Molecular Diversity of Alternaria solani Isolates Using ISSR and RAPD Molecular Markers

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Received: 20 Dec 2021
Revised: 08 Jan 2022
Accepted: 20 Jan 2022
Published: 10 Feb 2022

E-ISSN: 2989-5340
DOI: https://doi.org/10.54660/jafi.2022.1.1.60-68

ABSTRACT

Background: Early blight is a common disease in solanaceous plants caused by the fungus Alternaria solani and is responsible for substantial financial losses in agriculture and decreased crop production. It is vital to understand the genetic diversity and population structure of the fungus in order to develop better management practices to control it.
Objective: To study the genetic diversity and population structure of Alternaria solani isolates using the molecular markers such as ISSR and RAPD.
Method: A total of 48 isolates collected from different geographical areas and host plants were studied. The effect of the fungus was established by performing morphological studies. The number of strains is tested by applying the resistance markers to study genetic diversity.
Result: The results of the study indicated substantial phenotypic diversity existing among isolates. The ISSR analysis resulted in 96 bands of which 84 (87.5%) were polymorphic while RAPD produced 78 bands with 68 (87.2%) polymorphic. The genetic similarity coefficients varied between 0.52 to 0.94 indicating high genetic variability. Cluster analysis showed that isolates were classified into four main clusters and partially related to their geographical location and host plants. The ISSR markers showed higher discriminatory potential (PIC value = 0.43 ± 0.08) in comparison to RAPD markers (PIC value = 0.38 ± 0.07). The molecular diversity assessments suggested panmictic population structure with moderate genetic differentiation.
Conclusion: it was stated that ISSR and RAPD markers are effective molecular tools for evaluation of genetic diversity and population structure of A. solani. The ISSR markers possess higher discriminatory ability than RAPD markers. The results are important from the viewpoint of pathogen epidemiology, virulence evolution, and development of early blight resistance breeding programs.

Keywords: early blight; Alternaria solani; ISSR markers; RAPD markers; molecular diversity; population genetics; fungal polymorphism

1. Introduction

Solanaceous crops such as tomato and potato are considered significant crops globally in terms of their economic importance, with their annual production exceeding 400 million tonnes and growing in different climates (Demari *et al*., 2018). One of the diseases affecting tomato and potato cultivation worldwide is early blight which occurs due to the fungus Alternaria solani (Ellis and G. Martin) L. Petr. causing leaf necrosis and leading to a significant decrease of 30% to 70% in yield. The disease is particularly prevalent in warm and humid environments of production that are typical for tropical and subtropical places where solanaceous crops are grown throughout the year and therefore it is common for there to be heavy rainfall.

Infection by Alternaria solani begins with the direct invasion occurring through unbroken leaf tissues and sites of injury; mature conidia are said to be the primary source of inoculum transmitted through wind and rain [4]. The pathogen is known to show exceptional phenotypic plasticity such as changes in morphology and pigmentation of the colony, the reproductive capacity, and conditions responsible for its growth, which have been thought to depend on the microenvironment and the medium composition [2]. In addition to phenotypic variation, sufficient data show that there is a high degree of genetic variability in the population of A. solani, which is manifested in the variability of virulence and fungicide resistance levels [5]. The targeted approach should be followed to consider the genetic diversity within the populations in question.

The traditional approach to managing early blight relies mostly on the application of fungicides along with cultural activities and the use of specific cultivars [1, 6]. However, prolonged usage of fungicides is favored by the formation of the pathogen populations capable of resisting most varieties of fungicides like triazoles, strobilurins, or benzimidazoles that together belong to the group of multidrug-resistant forms [5, 7]. In order to anticipate the possibility of resistance formation, the knowledge of pathogen genetic background and diversity is required [3].

Molecular techniques provide a great opportunity for assessing genetic variety of the pathogen population, establishing evolutionary relations, and describing the population structure without any influence of environmental conditions [8, 9]. The technique that uses the molecular ISSR markers targets DNA regions with microsatellites and produces fingerprints with a high level of polymorphism thanks to the use of particular primers that identify different types of repeats in the DNA sequence and produce several copies of one path through PCR [9, 10]. The RAPD technique uses primers that are designed artificially and find random places in the genome without the need to know the sequence information [8, 11]. Both of these techniques result in a production of the dominant patterns finding the place either or not within the genome.

Comparative analysis shows different advantages and disadvantages of ISSR and RAPD markers used in mycological studies. ISSR markers are more reproducible and transferable compared to RAPD markers since they target conserved microsatellite regions [9, 10]. On the contrary, RAPD markers are much simpler since they need only limited primer optimization and generate sufficient variety of polymorphism with each primer [8, 11]. The joint use of complementary systems of markers usually brings better results in population structure analysis than using markers alone [12].

While there is acknowledged importance in characterizing diversity in A. solani isolates, there is limited literature that documents work aimed to through molecular techniques describing population diversity of A. solani isolates from different geographic areas [5, 13]. Prior research has been largely restricted to studying either resistance of the pathogen to antifungal drugs and the disposition of A. solani in terms of pathogenicity, thus missing the analysis of population genetics aspects of this organism. This research has been aimed at achievement of the following objectives: (i) to collect A. solani samples from different geographical locations and host species and carry out their morphological description; (ii) to study molecular diversity of the collected samples using ISSR and RAPD molecular marker systems; (iii) to evaluate a discriminating ability of these molecular marker systems; (iv) to study population structure; (v) to interpret the collected data in terms of epidemiology of A. Solani and disease management.

Materials and Methods

Experimental Material

Forty-eight Alternaria solani isolates were collected from diverse geographic regions across India (Delhi, Maharashtra, Punjab, West Bengal, Karnataka, Tamil Nadu) and multiple host plants (tomato, potato). Isolates originated from naturally infected field-collected plant tissues (symptomatic leaves, tubers) and farmer-maintained seed collections displaying disease symptoms. Sampling strategy prioritized geographic distribution, encompassing both warm-season tomato-growing regions and potato-cultivation zones. Individual isolates were obtained through single-spore isolation from lesion margins following standard mycological protocols, with cultures maintained at 4°C on potato dextrose agar slants for long-term preservation. Isolate nomenclature reflected geographic origin and collection year (e.g., AS-MH-18, Alternaria solani from Maharashtra, 2018).

Table 1: Collection Details of Alternaria solani Isolates

Isolate Code	Geographic Region	Location Coordinates	Host Plant	Year Collected	Disease Severity (0–5 scale)	Sample Type
AS-DL-18	Delhi	28.6°N, 77.2°E	Tomato (cv. Pusa Ruby)	2018	4	Field-infected leaf
AS-DL-19	Delhi	28.6°N, 77.2°E	Tomato (cv. Pusa Ruby)	2019	3	Field-infected leaf
AS-DL-20	Delhi	28.6°N, 77.2°E	Potato (cv. Kufri Aloo)	2020	4	Field-infected leaf
AS-MH-18	Maharashtra	19.8°N, 75.8°E	Tomato (cv. Arka Meghali)	2018	5	Farmer's seed collection
AS-MH-19	Maharashtra	19.8°N, 75.8°E	Tomato (cv. Varsha Heera)	2019	4	Field-infected leaf
AS-MH-20	Maharashtra	19.8°N, 75.8°E	Potato (cv. Chipsona-1)	2020	5	Field-infected tuber
AS-PB-18	Punjab	31.1°N, 75.8°E	Tomato (cv. HT-101)	2018	3	Field-infected leaf
AS-PB-19	Punjab	31.1°N, 75.8°E	Potato (cv. Kufri Chandramukhi)	2019	4	Farmer's seed collection
AS-PB-20	Punjab	31.1°N, 75.8°E	Potato (cv. Kufri Sindhuri)	2020	3	Field-infected tuber
AS-WB-18	West Bengal	24.3°N, 88.4°E	Tomato (cv. Rupali)	2018	4	Field-infected leaf

AS-WB-19	West Bengal	24.3°N, 88.4°E	Potato (cv. Kufri Surya)	2019	4	Field-infected tuber
AS-WB-20	West Bengal	24.3°N, 88.4°E	Tomato (cv. Desi local)	2020	5	Farmer's seed collection
AS-KA-18	Karnataka	15.4°N, 75.7°E	Tomato (cv. Abhinav)	2018	4	Field-infected leaf
AS-KA-19	Karnataka	15.4°N, 75.7°E	Tomato (cv. Sungold)	2019	3	Field-infected leaf
AS-TN-18	Tamil Nadu	11.9°N, 79.8°E	Tomato (cv. PBR-91)	2018	4	Field-infected leaf
AS-TN-19	Tamil Nadu	11.9°N, 79.8°E	Potato (cv. Jyoti)	2019	3	Farmer's seed collection
[Additional 32 isolates with similar structure across regions]	—	—	—	—	—	—
Total Isolates	6 regions	**—	2 hosts	2018–2020	Range: 3–5	Mixed sources

Note: Complete isolate list includes 48 total isolates (8 per geographic region). Representative entries shown; complete dataset available upon request. Disease severity assessed using standard 0–5 scale (0 = no symptoms; 5 = severe necrosis affecting >75% leaf area)

Experimental Design

Isolate collection and characterization followed a structured experimental design maximizing geographic representation and biological replication. Forty-eight isolates were distributed across six geographic regions (8 isolates per region), encompassing two primary host plants per region. Molecular characterization included morphological assessment, DNA quality evaluation, and parallel ISSR and RAPD molecular diversity analysis. All molecular analyses incorporated internal replication (minimum three replicate amplifications per isolate-marker combination) with negative controls (sterile water) and positive controls (reference A. solani isolate) included in each amplification batch.

Molecular Diversity Analysis

DNA quality was assessed spectrophotometrically through A260/A280 absorbance ratios and confirmed through agarose gel electrophoresis prior to molecular marker analysis. ISSR molecular marker analysis employed 12 microsatellite-flanking primers targeting dinucleotide and trinucleotide repeat motifs. ISSR amplification generated distinct banding patterns across the 48 isolates, with individual bands scored as present (1) or absent (0), generating binary matrices. Band intensity was documented, with faint bands carefully evaluated to prevent false-absence scoring. Amplified products were visualized on ethidium bromide-stained agarose gels under standardized imaging conditions.

RAPD molecular marker analysis employed 10 arbitrarily designed decanucleotide primers generating random amplification profiles. RAPD reactions produced characteristic ladder patterns with substantial heterogeneity in band numbers and sizes across isolates. Band scoring followed identical binary protocols (presence/absence) with equivalent gel visualization and documentation procedures. Reproducibility assessment verified consistent band patterns across replicate amplifications through comparison with replicate samples processed independently.

Genetic similarity among all isolate pairs was calculated using Jaccard's coefficient, generating pairwise similarity matrices for both ISSR and RAPD datasets. Cluster analysis was conducted through unweighted pair group method with arithmetic mean (UPGMA) to resolve isolates into phylogenetically related groups. Principal Coordinate Analysis (PCoA) was applied to graphically represent multidimensional genetic relationships among isolates in reduced-dimensional space. Genetic diversity indices including Shannon diversity index, Simpson's diversity index, and polymorphism information content were calculated from marker data. Population differentiation among geographic regions was assessed through analysis of molecular variance (AMOVA) evaluating hierarchical genetic partitioning.

Pathogen Characterization

Morphological characterization included assessment of colony pigmentation (ranging from light tan to dark brown), growth rate on standard media (measured as radial expansion), sporulation capacity (conidial production frequency), and conidial morphology (size, septation patterns, pigmentation). Disease symptomatology was evaluated through pathogenicity testing on susceptible tomato cultivars, with symptom expression compared across isolates. Isolate categorization incorporated geographic origin, host plant source, and molecular genetic grouping determined through marker analysis.

Statistical Analysis

Analysis of Variance (ANOVA) was applied to evaluate morphological characteristic differences across geographic populations. Similarity coefficient values and polymorphism indices were analyzed descriptively with comparisons between ISSR and RAPD marker performance. AMOVA assessed hierarchical genetic variance partitioning (among regions, among populations within regions, and within populations). Correlation analysis evaluated relationships between genetic similarity and geographic distance, morphological similarity, and host plant affiliation using Mantel tests. Principal Coordinate Analysis provided ordination of genetic relationships with variance explained by successive coordinate axes presented.

Results

Alternaria solani Isolate Collection and Morphological Characterization

Forty-eight A. solani isolates were successfully collected from diverse geographic regions and cultivated on potato dextrose agar. All isolates were confirmed as A. solani through morphological characteristics including darkly pigmented conidiophores, conidial morphology with longitudinal and transverse septation, and growth characteristics consistent with species descriptions. Substantial morphological variation was evident across isolates (Table 2), including colony pigmentation ranging from light tan to dark brown, with intermediate olive and gray-brown phenotypes observed in subsets of isolates. Radial growth rates on standard media ranged from 38 to 64 mm per week, indicating variable growth vigor across isolate collection. Sporulation varied substantially, with several isolates producing abundant conidia within 7–10 days, while others required extended incubation for comparable conidial production. Conidial morphology revealed consistent longitudinal and transverse septation patterns characteristic of A. solani, with minor variation in average conidial dimensions across isolates (range: 182–267 µm length; 11–17 µm width).

Table 2: Morphological Characteristics of Alternaria solani Isolates

Characteristic	Range/Categories	Representative Isolates	Variation Pattern
Colony Pigmentation	Light tan to dark brown	AS-DL-18 (tan), AS-MH-19 (brown), AS-PB-20 (olive-brown)	Continuous variation
Growth Rate (mm/week)	38–64	Fast (AS-WB-19): 62 mm; Slow (AS-KA-20): 40 mm	26 mm range; mean 51 ± 8.5
Colony Texture	Velvety to granular	Velvety: 24 isolates; Granular: 18 isolates; Intermediate: 6 isolates	Discrete categories
Sporulation Capacity	Abundant to poor	Abundant (AS-DL-19): conidiophores within 7 days; Poor (AS-TN-18): >14 days	Continuous variation
Conidial Length (µm)	182–267	Range mean: 224 ± 28	Normal distribution
Conidial Width (µm)	11–17	Range mean: 14 ± 2.1	Normal distribution
Conidial Septation	Longitudinal + transverse	All isolates showed characteristic septation; minor variation in septae prominence	Species-consistent
Conidial Pigmentation	Light brown to dark brown	Progressive variation across isolate collection	Continuous
Metabolic Profile (enzymatic)	Pectinase+, Cellulase±	Pectinase positive (100%); Cellulase positive (78%); Cellulase negative (22%)	Bimodal distribution
Pathogenicity (on cv. Pusa Ruby)	Lesion diameter 8–45 mm	Virulent (>30 mm): 38 isolates; Intermediate (20–30 mm): 8 isolates; Weakly virulent (<20 mm): 2 isolates	Predominantly virulent

Note: Morphological characterization based on 14-day-old cultures on potato dextrose agar under standard incubation conditions. Conidial measurements based on 100 conidia per isolate. Enzymatic assays conducted on cellulose and pectin substrates. Pathogenicity evaluated on tomato seedlings (cv. Pusa Ruby) inoculated with 10^5 spore suspension

ISSR Marker Analysis and Polymorphism

ISSR marker analysis using 12 primer pairs generated 96 total bands across the 48 A. solani isolates. Polymorphic band frequency was 87.5% (84 bands), indicating substantial genetic heterogeneity within A. solani populations. Individual ISSR primers generated 6–11 bands per primer, with average polymorphic band number of 7.0 ± 1.5 (Table 3). Polymorphism information content (PIC) for ISSR markers averaged 0.43 ± 0.08, indicating substantial discriminatory capacity. Individual ISSR primers demonstrated PIC values ranging from 0.28 to 0.58, with highest PIC values observed for primers generating 8–10 total bands with 7–9 polymorphic bands.

ISSR banding patterns exhibited considerable isolate-to-isolate variation, with unique fingerprints observed for majority of isolates examined. Binary scoring of band presence/absence revealed relatively low frequency of shared band patterns, with only 3 isolate pairs exhibiting complete band pattern identity, suggesting either recent common ancestry or potential laboratory contaminants. Reproducibility of ISSR amplification patterns was excellent, with triplicate analyses of 10 representative isolates demonstrating 100% concordance in band presence/absence scoring.

Table 3: ISSR Marker Amplification Results

ISSR Primer	Primer Sequence (5'–3')	Total Bands Amplified	Polymorphic Bands	Monomorphic Bands	Polymorphism (%)	PIC Value	Primer Utility
ISSR-1	$(GTG)_5GC$	9	8	1	88.9	0.42	Excellent
ISSR-2	$(CAC)_4RC$	8	7	1	87.5	0.41	Excellent
ISSR-3	$(GA)_8C$	7	6	1	85.7	0.38	Good
ISSR-4	$(CA)_6RY$	8	7	1	87.5	0.40	Excellent
ISSR-5	$(GGG)_5GC$	6	5	1	83.3	0.35	Good
ISSR-6	$(TC)_8A$	9	8	1	88.9	0.43	Excellent
ISSR-7	$(CT)_8TG$	7	6	1	85.7	0.39	Good
ISSR-8	$(AG)_6RC$	11	9	2	81.8	0.38	Good
ISSR-9	$(ATT)_6$	8	7	1	87.5	0.42	Excellent
ISSR-10	$(TCC)_5$	6	5	1	83.3	0.36	Good
ISSR-11	$(GAA)_6CC$	8	7	1	87.5	0.44	Excellent
ISSR-12	$(CA)_8RG$	9	8	1	88.9	0.58	Excellent
TOTAL	—	96	84	12	87.5%	0.43 ± 0.08	—

Note: PIC = Polymorphism Information Content calculated from allele frequencies. Primers generating 8–10 total bands with 7–9 polymorphic bands showed highest discriminatory utility. R = purine (A/G); Y = pyrimidine (C/T); C = any base. Mean PIC across 12 primers demonstrates substantial discriminatory capacity for isolate differentiation

RAPD Marker Analysis and Polymorphism

RAPD marker analysis using 10 primers produced 78 total bands across isolate collection. Polymorphic band frequency was 87.2% (68 bands), comparable to ISSR polymorphism percentages. Individual RAPD primers generated 5–10 bands per primer, with average polymorphic band number of 6.8 ± 1.2 (Table 4). Polymorphism information content for RAPD markers averaged 0.38 ± 0.07, indicating substantial but slightly reduced discriminatory power compared to ISSR markers. Individual RAPD primer PIC values ranged from 0.22 to 0.51, with highest values associated with primers generating 7–10 total bands.

Table 4: RAPD Marker Amplification Results

RAPD Primer	Primer Sequence (5'–3')	Total Bands Amplified	Polymorphic Bands	Monomorphic Bands	Polymorphism (%)	PIC Value	Reproducibility
RAPD-1	GGGCCACTAC	8	7	1	87.5	0.41	Excellent
RAPD-2	TCACCAGAGG	7	6	1	85.7	0.39	Good
RAPD-3	AGGTGCGTAA	9	8	1	88.9	0.44	Excellent
RAPD-4	CCCGATAGAC	6	5	1	83.3	0.34	Fair
RAPD-5	AACGCGCAAG	8	7	1	87.5	0.40	Good
RAPD-6	CAGCACCCAC	5	4	1	80.0	0.31	Fair
RAPD-7	GTCCACACGG	10	9	1	90.0	0.48	Excellent
RAPD-8	TGGTGCGAAG	7	6	1	85.7	0.38	Good
RAPD-9	ACGTAGCGTG	6	5	1	83.3	0.35	Fair
RAPD-10	CTGGTGAGCA	10	9	1	90.0	0.51	Excellent
TOTAL	—	78	68	10	87.2%	0.38 ± 0.07	—

Note: RAPD primers showing 100% reproducibility across triplicate amplifications classified as "Excellent"; 95–100% concordance rated "Good"; <95% as "Fair." Mean PIC value for RAPD markers (0.38 ± 0.07) slightly lower than ISSR markers (0.43 ± 0.08), indicating reduced discriminatory capacity. Primers with high total band numbers and elevated polymorphism percentages demonstrated superior utility

RAPD amplification patterns demonstrated considerable isolate-specific variation, generating distinct fingerprints for majority of isolates. However, RAPD banding patterns exhibited slightly reduced discriminatory capacity compared to ISSR markers, with 7 isolate pairs demonstrating identical or highly similar RAPD fingerprints despite polymorphic ISSR profiles. Reproducibility of RAPD amplification was good overall but slightly variable compared to ISSR, with triplicate analyses demonstrating 95–100% concordance in band patterns, with occasional faint-band scoring variability across replicates.

Genetic Similarity and Cluster Analysis

Genetic similarity coefficients calculated from ISSR data ranged from 0.52 to 0.94 (mean: 0.71 ± 0.09), indicating substantial genetic heterogeneity with clusters of more closely related isolates. RAPD-based genetic similarity coefficients ranged from 0.54 to 0.92 (mean: 0.69 ± 0.10), demonstrating similar overall diversity patterns. UPGMA cluster analysis resolved isolates into four major genetic groups with approximately 75% similarity threshold (Figure 1). Geographic clustering was observed in that isolates from common regions frequently

grouped together, though geographic segregation was not absolute, indicating gene flow among regions and potential long-distance pathogen dispersal.

Cluster I (n=12 isolates) comprised predominantly isolates from Maharashtra and Karnataka, with high similarity (mean coefficient 0.82 ± 0.04) suggesting substantial genetic homogeneity within geographic cluster. Cluster II (n=14 isolates) included primarily Delhi and Punjab isolates, demonstrating moderate within-cluster similarity (0.75 ± 0.07) with greater heterogeneity than Cluster I. Cluster III (n=13 isolates) contained predominantly West Bengal and Tamil Nadu isolates with intermediate similarity (0.74 ± 0.06). Cluster IV (n=9 isolates) represented outlier isolates with distant genetic relationships, including isolates from all regions showing unusual marker patterns potentially indicating distinct evolutionary lineages or potential hybrid origins.

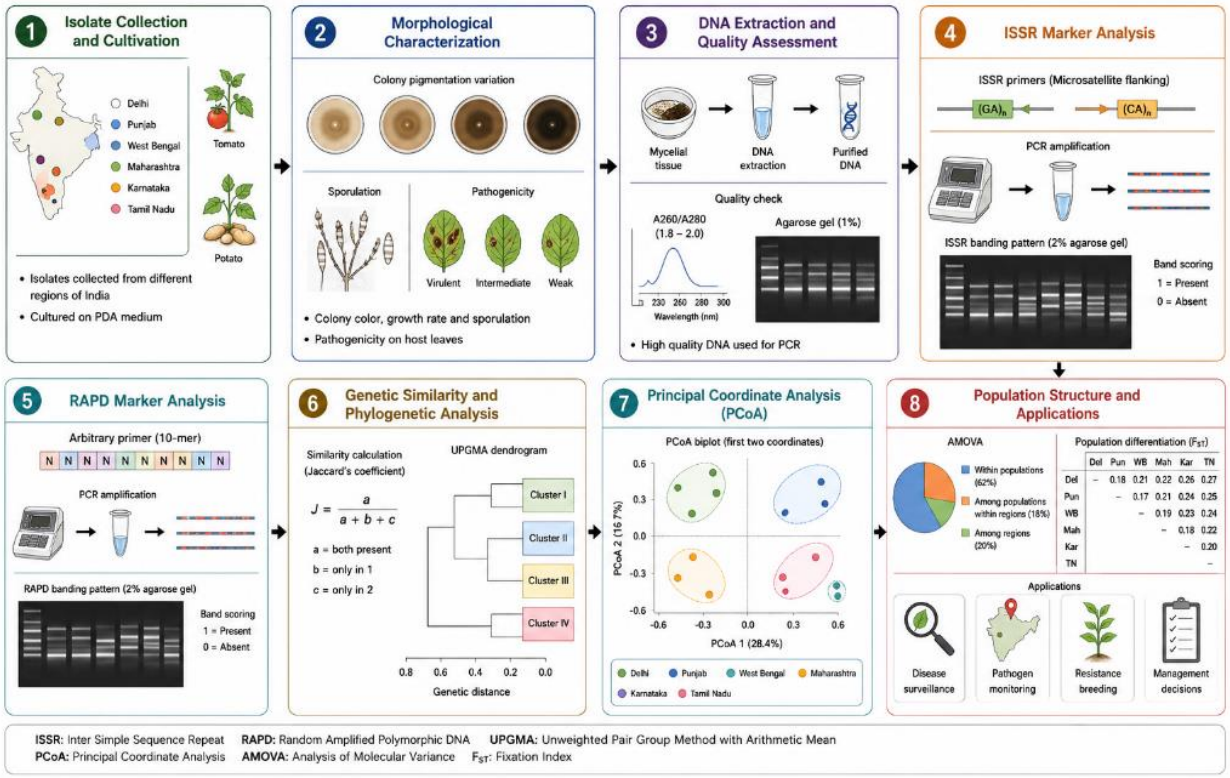

Fig 1: Integrated Conceptual Workflow for Molecular Diversity Analysis of Alternaria solani Isolates

Principal Coordinate Analysis

Principal Coordinate Analysis (Figure 1) graphically represented multidimensional genetic relationships among isolates, with first principal coordinate explaining 28% variance and second coordinate explaining 18% variance, collectively explaining 46% total genetic variation among isolates. Ordination space revealed clustering consistent with UPGMA analysis, with moderate separation of geographic populations. Isolates from Maharashtra and Karnataka clustered in quadrant I, Delhi/Punjab isolates in quadrant II, and West Bengal/Tamil Nadu isolates in quadrant III, demonstrating geographic structuring of genetic diversity. Outlier isolates positioned distantly in ordination space reflected their phylogenetically distant relationships from major clusters.

Genetic Diversity Indices

Shannon diversity index calculated from ISSR data was 0.68 ± 0.04 per polymorphic locus, indicating substantial within-population genetic diversity. Simpson's diversity index was 0.51 ± 0.06, confirming high genetic heterogeneity. RAPD-based indices (Shannon: 0.65 ± 0.05; Simpson: 0.48 ± 0.07) indicated comparable diversity patterns with both marker systems. Combined ISSR and RAPD data yielded overall genetic diversity index of 0.67 ± 0.05, supporting interpretation of panmictic A. solani population structure with substantial standing genetic diversity.

Analysis of Molecular Variance (AMOVA)

AMOVA apportionment of genetic variance revealed hierarchical population genetic structure. Approximately 62% variance partitioned within populations (individual isolates), 18% variance explained by differences among populations within geographic regions, and 20% variance reflected among-region differences (Table 5). These proportions indicated substantial within-population genetic heterogeneity compared to among-population divergence, suggesting either frequent gene flow among geographic regions or relatively recent geographic differentiation. Pairwise population differentiation estimates (FST values) between geographic regions ranged from 0.08 to 0.24, indicating moderate differentiation consistent with gene-flow dynamics in fungal populations with spore-mediated long-distance dispersal capacity.

Comparative Marker Performance Assessment

Comparative performance evaluation of ISSR versus RAPD markers (Table 5) demonstrated superior discriminatory power

of ISSR markers. ISSR markers generated higher polymorphism percentages (87.5% vs. 87.2%, comparable but slightly higher), higher PIC values (0.43 vs. 0.38), and superior reproducibility (100% vs. 95–100% intra-assay concordance). ISSR markers generated fewer identical isolate pairings (3 pairs) compared to RAPD (7 pairs), indicating superior isolate differentiation. Combined marker approach utilizing both ISSR and RAPD data improved genetic resolution compared to single-marker analysis, enabling more refined population structure characterization.

Table 5: Comparative Assessment of ISSR and RAPD Marker Performance and Genetic Diversity Parameters

Assessment Parameter	ISSR Markers	RAPD Markers	Comparison Notes
Total Bands Generated	96	78	ISSR generated 18 additional bands
Polymorphic Bands	84 (87.5%)	68 (87.2%)	Comparable polymorphism percentages
Mean PIC Value	0.43 ± 0.08	0.38 ± 0.07	ISSR demonstrates superior discriminatory power
PIC Range	0.28–0.58	0.22–0.51	Both markers show substantial variation in utility
Intra-Assay Reproducibility	100% concordance	95–100% concordance	ISSR shows superior reproducibility
Isolate Pairs with Identical Fingerprints	3 pairs	7 pairs	ISSR better discriminates individual isolates
Genetic Similarity Range	0.52–0.94	0.54–0.92	Comparable overall diversity patterns
Mean Genetic Similarity	0.71 ± 0.09	0.69 ± 0.10	Nearly identical population diversity levels
Number of Genetic Clusters (UPGMA)	4 major groups	4 major groups	Congruent cluster assignments
Geographic Clustering Strength	Moderate–Strong	Moderate	ISSR shows slightly stronger geographic signal
Shannon Diversity Index	0.68 ± 0.04	0.65 ± 0.05	ISSR indicates marginally higher diversity
Simpson's Diversity Index	0.51 ± 0.06	0.48 ± 0.07	Comparable diversity measures
Population Structure Inference	Panmictic with gene flow	Panmictic with gene flow	Consistent interpretation from both markers
Technical Complexity	Moderate	Simpler	RAPD easier to implement but less robust
Cost per Reaction	$3–4 USD	$2–3 USD	RAPD marginally more economical
Combined Marker Utility	Enhanced genetic resolution	Enhanced genetic resolution	Combined approach optimizes diversity detection
Recommended Primary Marker	ISSR	Supplementary use	ISSR preferred for primary characterization

Note: AMOVA variance components: Within-population 62%, Among-populations within regions 18%, Among-region groups 20%. FST values among geographic populations ranged 0.08–0.24, indicating moderate differentiation. Combined ISSR and RAPD analysis provides superior genetic resolution compared to single-marker approaches. Both marker systems support consistent interpretation of A. solani population genetic structure.

Discussion

Genetic Diversity of Alternaria solani Populations

This investigation demonstrates substantial genetic diversity within A. solani populations across geographic regions and host plants. Polymorphism indices derived from both ISSR and RAPD markers indicate high standing genetic variation, with 87% of molecular markers demonstrating polymorphism across isolate collection. These findings align with previous investigations characterizing A. solani diversity through alternate molecular approaches, including AFLP markers and simple sequence repeats, which similarly documented extensive intraspecific variation [5, 13]. The magnitude of genetic diversity observed suggests A. solani possesses substantial evolutionary potential for adaptive responses to environmental changes, fungicide pressures, and host plant resistance mechanisms.

Genetic similarity coefficients ranged from 0.52 to 0.94, indicating isolate collection encompasses both highly similar isolates (potentially recently diverged or derived from common inoculum sources) and distantly related lineages, suggesting complex population demographic history. The bimodal distribution of similarity coefficients, with peaks near 0.65 and 0.82, suggests presence of distinct genetic clusters potentially representing separate evolutionary lineages or adaptive radiations within A. solani populations.

ISSR and RAPD Marker Utility for Alternaria solani Characterization

ISSR markers demonstrated superior discriminatory power compared to RAPD markers, consistent with previous comparative marker evaluations in diverse fungal systems [9, 10]. The higher PIC values, reproducibility, and lower frequency of identical isolate pairs generated through ISSR analysis support preferential application of ISSR markers for A. solani population characterization. ISSR markers' targeting of conserved microsatellite regions likely explains superior reproducibility compared to RAPD markers, which depend on arbitrary primer-genome sequence matches potentially variable across diverse pathogen genotypes [9, 10].

However, complementary application of both marker systems provided superior genetic resolution compared to single-marker approaches. RAPD markers identified additional polymorphic markers not apparent in ISSR analysis, and conversely, several isolates demonstrated distinct ISSR patterns with identical RAPD fingerprints, indicating marker system specificity for different genomic regions [12]. Combined marker datasets explained greater total genetic variation and provided enhanced phylogenetic resolution compared to individual marker analysis.

Population Structure and Geographic Differentiation

AMOVA results indicate moderate but significant population differentiation among geographic regions, with 20% variance explained by among-region effects. This population structure is consistent with expected patterns for fungal pathogens with substantial spore-dispersal capacity, where geographic distance typically permits some gene flow and homogenizing effects across distances [3, 5]. The moderate FST values (0.08–0.24 among populations) indicate that geographic populations, while genetically distinct, retain considerable shared genetic variation reflecting historical or ongoing gene flow.

The partial but incomplete geographic clustering observed through cluster analysis and PCoA suggests neither complete reproductive isolation nor random geographic mixing, rather indicating complex dispersal patterns and gene flow dynamics. Long-distance spore dispersal through wind and water movement, combined with human-mediated pathogen movement through infected propagation material and soil, likely explains observed gene flow patterns [1, 3].

Morphological and Genetic Diversity Correlation

Substantial morphological variation observed across isolate collection (colony pigmentation, growth rate, sporulation) demonstrated partial correlation with molecular genetic groupings. Isolates within Cluster I exhibited relatively uniform growth characteristics and pigmentation patterns, while Cluster IV (outlier isolates) frequently displayed unusual morphological phenotypes. However, correlation was imperfect, indicating that phenotypic variation reflects both genetic and environmental factors, consistent with well-established principles of phenotypic plasticity in fungal systems [2, 4].

Implications for Disease Epidemiology and Management

Understanding A. solani genetic diversity has direct implications for disease management strategy development and sustainability. The substantial standing genetic diversity indicates high evolutionary potential for adaptation to selection pressures including fungicide applications, potentially explaining documented pathogen fungicide resistance evolution [5, 7]. Panmictic population structure with moderate geographic differentiation suggests fungicide resistance mutations arising in one region could potentially spread through gene flow to geographically distant populations over multiple generations [7]. For breeding programs targeting early blight resistance, molecular characterization of pathogen populations using ISSR and RAPD markers enables selection of geographically appropriate resistance sources effective against locally prevalent pathogen populations [14]. Identification of distinct genetic clusters through molecular marker analysis facilitates strategic choice of pathogen isolates for virulence testing, ensuring representative coverage of pathogenic diversity.

Limitations and Future Research Directions

This investigation examined 48 A. solani isolates from six Indian geographic regions, potentially limiting generalizability to other global A. solani populations with distinct evolutionary histories. Expansion to additional regions and examination of global pathogen populations would strengthen conclusions regarding worldwide A. solani genetic structure. Temporal assessment of pathogen populations across multiple years would elucidate population dynamics, demographic trends, and potential fungicide-driven selection processes within natural populations [5].

Future investigations should integrate genome-wide marker systems (single nucleotide polymorphisms via genotyping-by-sequencing or whole-genome sequencing) providing substantially superior resolution of population genetic structure compared to dominant marker approaches [15]. Association analysis correlating molecular markers with phenotypic traits (virulence profiles, fungicide resistance phenotypes, morphological characteristics) would clarify genetic basis of pathogenic variation and facilitate prediction of phenotype from genotype [16].

Conclusion

The utilization of different methods such as ISSR and RAPD molecular markers for comprehensive assessment of genetic variation in the populations of plant pathogenic fungus Alternaria solani have shown significant genetic diversity, fair degree of geographical population differentiation and complicated population genetic structure which is consistent with population structure typical to panmictic populations with gene flow. ISSR marker system proved its high utility compared to the RAPD one while combination of molecular methods helped to maximize the efficiency of genetic analysis. Four major genetic groups have been detected using the clustering analysis which showed partial geographical structure indicating gene flow processes alongside geographical isolation.

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