate *Ascochyta* from *Phyllosticta* (Pem et al., 2021).

Cultural characteristics of the anamorph on artificial media like Czapek's Dox agar or chickpea seed meal dextrose agar (CSMDA) also exhibit high variability. Radial growth rates of isolates collected from various districts in Pakistan range from 2.6 to 6.7 cm over a 12-day incubation period at 20 degrees Celsius (Kumar, 2023). Colony colors vary across a spectrum of greys and browns, a trait that has been linked to specific genetic clusters through principal component analysis (PCA), indicating

that morphological diversity is a window into the underlying molecular heterogeneity of the population (Rathod, 2022).

3.2 The Teleomorph Stage

The sexual stage, *Didymella rabiei*, is characterized by the formation of pseudothecia on overwintered chickpea residue. These structures are slightly larger and darker than pycnidia and contain asci, each housing eight hyaline, two-celled, asymmetrically septate ascospores (Chen et al., 2024). The teleomorph is heterothallic, requiring the encounter of two compatible mating types (MAT1-1 and MAT1-2) to complete the sexual cycle (Motagi et al., 2020). In Pakistan, while the teleomorph's role in the disease cycle is recognized, its field observation is less frequent than the anamorph, although molecular evidence of historical recombination suggests that sexual reproduction remains a critical driver of the pathogen's long-term evolution (Vandana et al., 2020).

Table 2. Morphological and cultural characteristics of Ascochyta rabiei isolates.

Morphological Feature	Range of Measurement/Description	Source
Pycnidia Size (Pakistan)	91 x 85 to 225 x 224 micrometers squared	(Iqbal et al., 2004)
Pycnidiospore Size	8 x 4 to 13 x 5 micrometers squared	(Iqbal et al., 2004)
Radial Growth Rate	2.6 to 6.7 cm	(Iqbal et al., 2004; Jamil et al., 2000)
Mating Type Locus	MAT1-1 and MAT1-2	(Ali et al., 2012; Manjunatha et al., 2022)
Spore Type	Uniseptate (2-4%), Unicellular (majority)	(Jamil et al., 2010)

4. Environmental Determinants of Diversity and Pathogenesis

The progression of Ascochyta blight in the diverse agro-ecological zones of Pakistanfrom the arid Thal desert to the humid Pothwar plateauis dictated by a strict set of environmental parameters. Molecular diversity allows the pathogen to adapt to these varying conditions, ensuring its survival across thermal and moisture gradients (Jadon et al., 2020). The epidemiology of Ascochyta blight is closely linked to environmental conditions that facilitate spore dispersal and infection. Figure 1 presents the life cycle of Ascochyta rabiei, illustrating how the pathogen survives in residues and spreads th rough rain splash and airborne spores during favorable climatic conditions.

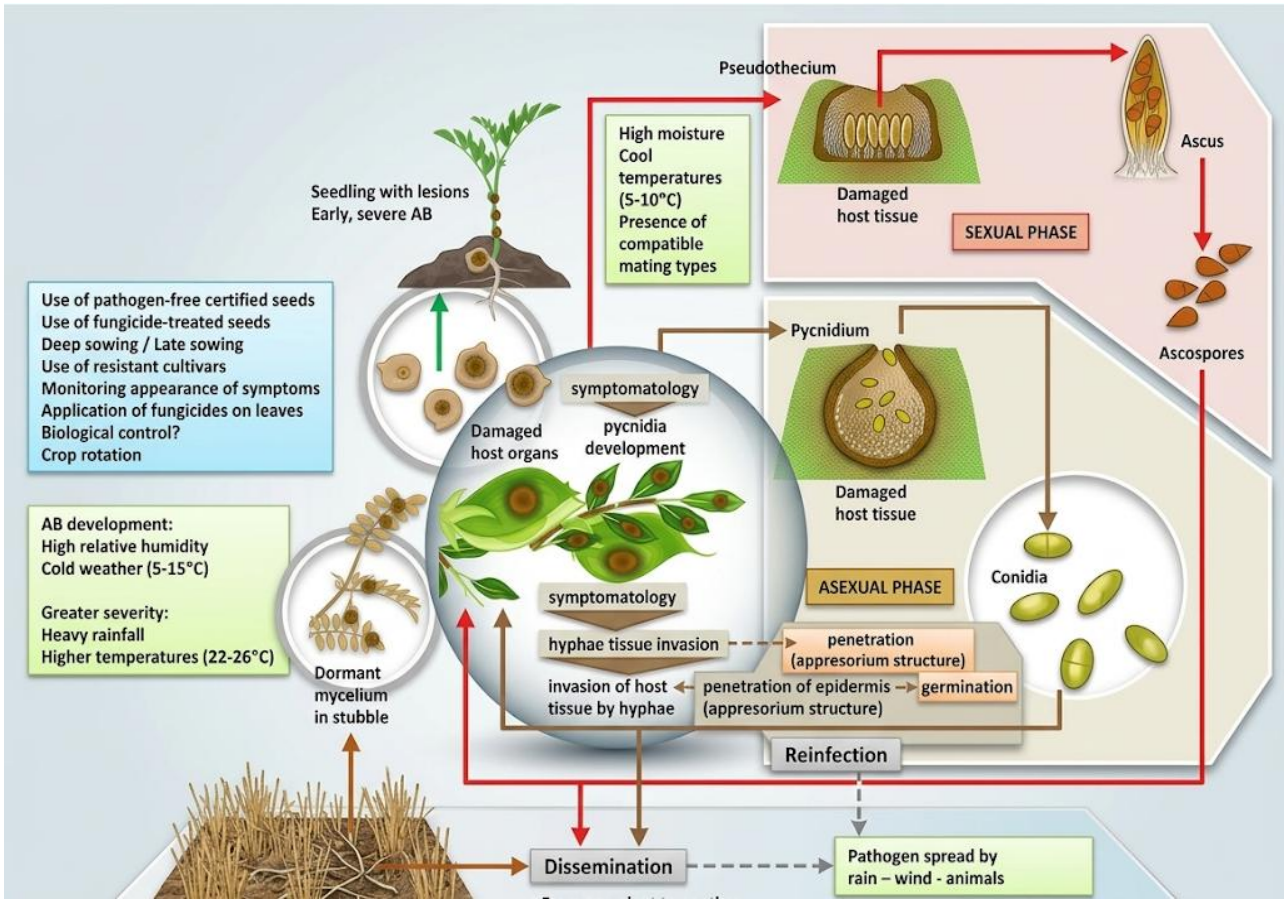

Figure 1: Disease Cycle of Ascochyta rabiei in Chickpea

4.1 Temperature and Humidity Correlations

The optimal temperature for *A. rabiei* infection and subsequent lesion development is typically between 15 and 25 degrees Celsius. However, the fungus exhibits remarkable thermal resilience; it can survive in crop residues at temperatures as high as 35 degrees Celsius provided that humidity levels are elevated (Jayavelu et al., 2025). In the Thal region, research has demonstrated that disease

incidence is positively correlated with relative humidity and wind speed, while disease severity is strongly tied to rainfall and negatively correlated with maximum temperature (Nigam, 2024).

Specific studies in districts like Layyah and Bhakkar have shown that even a brief period (48 hours) of optimum moisture and temperature can lead to a 40-70% yield loss. The relationship between temperature and pathogenicity is a critical factor in the development of disease forecasting models (Priya et al., 2024). In northern India and Pakistan, temperatures between 22 and 26 degrees Celsius during the reproductive stage of the chickpea, when combined with heavy rainfall, exacerbate the disease's severity, leading to rapid tissue disintegration and the death of the entire plant (Lavanya, 2025).

4.2 Dispersal Mechanics

The dispersal of *A. rabiei* in the Pakistani landscape involves both short-range and long-range mechanisms. Short-range dispersal is primarily achieved through "spore splash," where rain droplets hit pycnidial lesions, dislodging conidia and carrying them to adjacent plants (Ahmad et al., 2023). Long-range dispersal is the domain of ascospores released from the teleomorph, which can be carried by wind currents over several kilometers (Geoffrey, 2024). In the vast, flat terrain of the Thal region, wind speed is a significant predictor of disease incidence, as it facilitates the spread of both ascospores and wind-borne conidia from primary foci across thousands of hectares of contiguous chickpea fields (Neupane et al., 2020).

5. Pathogenic Variability and the Pathotype Classification

One of the greatest challenges for chickpea breeders in Pakistan is the extreme pathogenic variability of *A. rabiei*. The pathogen does not exist as a single uniform entity but as a collection of diverse pathotypes (or physiological races) that differ in their ability to infect and cause disease across a spectrum of chickpea genotypes (J Parveen et al., 2024).

5.1 Identification of Pathotypes in Pakistan

Extensive surveys and glasshouse trials have been conducted to classify Pakistani isolates based on their virulence on host differentialscultivars with known resistance or susceptibility profiles (Sarwar et al., 2013).

Furthermore, recent work has identified a Pathotype IV, a highly virulent class that has been reported in Syria and potentially exists within the aggressive populations of northern Pakistan (Manjunatha et al., 2022). Studies using 18 Pakistani isolates compared against Syrian pathotypes III and IV revealed

that some local isolates exhibit a level of aggressiveness comparable to the international pathotype IV standards, emphasizing the constant evolution of virulence genes within the local population (Khan et al., 2021).

5.2 Regional Distribution of Virulence

The distribution of these pathotypes in Pakistan is not uniform. Highly aggressive isolates (Pathotype III) are most frequently encountered in Northern Punjab (e.g., Attock and Sialkot) and the Khyber Pakhtunkhwa province (e.g., Peshawar), where the climate is more conducive to the pathogen's rapid multiplication and selection for virulence (Idrees et al., 2022). In the Thal region, while Pathotype II is dominant, the emergence of virulent strains like 'LAY-01' and 'BKR-01' from Layyah and Bhakkar, respectively, highlights that high-risk genotypes are spreading even into the more arid zones of the country (Monecke et al., 2020).

Table 3. Frequency and aggressiveness of Ascochyta rabiei pathotype classes in Pakistan.

Pathotype Class	Aggressiveness	Frequency in Pakistan (130 Isolates)	Dominant Region	Source
Pathotype I	Low	4 isolates	Islamabad/Central Punjab	(Jamil et al., 2000)
Pathotype II	Moderate	79 isolates	Southern/Central Punjab	(Jamil et al., 2000)
Pathotype III	High	47 isolates	Northern Punjab/KPK	(Jamil et al., 2000)

6. Molecular Marker Systems for Diversity Assessment

The limitations of biological pathotypingspecifically its dependence on host genotype and environmental conditionshave necessitated the use of molecular DNA markers to accurately assess the genetic diversity of *A. rabiei* (Alhasnawi et al., 2024). In Pakistan, several marker systems have been employed, ranging from early RAPD assays to modern high-throughput genotyping-by-sequencing (Amiteye, 2021).

6.1 Random Amplified Polymorphic DNA (RAPD)

RAPD markers have been a cornerstone of molecular research on *A. rabiei* in Pakistan due to their sensitivity and ease of application across large sample sizes. Analysis of Pakistani isolates using 10-mer oligonucleotide primers (such as OPA, OPB, and OPJ series) has revealed extensive genomic diversity (Rana et al., 2023). In one study of 21 isolates, 49 polymorphic bands were identified, resulting in the grouping of isolates into three main clusters (A, B, and C) (Babu et al., 2020).

Crucially, RAPD analysis has shown that genetic similarity does not always correlate with geographic origin or pathotype classification. For instance, two highly virulent (HV) isolates from Islamabad were found to share only 50% genetic homology, while an LV and an HV isolate from the same area showed 80% similarity (Mahboob et al., 2023). This lack of correspondence suggests that virulence is a complex trait controlled by a few genes that may move between different genetic backgrounds via recombination or horizontal gene transfer (Azra et al., 2025).

6.2 Simple Sequence Repeats (SSR) and Microsatellites

SSR markers, or microsatellites, offer a more stable and co-dominant alternative to RAPD. These markers target tandem repetitive DNA sequences (1-6 bp) and are highly polymorphic within the *A. rabiei* genome. Research in Pakistan has utilized SSR markers like ArH05T, ArA06T, and ArH02T to characterize isolates and identify microsatellite haplotypes (Ali et al., 2013).

In a comparative study between Pakistani and US populations, SSR analysis identified three distinct genetic clusterstwo for Pakistan and one for the US. However, a small degree of crossover was noted, with some US isolates sharing the genetic background of one Pakistani cluster (Fatima et al., 2023). This finding provides molecular evidence for inter-continental migration, likely mediated by the trade of infected chickpea germplasm. Globally, SSR gene diversity in *A. rabiei* is estimated at around 0.29, though local populations in countries with active sexual cycles can exhibit much higher levels of heterozygosity (Farahani et al., 2025).

6.3 Inter-Simple Sequence Repeat (ISSR) and Universal Rice Primers (URP)

ISSR markers have been used in Pakistan primarily to screen local genotypes for resistance-linked QTLs. While SSRs target the repeat itself, ISSRs amplify the genomic region between two microsatellite loci, providing a broader view of genomic variation (Mogali et al., 2020).

A more innovative approach in recent years has involved the use of Universal Rice Primers (URP), which are derived from repeat sequences of the weedy rice genome but are applicable to diverse taxa including fungi. URP markers have proven exceptionally effective at differentiating highly aggressive

Pakistani isolates from Syrian ones (Mohd Hanafiah et al., 2020). In a study of 24 isolates, URP-PCR profiling differentiated pathotypes and geographic populations more clearly than SSR markers, which showed identical banding patterns for some isolates across countries (Choudhury et al., 2023). This highlights the utility of URPs in identifying the fine-scale molecular differences that define national pathogen populations (Rabuma et al., 2020).

Table 4. Utility and specific findings of molecular marker systems in Pakistan.

Marker System	Specific Findings in Pakistan	Significance	Source
RAPD	High polymorphism; identified 3 clusters in 21 isolates	Resolves genetic variation not visible in pathotyping	(Sarwar et al., 2013)
SSR (Microsatellite)	Revealed links between Pakistani and US populations	Evidence for long-distance migration/gene flow	(Ali et al., 2012)
URP (Universal Rice Primers)	Differentiated Pakistani from Syrian HV pathotypes	High-resolution genomic fingerprinting	(Ali et al., 2013)
ISSR	Used for mapping QTLs on LG2, 4, and 6	Essential for marker-assisted selection (MAS)	(Shah et al., 2008)
STMS	Ta2, Ta146, and Ts54 found most effective for screening	Reliable markers for resistance breeding	(Dogan et al., 2023)

7. Mating Type Distribution and the Potential for Recombination

The genetic diversity of *A. rabiei* in Pakistan is inextricably linked to its mode of reproduction. While the asexual cycle ensures rapid multiplication during the growing season, the sexual cycle provides the mechanism for generating new genotypes through recombination (Saqib et al., 2024).

7.1 Mating Type Frequency in Pakistan

The distribution of mating types in the Pakistani *A. rabiei* population has been a subject of significant molecular investigation. As a bipolar, biallelic species, an equal (1:1) frequency of MAT1-1 and MAT1-2 is expected in populations that are randomly mating (panmictic) (Farahani et al., 2021). However, molecular assays using MAT-specific primers have revealed a skewed distribution in Pakistan, with a reported ratio of 3 MAT1-2 for every 1 MAT1-1 isolate (Bencheqroun et al., 2022).

This uneven distribution suggests that sexual reproduction may be relatively rare in the current Pakistani environment, potentially due to the physical isolation of mating types or unfavorable climatic conditions for the formation of pseudothecia during the winter (Weyl et al., 2020). In contrast, populations in countries like Turkey, Uzbekistan, and the United States often show a near-perfect 1:1 ratio, which correlates with the frequent observation of the teleomorph and high levels of genotypic diversity (Singh Saharan et al., 2023).

7.2 Implications of Historical Recombination

Despite the current skew in mating types, statistical analysis of microsatellite data in Pakistani populations indicates the existence of "panmixia" or historical random mating. This suggests that the genetic diversity currently observed is at least partly the result of past recombinational events (Phillips et al., 2021). The risk of renewed sexual cycles remains high, especially if new mating types are introduced through germplasm exchange. The presence of both mating types in neighboring regions means that a shift in climatespecifically toward wetter, cooler winters in the Pothwar or northern zonescould trigger a surge in sexual reproduction, leading to the rapid emergence of even more aggressive pathotypes that could bypass existing resistance in Pakistani chickpea cultivars (Kumar et al., 2024).

Table 5. Comparative mating type frequencies and genetic diversity across regions.

Country/Region	MAT1-1 Frequency	MAT1-2 Frequency	Gene Diversity (Hexp)	Source
Pakistan	~25%	~75%	N/A (Panmixia signature)	(Ali et al., 2012)
India (Punjab)	0%	100%	Low	(Manjunatha et al., 2022)
Ethiopia	N/A	N/A	0.61	(Getaneh et al., 2021)
Australia	100%	0%	0.066	(Manjunatha et al., 2022)
Turkey/Uzbekistan	~50%	~50%	High	(Farahani et al.,

				2025)

8. Population Genetics and Gene Flow

Molecular analysis has provided profound insights into the population structure and gene flow of *A. rabiei* within Pakistan. The movement of the pathogen across the vast chickpea-growing regions of Punjab and KPK is a major factor in its ability to adapt and survive (Yadav et al., 2023). Molecular population studies reveal extensive genetic exchange among A. rabiei populations across Pakistan. Figure 2 illustrates the population structure and gene flow patterns among p athogen populations, highlighting the interconnected nature of chickpea-growing regions.

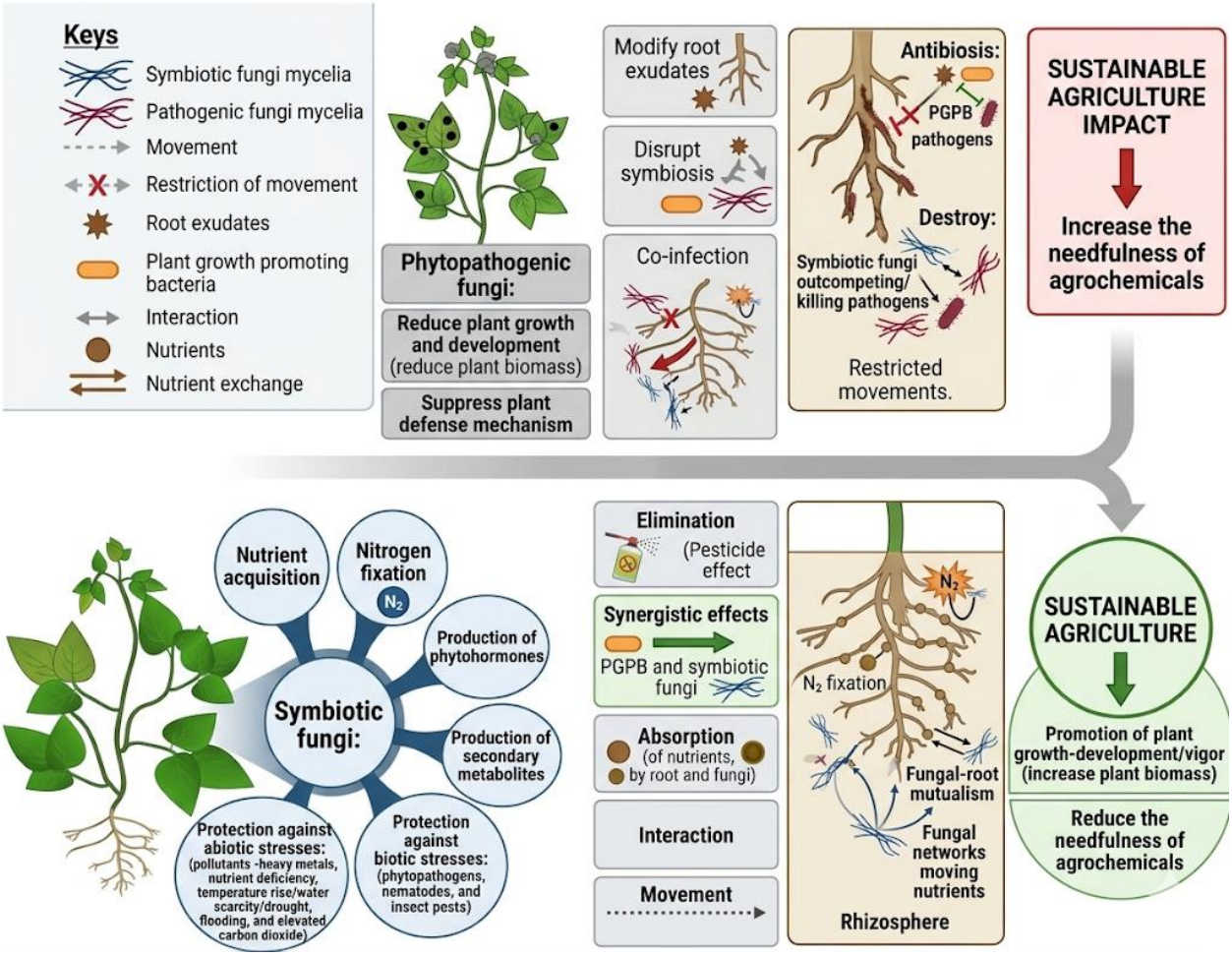

Figure 2: Population Structure and Gene Flow of Ascochyta rabiei in Pakistan

8.1 Genetic Structure and Clustering

Using Bayesian analysis tools like STRUCTURE, researchers have been able to categorize Pakistani isolates into distinct genetic groups that operate independently of administrative boundaries (Shahid et al., 2025). For example, studies have consistently found that isolates within a single geographic population (such as those from a single district like Peshawar) often exhibit more genetic variation than is found between populations separated by hundreds of kilometers (Song et al., 2021).

This high level of intra-population variationoften exceeding 90%is typical of a pathogen with high dispersal capability. It indicates that the Pakistani population of *A. rabiei* is a large, interconnected meta-population where genetic material is constantly being reshuffled and redistributed (Bindra et al., 2023).

8.2 Evidence for Long-Distance Gene Flow

The mechanism for this interconnection is likely twofold: the transport of wind-blown spores and the domestic trade of chickpea seeds. Since 90% of the chickpea area in Punjab is rainfed and concentrated in the Thal, there is a continuous "green bridge" that allows the fungus to travel from one field to the next during the monsoon and subsequent rabi season (Ali & Malik, 2014). Gene flow analysis, measured by the number of migrants (Nm), has shown values as high as 6.2 between clusters, suggesting that geographical isolation is virtually non-existent in the Pakistani context (Masombuka, 2023). This high gene flow ensures that any new virulence mutation or recombined genotype that arises in one part of the country can quickly spread to others, making localized disease management strategies less effective (Motagi et al., 2020).

9. Genomics and the Search for Durable Resistance

The molecular diversity of the pathogen is mirrored by the genetic complexity of the host's resistance response. Breeding for resistance to *A. rabiei* in Pakistan has moved from traditional phenotype-based selection to the identification of molecular markers and genomic regions that confer stable protection (Shah et al., 2024).

9.1 Quantitative Trait Loci (QTL) Mapping

Resistance to AB in chickpea is predominantly a quantitative trait, although some studies suggest the involvement of major genes. In Pakistan, research has focused on identifying QTLs on specific linkage groups (LG) (Kaur et al., 2025).

In a study of local Pakistani genotypes and mutants, STMS and SCAR markers on LG 4b showed the strongest linkage with blight resistance, providing a reliable tool for marker-assisted breeding (MAB)

programs. Specifically, the gene *arr6* on chromosome 4 has been identified as a single recessive gene conditioning resistance in certain kabuli crosses, mapping near the markers CGMM072 and NCPGR247 (Raman et al., 2022).

9.2 Single Nucleotide Polymorphism (SNP) and Association Mapping

The advent of Genotyping-by-Sequencing (GBS) has allowed Pakistani researchers to identify thousands of SNP markers associated with AB resistance. GWAS analysis of advanced breeding lines and cultivars has pinpointed 21 different genomic regions on Ca2 and Ca4 that are significantly associated with resistance, explaining 11-39% of the phenotypic variation (Saleem et al., 2022). These regions harbor candidate genes encoding for serine/threonine-protein kinases, Myb proteins, and quinone oxidoreductasesenzymes that are integral to the plant's immune signaling and oxidative burst during fungal attack (Yunus et al., 2025).

Association mapping of 80 Pakistan-adapted genotypes identified 43 marker-trait associations (MTAs) for disease severity. The identifying of six highly resistant genotypes within this local germplasm serves as an excellent resource for breeders to integrate favorable SNPs into high-yielding varieties like 'Noor 23' or 'Bittle 2016' (Mahmood et al., 2022).

10. Host-Pathogen Molecular Interactions

The interaction between *A. rabiei* and chickpea is a sophisticated molecular battle. Resistant genotypes in Pakistan have developed a suite of mechanisms to counter the pathogen's necrotrophic strategy (Ilyas et al., 2022).

10.1 Physiological Mechanisms of Resistance

Resistance is often characterized by a rapid transcriptional reprogramming of the host. Within 24 hours of inoculation, resistant plants initiate cell-wall remodeling and activate secondary metabolite pathways, such as the production of phytoalexins (e.g., medicarpin and maackiain) (Narváez-Barragán et al., 2022). By 72 hours post-inoculation, there is a significant upsurge in signaling components, including those related to jasmonic acid, ethylene, and abscisic acid pathways (Wan et al., 2021).

10.2 Pathogen Strategy and Virulence Factors

Conversely, *A. rabiei* employs a battery of effectors and toxins to kill host tissue and thrive on the nutrients released. The high genetic diversity of the pathogen ensures a "reservoir" of these effectors, allowing different pathotypes to exploit different weaknesses in the chickpea immune system (Farahani et al., 2025). Understanding the structural diversity of these pathogen-associated molecular patterns (PAMPs) and host-specific toxins (HSTs) is the next frontier for Pakistani molecular plant pathology (Haq et al., 2022).

11. Regional Diversity Case Studies: Thal vs. Pothwar

The geographic dichotomy between the Thal and Pothwar regions provides a natural laboratory for studying how molecular diversity adapts to environment (Thombre et al., 2022).

11.1 The Thal Desert Region

In the Thal (encompassing Bhakkar, Layyah, and Mianwali), chickpea is the lifeblood of the economy. Survey data from 2020 to 2022 showed that Layyah consistently recorded the highest disease magnitude, with a prevalence of 76%, incidence of 84.2%, and severity of 59.41% (Farley et al., 2021). This is largely due to the district's unique environmental profile, which combines high relative humidity with higher-than-average wind speeds that facilitate the secondary spread of conidia (Tong et al., 2023). Molecular profiling of Thal isolates reveals a high prevalence of Pathotype II, but the occasional introduction of highly virulent strains from other regions creates a volatile disease landscape (Idrees et al., 2022).

11.2 The Pothwar Plateau and Salt Range

The Pothwar region (Attock, Chakwal, and Rawalpindi) is more humid and temperate. This region is often the source of Pathotype III isolates, as the cooler, wetter springs are more conducive to the sexual cycle and the generation of genetic diversity (Pawełkowicz et al., 2025). Research at the Barani Agriculture Research Institute (BARI) in Chakwal has focused on enhancing yields through balanced fertilization (phosphorus and sulfur), which can indirectly improve the plant's resilience to biotic stress (Vandana et al., 2020). However, the high diversity of the pathogen in this region means that resistance is often shorter-lived, as the fungus quickly evolves to overcome the selection pressure of new varieties (Abassy, 2024).

Table 6. Magnitude of Ascochyta blight in different districts of the Thal region.

District	Prevalence	Incidence	Severity	Primary	Source

	(%)	(%)	(%)	Pathotype	
Layyah	76	84.20	59.41	Pathotype II/III	(et al., 2024)
Jhang	64	N/A	N/A	Pathotype II	(et al., 2024)
Bhakkar	56	N/A	N/A	Pathotype II	(et al., 2024)
Mianwali	40	N/A	N/A	Pathotype II	(et al., 2024)
Muzaffargarh	34 - 40	20.55	8.55	Pathotype I/II	(et al., 2024)

12. Integrated Management Strategies and Future Perspectives

Managing a pathogen as diverse as *A. rabiei* requires a multi-pronged approach that integrates molecular insights with field-level management (Alhasnawi et al., 2024).

12.1 Chemical and Cultural Controls

While host resistance is the most sustainable approach, it must be supported by other measures. Seed treatment with fungicides remains critical to prevent the early introduction of inoculum (Yadav et al., 2023). Cultural practices such as inter-cropping with cereals (e.g., wheat or barley) and increasing plant spacing can alter the micro-climate within the canopy, reducing humidity and hindering the splash-dispersal of spores. Deep sowing (up to 10 cm) has also been shown to reduce seedling infection by placing the seed away from surface residues (Ghazanfar et al., 2010).

12.2 Biological Control Innovations

The use of *Trichoderma harzianum* and other biocontrol agents is gaining traction in Pakistan as a way to manage soil-borne and residue-borne inoculum. *T. harzianum* competes with *A. rabiei* on crop residues, reducing the survival of pycnidia and pseudothecia over the summer months (Saqib et al., 2024). Additionally, botanical extracts from *Tagetes erectus* (marigold) and other plants offer a biological alternative to synthetic fungicides, significantly suppressing fungal colony growth by 55-73% in laboratory settings (Rabuma et al., 2020).

12.3 Molecular Breeding and Genomic Selection

The future of chickpea production in Pakistan lies in the deployment of varieties with "stacked" or "pyramided" resistance genes. By utilizing identified markers like Ta2, Ta146, and Ts54, breeders can create genotypes that possess multiple layers of defense against different pathotypes (Kaur et al., 2025). Genomic selection, which uses the entire profile of SNP markers to predict the resistance of a breeding line, offers a faster and more accurate method for developing durable resistance than traditional phenotype-based breeding (Mahboob et al., 2023).

13. Synthesis and Strategic Recommendations

The molecular diversity of *Ascochyta rabiei* in Pakistan is a dynamic and formidable challenge that requires a deep, ongoing commitment to molecular research and surveillance. The high intra-population variability, evidence of historical sexual recombination, and significant gene flow across regions create a scenario where resistance genes are constantly under siege (Bencheqroun et al., 2022).

To ensure the long-term sustainability of chickpea production in Pakistan, several strategic priorities are essential:

1. **Continuous Pathogen Monitoring:** Systematic annual surveys and molecular fingerprinting (using URP and SSR markers) are necessary to track the movement of aggressive pathotypes and identify the emergence of new virulent classes like Pathotype IV (Choudhury et al., 2023).
2. **Genomic Integration:** National breeding programs should fully integrate MAS and genomic selection, focusing on the highly effective QTLs identified on chromosome 4 (Phillips et al., 2021).
3. **Climate-Smart Management:** Disease forecasting models should be developed that incorporate regional environmental data (humidity, rainfall, wind) to provide farmers with early warnings and optimize the timing of fungicide applications (Kumar et al., 2024).
4. **Seed Health Programs:** Strengthening the national seed certification system to ensure the distribution of disease-free, high-quality seeds is vital for disrupting the pathogen's primary dispersal route (Manjunatha et al., 2022).

5. **Biological Synergy:** Further research into the use of biocontrol agents like *Trichoderma* and botanical extracts can provide sustainable, low-cost options for managing inoculum loads in the Thal and Pothwar regions (Mogali et al., 2020).

Ultimately, the goal is to move from a reactive management postureresponding to epidemics after they occurto a proactive, molecularly-informed strategy that anticipates the pathogen's next evolutionary move. By leveraging the genetic diversity of both the host and the pathogen, Pakistan can secure the future of this ancient and essential crop (Getaneh et al., 2021).

Conclusion

The molecular diversity of Ascochyta rabiei in Pakistan presents a dynamic and persistent threat to chickpea production, characterized by high intra-population genetic variation, substantial pathogenic variability, and extensive gene flow across agro-ecological zones, and the evolutionary potential facilitated by historical recombination events despite current mating type imbalances. This diversity enables the pathogen to rapidly adapt to resistant cultivars and diverse environmental conditions, resulting in recurrent epidemics and significant economic losses, particularly in concentrated production areas like the Thal desert and more humid northern regions. Molecular marker systems (RAPD, SSR, ISSR, URP) have proven invaluable in elucidating population structure, tracing migration, and differentiating aggressive pathotypes, while genomic tools such as QTL mapping and SNP-based association studies have identified promising resistance loci for marker-assisted and genomic selection programs. To achieve durable disease management and ensure long-term sustainability of chickpea as a key food security crop in Pakistan, a proactive, integrated strategy is imperative. This includes ongoing national pathogen surveillance and fingerprinting, pyramiding multiple resistance genes, strengthening seed health certification, incorporating biocontrol and cultural practices, and developing climate-informed forecasting models. By bridging molecular insights with practical field interventions, Pakistan can anticipate and mitigate the pathogen's evolutionary advances, thereby securing the future productivity and resilience of this ancient pulse crop.

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