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GENETIC DIVERSITY AND POPULATION STRUCTURE OF *XANTHOMONAS ORYZAE* PV. *ORYZAE* ISOLATES FROM ANDHRA PRADESH AND TELANGANA INDIA

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ABSTRACT

Bacterial Blight (BB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), remains a major constraint to rice productivity across South Asia. Understanding the genetic diversity and population structure of *Xoo* at regional scales is critical for deploying durable resistance and designing effective surveillance. Here, we characterized 148 *Xoo* isolates collected across Andhra Pradesh and Telangana during the rice-growing season of *kharif*-2024 using insertion sequence–based PCR (IS-PCR) fingerprinting with the JEL-3 (J3) primer targeting IS1112/IS1113-flanked genomic regions. JEL-3 IS-PCR generated 916 reproducible fragments (4–11 bands per isolate), yielding a binary matrix of 21 reproducible loci. Hierarchical clustering (UPGMA; Manhattan distance) resolved two major clusters: Cluster I comprised Telangana isolates exclusively, whereas Cluster II contained both Andhra Pradesh and Telangana isolates, indicating strong but incomplete geographic structuring (bootstrap = 100% at the primary split). Principal coordinate analysis (PCoA) corroborated this structure, with Axis 1 and Axis 2 explaining 83.42% and 16.50% of the variation, respectively and showing complete separation of TS (compact) and AP (more dispersed) populations. Minimum spanning network (MSN) analysis indicated greater genotypic connectivity and branching in AP, consistent with higher within-population diversity. Analysis of molecular variance (AMOVA) attributed 83.42% of total variation to among-population differences (ÖST = 0.8342, P < 0.001). Pairwise Nei's GST between AP and TS was 0.357, indicating strong differentiation. Genetic diversity indices revealed higher genotypic richness and evenness in Telangana (15 MLGs; H = 2.52; E5 = 0.860) than Andhra Pradesh (10 MLGs; H = 1.77; E5 = 0.709), despite the latter's larger sample size. A Mantel test detected no significant isolation-by-distance (r = 0.042, P = 0.312), implying that geographic distance alone does not explain the observed structure. Collectively, our results demonstrate pronounced geographic population structure in *Xoo* across AP and TS, with Telangana exhibiting a more diverse and evenly distributed set of multilocus genotypes. These findings have implications for regional BB management, resistance deployment strategies, and the design of pathogen monitoring frameworks in the rice ecosystems of peninsular India.

***Key words*:** *Xanthomonas oryzae* pv. *oryzae*, Bacterial Blight, genetic diversity, UPGMA, PCoA, AMOVA.

Introduction

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population and its stable production is essential for food security in Asia. In India, rice is cultivated across diverse agro-ecological zones, with the eastern and southern regions contributing substantially to national output. However, biotic stresses, particularly Bacterial Blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), continue to impose significant yield losses, especially under high-input, irrigated ecosystems and during periods of conducive weather (Nino-Liu *et al*., 2006).

BB epidemics are driven by complex interactions among pathogen populations, host resistance genes and agronomic practices. *Xoo* exhibits considerable genetic and pathotypic diversity, which complicates resistance breeding and the durability of deployed resistance genes. In South and Southeast Asia, multiple *Xoo* races/ pathotypes have been reported, and their frequencies often shift following the introgression of major resistance genes (e.g., *Xa4*, *xa5*, *xa13*, *Xa21*, *Xa23*) into elite cultivars (Leach *et al*., 1992; Adhikari *et al*., 1999; Vera Cruz *et al*., 2000). Consequently, region-specific characterization of *Xoo* populations is vital to guide resistance gene pyramiding, monitor pathogen evolution, and inform integrated disease management.

Molecular fingerprinting has been extensively used to investigate the genetic structure of *Xoo* populations. Insertion sequence (IS) elements, particularly IS1112 and IS1113, are abundant in the *Xoo* genome and provide highly polymorphic markers for strain differentiation. Leach *et al*. (1992) first demonstrated that IS-PCR using JEL primers effectively resolved genetic relationships among *Xoo* isolates and enabled the characterization of population diversity. Subsequent studies employing IS-PCR and other molecular marker systems, including rep-PCR, AFLP, MLVA, and, more recently, whole-genome sequencing and SNP-based analyses, have revealed substantial geographic population structure, clonal expansion of successful lineages, and genomic diversification associated with pathogen adaptation and evolution (Adhikari *et al*., 1999; Vera Cruz *et al*., 2000; Triplett *et al*., 2016). Andhra Pradesh and Telangana together constitute a major rice bowl of peninsular India, with extensive irrigated area in coastal deltas (e.g., Godavari–Krishna basins) and expanding rice cultivation in northern Telangana. BB is frequently reported in these regions, yet comprehensive population-level analyses of *Xoo* using standardized, reproducible markers remain limited. Understanding whether *Xoo* populations in these neighboring states are genetically differentiated and how diversity is distributed within and among districts is essential for altering the resistance deployment and surveillance.

In this study, we collected 148 *Xoo* isolates from infected rice fields across Andhra Pradesh and Telangana and applied JEL-3 (J3) IS-PCR to generate binary fingerprinting profiles and analyzed the resulting multilocus dataset using hierarchical clustering (UPGMA), principal coordinate analysis (PCoA), minimum spanning network (MSN), analysis of molecular variance (AMOVA), pairwise Nei's GST, diversity indices and isolation-by-distance (Mantel test).

Materials and Methods

Study area and sampling design

Field surveys were conducted during the rice-growing season (*Kharif* -2024) in the major rice-producing districts of Andhra Pradesh and Telangana, India. Symptomatic rice leaves showing typical BB lesions (water-soaked margins, wavy lesion edges, yellowing) were collected from farmers' fields. A total of 148 *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates were obtained from infected rice fields representing diverse agro-ecological conditions. The collection comprised of 107 isolates from Andhra Pradesh and 41 isolates from Telangana. At each sampling location, geographic coordinates (latitude and longitude) were recorded in the field using a handheld Global Positioning System (GPS) receiver and the spatial distribution of the isolates was visualized in R version 4.5.1. (R Core Team, 2025).

Isolation, DNA Extraction and JEL-3 (IS-PCR) Fingerprinting

Pure cultures of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) were obtained by single-colony isolation from typical colonies grown on Modified Wakimoto Agar (MWA). Genomic DNA was extracted from *Xanthomonas oryzae* pv. *oryzae* isolates following the modified protocol of George *et al*. (1997) as described by Ladhalakshmi and Rabindran (2012) and Yugander *et al*. (2017). Briefly, 3-day-old bacterial cultures grown on Modified Wakimoto's Agar (MWA) at 28 ± 2°C were suspended in DNA extraction buffer (400 mM Tris-HCl, pH 8.0; 60 mM EDTA, pH 8.0; 150 mM NaCl; 1% SDS) and lysed at room temperature for 10 min. Cellular debris was precipitated with potassium acetate (pH 4.8), followed by centrifugation. The supernatant was purified using chloroform:isoamyl alcohol (24:1), and genomic DNA was precipitated with chilled isopropanol. The DNA pellet was washed with 70% ethanol, air-dried, dissolved in 50–100 µL DNase-free water or TE buffer, and stored at -20°C until further use. DNA quality and concentration were assessed by agarose gel electrophoresis and spectrophotometry, working DNA solutions (20–50 ng μL^{-1}) were stored at -20°C.

Genetic diversity was assessed by insertion sequence-based PCR (IS-PCR) using the JEL-3 (J3) primer (52 – GCTCAGGTCAGGTCGCCTGG–32), which amplifies genomic regions flanked by the insertion sequence elements IS1112 and IS1113 (Leach *et al*., 1992). PCR amplification was performed in a 25 µL reaction mixture containing genomic DNA, 1X PCR buffer, $MgCl_2$, dNTPs, JEL-3 primer, Taq DNA polymerase and nuclease-free water. Amplification was carried out under

optimized cycling conditions, and PCR products were separated by electrophoresis on a gel containing 0.5% agarose and 0.75% Synergel agarose in 1× TAE buffer, stained with ethidium bromide, visualized under UV illumination, and documented using a gel documentation system. Only clear and reproducible bands were scored for subsequent analyses.

Scoring of binary matrix

Only clear and reproducible amplification bands were scored, and the resulting profiles were converted into a binary matrix by assigning band presence as 1 and absence as 0 for subsequent analyses. Fragment sizes were assigned to discrete loci based on the fragment size of 100-bp DNA ladder and only consistently reproducible bands were retained. The final binary matrix was comprised of 21 JEL-3 loci across 148 *Xanthomonas oryzae* pv. *oryzae* isolates (Leach *et al*., 1992; Vos *et al*., 1995).

Data analysis environment

All spatial processing, statistical analyses and visualizations were performed in R version 4.5.1 (R Core Team, 2025). Key packages used were, sf and ggplot2 for mapping and plotting (Pebesma, 2018; Wickham, 2016); phangorn and ape for UPGMA and bootstrapping (Schliep, 2011; Paradis and Schliep, 2019); vegan for Principal Coordinate Analysis (PCoA) and Mantel tests (Oksanen *et al*., 2001); and poppr for Minimum Spanning Network (MSN) analysis, Analysis of Molecular Variance (AMOVA), Nei's genetic differentiation (GST) and estimation of genetic diversity indices (Kamvar *et al*., 2014), while pairwise geographic distances for the isolation-by-distance (IBD) analysis were calculated using the geosphere package (Hijmans *et al*., 2019)

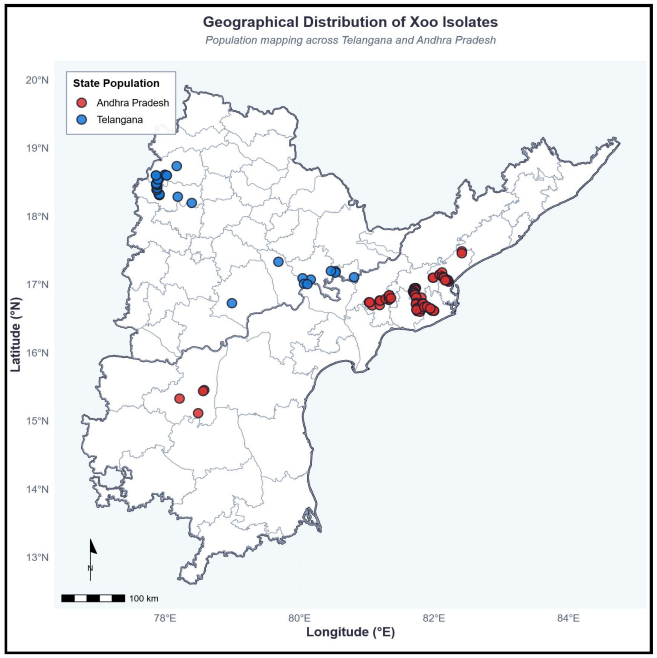


Fig. 1: Geographical distribution of 148 *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates collected from major rice-growing districts of Andhra Pradesh and Telangana.

Results

Geographical distribution of *Xanthomonas oryzae* pv. *oryzae* isolates

A total of 148 *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates were collected from BB-infected leaf samples collected from rice fields across the major rice-growing regions of Andhra Pradesh and Telangana (Fig. 1). Among the collected isolates, 107 (72.3%) were from Andhra Pradesh and 41 (27.7%) were from Telangana. In Andhra Pradesh, the isolates were collected from six districts: West Godavari (42 isolates), Eluru (23), Konaseema (19), East Godavari (15), Kakinada (4), and Nandyal (4). West Godavari contributed the highest number of isolates, followed by Eluru and Konaseema. In Telangana, isolates were collected from four districts: Kamareddy (15 isolates), Nizamabad (12), Khammam (10), and Karimnagar (4), with Kamareddy representing the largest sampling locations within the state.

The geographical distribution map (Fig. 1) demonstrated that the sampling locations were well distributed across the principal rice-growing districts of both states. A higher density of sampling sites was observed in the Godavari delta and adjoining coastal regions of Andhra Pradesh, reflecting the extensive cultivation of rice and the frequent occurrence of bacterial leaf blight in these areas. In Telangana, sampling sites were primarily concentrated in the northern and central rice-growing districts. The broad spatial coverage of the sampling locations ensured a representative collection of *Xoo* isolates and provided a robust foundation for subsequent analyses of genetic diversity and population structure.

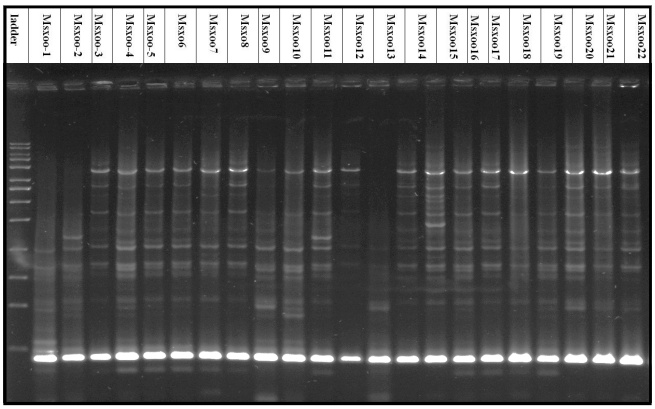


Fig. 2: Gel electrophoresis image showing JEL-3 DNA fingerprinting patterns of regional *Xanthomonas oryzae* pv. *oryzae* isolates.

JEL-3 (IS-PCR) fingerprinting profiles

DNA fingerprinting of the 148 *Xoo* isolates using the JEL-3 (J3) insertion sequence primer generated highly polymorphic and reproducible amplification profiles (Fig. 2). The J3 primer successfully amplified genomic regions flanked by the insertion sequence elements IS1112 and IS1113, producing distinct DNA fingerprinting patterns among the isolates.

A total of 916 reproducible amplification fragments were obtained across all isolates. The number of amplified fragments per isolate ranged from 4 to 11, indicating substantial genetic polymorphism within the *Xoo* population. The highest number of fragments (11 bands) was observed in isolates MSXoo #11, #15 and #20, whereas the lowest number (4 bands) was recorded in isolates MSXoo #99, #100, #104–110, #115–119 and #121–126. The remaining isolates exhibited intermediate banding profiles, demonstrating considerable variation in the distribution of insertion sequence elements within the *Xoo* genome.

The representative JEL-3 fingerprinting profile (Fig. 2) clearly illustrates the polymorphic amplification patterns generated among representative isolates. Differences in both the number and size of amplified fragments confirmed the effectiveness of the J3 primer in discriminating genetically distinct *Xoo* isolates. The reproducibility and high level of polymorphism observed among the amplification profiles demonstrate the suitability of JEL-3 IS-PCR for assessing genetic diversity within *Xoo* populations (Leach *et al*., 1992).

All reproducible amplification fragments were scored as binary characters based on their presence (1) or absence (0), generating a multilocus binary dataset for the 148 *Xoo* isolates. The final informative dataset comprised 21 JEL-3 loci used in all downstream analyses.

UPGMA cluster analysis

Hierarchical cluster analysis of the multilocus JEL-3 (IS-PCR) binary dataset, based on Manhattan genetic distances and the UPGMA method, grouped the 148 *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates into two major clusters (Fig. 3). The principal division was supported by 100% bootstrap support, indicating high confidence in the observed genetic separation (Felsenstein, 1985).

Cluster I comprised Telangana isolates exclusively and was further subdivided into several well-supported subclusters, indicating genetic differentiation within the Telangana population. Cluster II comprised isolates from both Andhra Pradesh and Telangana and exhibited several subordinate subclusters, reflecting considerable genetic variability among the isolates. Isolates from geographically close locations occasionally showed close genetic relationships; however, geographically close locations did not consistently correspond to genetic similarity.

Overall, the UPGMA dendrogram indicated partial association between genetic structure and geographical origin. The exclusive grouping of Telangana isolates in Cluster I, together with the mixed representation of Andhra Pradesh and Telangana isolates in Cluster II, suggests regional differentiation within the *Xoo* population, although complete geographic separation was not evident. The formation of multiple subclusters further indicates substantial genetic diversity among the studied isolates.

In addition to UPGMA dendrogram provided a hierarchical representation of the genetic relationships among the *Xoo* isolates. Principal Coordinate Analysis (PCoA) was subsequently performed to obtain an independent multivariate visualization of the genetic distance matrix. This complementary analysis enabled a clearer assessment of population structure and the extent

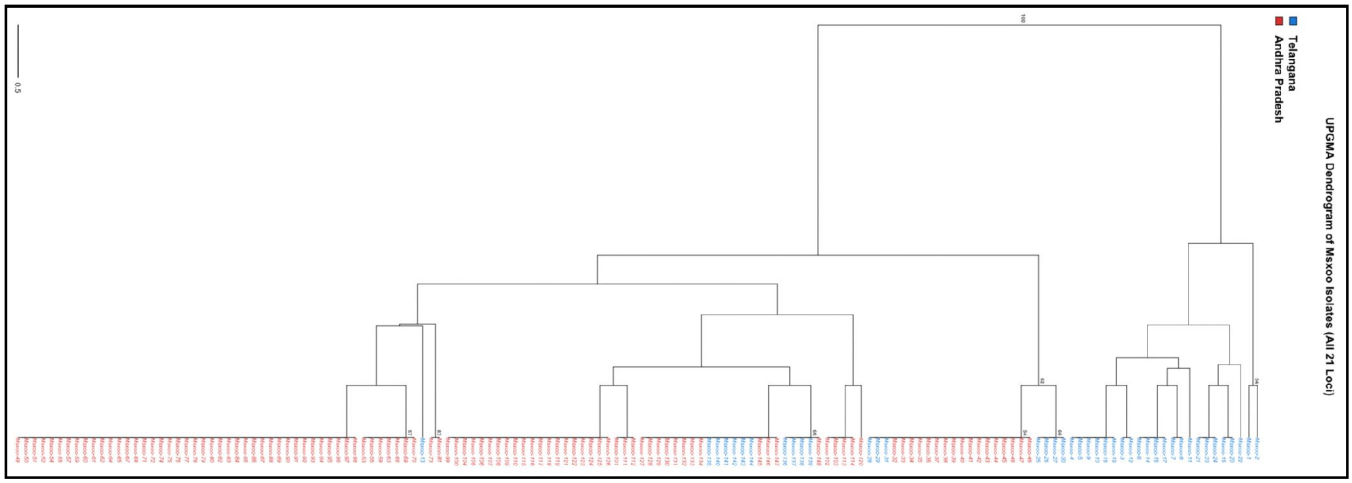


Fig. 3: UPGMA dendrogram showing the genetic relationships among 148 *Xanthomonas oryzae* pv. *oryzae* isolates based on JEL-3 (IS-PCR) binary marker profiles.

of genetic differentiation between isolates from Andhra Pradesh and Telangana.

Principal Coordinate Analysis (PCoA)

The Principal Coordinate Analysis (PCoA) ordination revealed a pronounced regional population structure that was largely congruent with the UPGMA clustering topology (Fig. 4). The first two principal coordinate axes explained 83.42% and 16.50% of the total genetic variation, respectively, together accounting for 99.92% of the overall molecular variation. The separation of isolates along the first principal coordinate indicated marked genetic differentiation between the *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) populations of Andhra Pradesh and Telangana. The majority of the Telangana isolates (n = 41) formed a compact cluster on the negative side of PCoA Axis 1, reflecting relatively uniform multilocus JEL-3 fingerprinting profiles. However, a small subset of Telangana isolates was positioned within the positive coordinate space and clustered closely with Andhra Pradesh isolates, resulting in limited overlap between the two regional populations. In contrast, the Andhra Pradesh isolates (n = 107) were distributed over a broader ordination space, particularly along PCoA Axis 2, indicating comparatively greater within-population genetic variability. Despite the limited overlap observed for a few isolates, the overall ordination pattern clearly supported the existence of distinct regional population structure while highlighting shared multilocus genotypes among a small proportion of isolates.

To further resolve the genetic relationships and connectivity among the isolates, Minimum Spanning Network (MSN) analysis was performed based on the multilocus genotypes (MLGs) identified from the JEL-3 (IS-PCR) profiles. An MLG represents a unique combination of marker states across the analyzed loci and serves as a genetic unit for assessing relationships among isolates. The MSN provided a graphical representation of the genetic connections and differentiation among the identified MLGs. The network revealed that most MLGs were region-specific, whereas only a few MLGs were shared between Andhra Pradesh and Telangana, suggesting limited genetic exchange between the two regional *Xoo* populations.

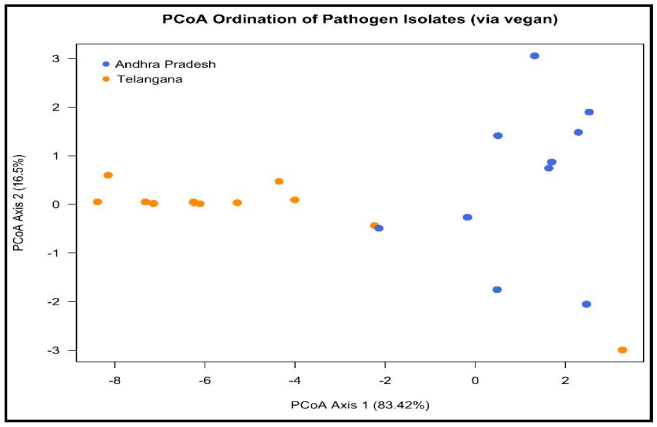


Fig. 4: Principal Coordinates Analysis (PCoA) ordination diagram of the 148 *Xanthomonas oryzae* pv. *oryzae* isolates from Telangana and Andhra Pradesh based on binary multilocus genotypes across 21 evaluated loci.

Minimum Spanning Network (MSN) analysis

The Minimum Spanning Network (MSN) resolved the 148 *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates into 22 multilocus genotypes (MLGs) based on the JEL-3 (IS-PCR) fingerprinting profiles (Fig. 5). The network was largely consistent with the population structure inferred from the UPGMA dendrogram and Principal Coordinate Analysis (PCoA), revealing distinct yet interconnected genetic relationships among the identified MLGs.

The network comprised nodes of varying sizes, representing the frequency of isolates sharing identical MLGs. MLG.4 was the largest and most centrally positioned node, indicating that it was the most common genotype and acted as the primary genetic hub connecting several related MLGs, including MLG.3, MLG.5, MLG.9, MLG.20, MLG.21, and MLG.22. Additional frequent genotypes included MLG.1 and MLG.2, whereas the remaining MLGs were represented by smaller nodes, reflecting less common or unique multilocus genotypes.

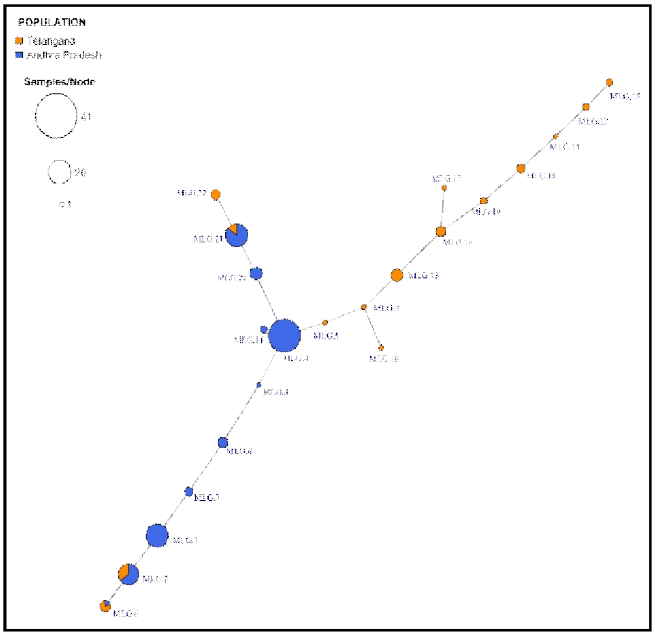


Fig. 5: Minimum spanning network (MSN) based on 21 loci showing the genetic relationships and geographic distribution of the 22 unique multilocus genotypes (MLGs) of *Xanthomonas oryzae* pv. *oryzae* isolates from Andhra Pradesh and Telangana.

Table 1: Genetic diversity indices of *Xanthomonas oryzae* pv. *oryzae* populations from Andhra Pradesh and Telangana based on JEL-3 (IS-PCR) binary marker data.

Population	n	Um	SH	ST	SI	GE
Telangana	41	15	2.52	10.85	0.892	0.860
Andhra Pradesh	107	10	1.77	4.45	0.721	0.709
Total collection	148	22	2.42	7.38	0.864	0.623

Um: Unique MLGs; SH: Shannon Index (H); ST: Stoddart & Taylor Index (G); SI: Simpson's Index (λ); GE: Genotype Evenness (E5)

Most MLGs were predominantly associated with a single regional population, although a few nodes contained isolates from both Andhra Pradesh and Telangana, indicating the occurrence of shared MLGs between the two regions. The Andhra Pradesh population occupied a comparatively larger and more branched portion of the network, whereas the Telangana population was represented by a relatively compact arrangement of interconnected MLGs. Despite the regional clustering, all MLGs remained connected through a single spanning network, suggesting overall genetic relatedness among the *Xoo* isolates. Collectively, the MSN supported the regional population structure identified by the UPGMA dendrogram and PCoA while providing a detailed representation of the relationships among individual MLGs.

While the Minimum Spanning Network (MSN) provided a graphical representation of the relationships among multilocus genotypes and suggested limited genetic connectivity between Andhra Pradesh and Telangana, it did not quantify the magnitude or statistical significance of population differentiation. Therefore, Analysis of Molecular Variance (AMOVA) and Nei's genetic differentiation coefficient (GST) were employed to partition genetic variation within and between populations and to estimate the extent of regional genetic divergence. These complementary analyses provided statistical support for the population structure inferred from the clustering, ordination, and network analyses.

Population Differentiation Based on AMOVA and Nei's GST

Analysis of Molecular Variance (AMOVA) and genetic differentiation statistics consistently revealed substantial population structuring between the *Xanthomonas oryzae* pv. *oryzae* (Xoo) populations from Andhra Pradesh and Telangana. Hierarchical AMOVA indicated that 83.42% of the total molecular variation was partitioned among populations, whereas 16.58% occurred within populations. The corresponding fixation index, Φ_{ST} = 0.8342, was highly significant (P < 0.001), demonstrating pronounced regional genetic subdivision. AMOVA estimates population differentiation from molecular-marker distance data and the high among-population component indicates that the two state populations possess markedly different multilocus compositions.

Table 2: Mantel test results for isolation-by-distance (IBD) among 148 *Xanthomonas oryzae* pv. *oryzae* isolates based on JEL-3 (IS-PCR) binary marker data.

Statistic	Value	P-value
Mantel *r*	0.042	0.312

Pairwise genetic differentiation based on Nei's fixation index (F_{ST}= 0.357) similarly revealed substantial molecular divergence between the Andhra Pradesh and Telangana populations. However, the presence of a small number of shared multilocus genotypes, along with the mixed composition of UPGMA Cluster II, suggests that the two populations are not completely genetically isolated. These findings may indicate limited epidemiological connectivity or the persistence of widespread clonal lineages across neighboring rice-growing regions. Overall, the high and significant Φ_{ST} and F_{ST} values confirm that the two regional *Xoo* populations possess distinct multilocus genetic compositions, while still exhibiting some degree of genetic overlap.

Furthermore, genetic diversity indices were calculated to characterize the diversity within each population, followed by isolation-by-distance (IBD) analysis to assess whether geographic distance contributed to the observed population structure.

Genetic diversity indices

Genetic diversity analysis identified 22 multilocus

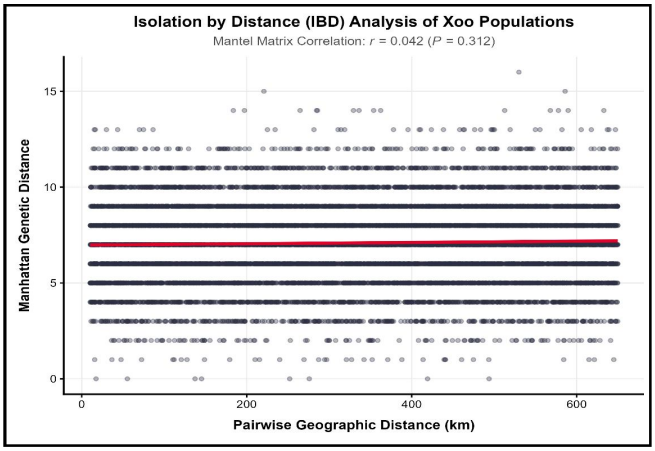


Fig. 6: Isolation-by-distance (IBD) analysis showing the relationship between pairwise geographic distance (km) and Manhattan genetic distance among 148 *Xanthomonas oryzae* pv. *oryzae* isolates based on JEL-3 (IS-PCR) binary marker data. The Mantel test yielded a correlation coefficient of r = 0.042 with a P-value = 0.312.

genotypes (MLGs) among the 148 Xoo isolates based on the 21 JEL-3 (IS-PCR) loci (Table 1). Telangana (n = 41) comprised of 15 MLGs, whereas Andhra Pradesh (n = 107) contained 10 MLGs, indicating greater genotypic richness in Telangana despite its smaller sample size. The overall collection exhibited a Shannon diversity index (H) of 2.42, Stoddart and Taylor's diversity index (G) of 7.38, Simpson's diversity index (ë) of 0.864, and genotype evenness (E5) of 0.623.

Among the regional populations, Telangana showed higher genetic diversity (H = 2.52, G = 10.85, λ = 0.892, E5 = 0.860) than Andhra Pradesh (H = 1.77, G = 4.45, λ = 0.721, E5 = 0.709). Although Andhra Pradesh contributed a larger number of isolates, the lower diversity and evenness indices indicate that its population was dominated by relatively few multilocus genotypes. In contrast, the Telangana population exhibited a more even distribution of genotypes, reflecting comparatively greater genotypic diversity.

Isolation-by-distance (IBD) analysis

The Mantel test revealed a non-significant correlation between pairwise geographic and genetic distances among the 148 *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates (Mantel r = 0.042, P = 0.312; Table 2). The correlation between geographic and genetic distances is presented in Fig. 6.

Discussion

Understanding the genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is essential for understanding pathogen evolution and developing effective strategies for the management of bacterial blight of rice. In the present study, JEL-3 (IS-PCR) fingerprinting targeting the insertion sequence elements effectively discriminated *Xoo* isolates collected from Andhra Pradesh and Telangana. The JEL-3 primer-based molecular marker system generated highly polymorphic fingerprinting profiles, producing 916 reproducible amplification fragments that were scored into 21 loci and resolved into 22 multilocus genotypes (MLGs). These findings demonstrate the suitability of JEL-3 as a reliable molecular marker for population genetic studies of *Xoo* and agree with earlier reports describing the effectiveness of IS-based PCR for assessing genetic diversity in *Xoo* populations (Leach *et al*., 1992; Adhikari *et al*., 1999; Ladhalakshmi and Rabindran, 2012; Mishra *et al*., 2013; Yugander *et al*., 2022; Noer *et al*., 2021).

The combined results of UPGMA clustering, Principal Coordinate Analysis (PCoA), and Minimum Spanning Network (MSN) consistently demonstrated pronounced regional population structure, but with incomplete geographic separation. The UPGMA dendrogram resolved two major clusters with 100% bootstrap support at the primary node Cluster I comprised Telangana isolates exclusively, whereas Cluster II contained both Andhra Pradesh and Telangana isolates, indicating strong but not absolute geographic structuring. PCoA reinforced this pattern, with Telangana isolates forming a relatively compact group that partially overlapped with a more dispersed Andhra Pradesh cluster. Similarly, the MSN grouped most multilocus genotypes (MLGs) by geographic origin, yet a limited number of shared MLGs between Andhra Pradesh and Telangana implied restricted genetic connectivity across state boundaries. The concordance among these complementary analyses provides robust evidence of regional genetic differentiation within Xoo populations, together with the presence of shared or closely related genotypes across the two states. Comparable patterns of strong yet incomplete geographic structuring have been reported in Xoo populations from Nepal, India, China, and Indonesia (Adhikari *et al*., 1999; Chen *et al*., 2012; Mishra *et al*., 2013; Noer *et al*., 2021), as well as in other Xanthomonas species using repetitive sequence–based fingerprinting approaches (Scortichini and Rossi, 2003; Chen *et al*., 2021; Zhong-Ting *et al*., 2023).

Population genetic analyses further supported this regional subdivision. AMOVA attributed 83.42% of the total molecular variation to differences between Andhra Pradesh and Telangana, whereas only 16.58% occurred within populations, resulting in a highly significant fixation index (Φ_{ST} = 0.8342; $P < 0.001$). Likewise, Nei's genetic differentiation coefficient (GST = 0.8342) indicated strong genetic divergence between the two populations. These findings suggest restricted gene flow and long-term regional differentiation of *Xoo* populations. Similar patterns have been reported in other *Xanthomonas* species using rep-PCR and VNTR markers, where AMOVA and genetic differentiation analyses demonstrated significant regional subdivision associated with local evolutionary processes (Munhoz *et al*., 2011; Oliveira *et al*., 2023; Siddique *et al*., 2025). Such observations are consistent with the population genetic framework proposed for plant-pathogenic microorganisms, in which limited gene flow promotes regional divergence (McDonald and Linde, 2002; Grünwald *et al*., 2017).

The genetic diversity indices revealed contrasting patterns between the two populations. Although Andhra Pradesh contributed a larger number of isolates, Telangana exhibited greater multilocus genotypic richness, higher

Shannon and Simpson diversity, greater Stoddart and Taylor diversity, and higher genotype evenness. In contrast, the Andhra Pradesh population was dominated by relatively few multilocus genotypes, suggesting greater clonal expansion despite its broader genetic dispersion in the PCoA. This distinction indicates that ordination-based dispersion reflects genetic relationships among isolates, whereas diversity indices quantify the richness and frequency distribution of multilocus genotypes. Similar regional differences in genotypic diversity and clonality have been reported for *Xoo* populations from Nepal, China, Indonesia, and India (Adhikari *et al*., 1999; Chen *et al*., 2012; Mishra *et al*., 2013; Noer *et al*., 2021), as well as for other *Xanthomonas* pathogens (Oliveira *et al*., 2023; Siddique *et al*., 2025). The predominance of a limited number of multilocus genotypes in Andhra Pradesh is consistent with the clonal population structure commonly reported in bacterial plant pathogens (McDonald and Linde, 2002; Grünwald *et al*., 2017).

Despite the pronounced regional differentiation observed across all population genetic analyses, the Mantel test detected no significant relationship between pairwise genetic and geographic distances (r = 0.042, *P* = 0.312), indicating the absence of a significant isolation-by-distance (IBD) pattern. This result suggests that geographic distance alone does not explain the observed genetic structure of *Xoo* in the study region. Similar findings have been reported for *Xanthomonas citri* pv. *citri*, where MLVA-based studies demonstrated that population structure is influenced more by localized evolutionary processes and epidemiological connectivity than by simple geographic separation (Pruvost *et al*., 2014, Pruvost *et al*., 2019). More generally, restricted gene flow, host selection, and local adaptation are recognized as major drivers of population differentiation in plant-pathogenic microorganisms (McDonald and Linde, 2002; Peakall and Smouse, 2012; Grünwald *et al*., 2017).

Overall, this study provides a comprehensive assessment of the population genetics of *Xoo* in southern India by integrating JEL-3 fingerprinting with hierarchical clustering, multivariate ordination, network analysis, population differentiation statistics, diversity indices, and isolation-by-distance analysis. The marked genetic differentiation between Andhra Pradesh and Telangana populations, together with their contrasting patterns of genotypic diversity, establishes an important molecular epidemiological baseline for bacterial blight surveillance. These findings will facilitate future studies on pathogen evolution and support region-specific disease monitoring, resistance gene deployment, and breeding programmes.

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