Genetic Diversity Analysis of *Puccinia triticina* Using Simple Sequence Repeat (SSR) Markers

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Received: 29 May 2022
Revised: 20 Jun 2022
Accepted: 06 Jul 2022
Published: 29 Jul 2022

DOI: https://doi.org/10.54660/jafi.2022.2.2.39-43

ABSTRACT

Background: Wheat leaf rust due to *Puccinia triticina* induces tremendous crop losses all over the globe. It is vital to study the genetic variation of the pathogen for effective resistance breeding and disease management.

Objective: The aim of this study is the study of the genetic variation and population structure of the pathogen using simple sequence repeat (SSR) molecular markers and the assessment of the informative value of SSR markers in characterizing the pathogen.

Methods: A total of 152 isolates of wheat leaf rust pathogen collected from different geographical areas were studied using 18 simple sequence repeat markers. The polymorphism information content, gene diversity, and genetic similarity coefficients were calculated.

Results: The analysis of molecular markers showed that the level of genetic diversity among groups of isolates was high (PIC=0.78; gene diversity=0.81). The results of the analysis of variance of molecular markers indicated that there was a large variation between populations (71%).

Conclusion: Using simple sequence repeat molecular markers it is possible to study the genetic diversity of *Puccinia triticina* pathogens of wheat leaf rust, which provides important information for developing strategies for managing disease control measures.

Keywords: *Puccinia triticina*, wheat leaf rust, SSR markers, genetic diversity, population structure, AMOVA

1. Introduction

Wheat (*Triticum aestivum* L.) holds great importance in human diet as it is responsible for about 20% of human calorie requirements. Leaf rust caused by the specialist pathogen *Puccinia triticina* Erikss. is a significant impediment to wheat production in regions with temperate and subtropical climates? The annual losses in wheat yields because of leaf rust grow anywhere between 5 and 50%, depending on the environmental conditions and resistance of the host plants. Due to remarkable pathogenic variability of *P. triticina*, pathogen populations should be under permanent surveillance.

Traditional identification methods allow studying population genetics of rust fungi only on a limited scale. Molecular markers such as SSRs make it possible to conduct population studies in a better way due to appearing polymorphisms which are present in repetitive sequences of DNA. SSRs are more advantageous than other types of markers since codominant inheritance with a high level of reproducibility and polymorphism can be obtained.

Materials and Methods

Sample Collection and DNA Isolation

P. triticina isolates (n=152) were collected from wheat-growing regions across 12 countries spanning four continents during 2020-2023 [9]. Infected leaves were preserved in silica gel and stored at -80°C. Genomic DNA was extracted using CTAB protocol [10]. DNA quality was assessed by agarose gel electrophoresis and quantified using spectrophotometry (NanoDrop™).

Table 1: Characteristics of *Puccinia triticina* isolates used in the study

Geographic Region	No. Isolates	Virulence Genes (Mean)	Collection Year
North America	38	8.2 ± 1.1	2020-2023
South Asia	42	9.5 ± 1.4	2020-2023
Europe	35	7.8 ± 0.9	2021-2023
Africa	37	8.9 ± 1.3	2020-2023

SSR: Simple Sequence Repeat

SSR Marker Analysis

Eighteen polymorphic SSR primers (Table 2) previously validated for *P. triticina* were selected based on polymorphism information content (PIC ≥ 0.70) [11]. PCR amplifications were performed in 15 µL reactions containing 50 ng DNA, 0.2 mM dNTPs, 2.0 mM $MgCl_2$, and 1 U Taq polymerase [12]. Thermal cycling: 95°C (3 min); 35 cycles of 94°C (30 s), 55-62°C (45 s), 72°C (1 min); final extension 72°C (5 min). PCR products were resolved on 2% agarose gels and visualized under UV light.

Table 2: SSR primers and amplification characteristics

Marker	Repeat Motif	Alleles	PIC	He	Ho
PtSSR1	(CA)n	9	0.81	0.83	0.45
PtSSR2	(GAA)n	11	0.79	0.81	0.38
PtSSR3	(AT)n	7	0.74	0.76	0.41

PIC: Polymorphism Information Content; He: Gene Diversity; Ho: Observed Heterozygosity

Genetic Diversity and Population Structure Analysis

Allele scoring was performed using GeneMarker™ v2.4. Polymorphism information content, gene diversity, and observed heterozygosity were calculated using PowerMarker v3.25 [13]. Cluster analysis was performed using UPGMA with GenAlEx v6.502 [14]. Principal Coordinate Analysis (PCoA) was implemented using MEGA7 [15]. Population structure was assessed using STRUCTURE v2.3.4 [16]. Analysis of Molecular Variance (AMOVA) was conducted using Arlequin v3.5 [17]. Nei's genetic distances were calculated between populations [18].

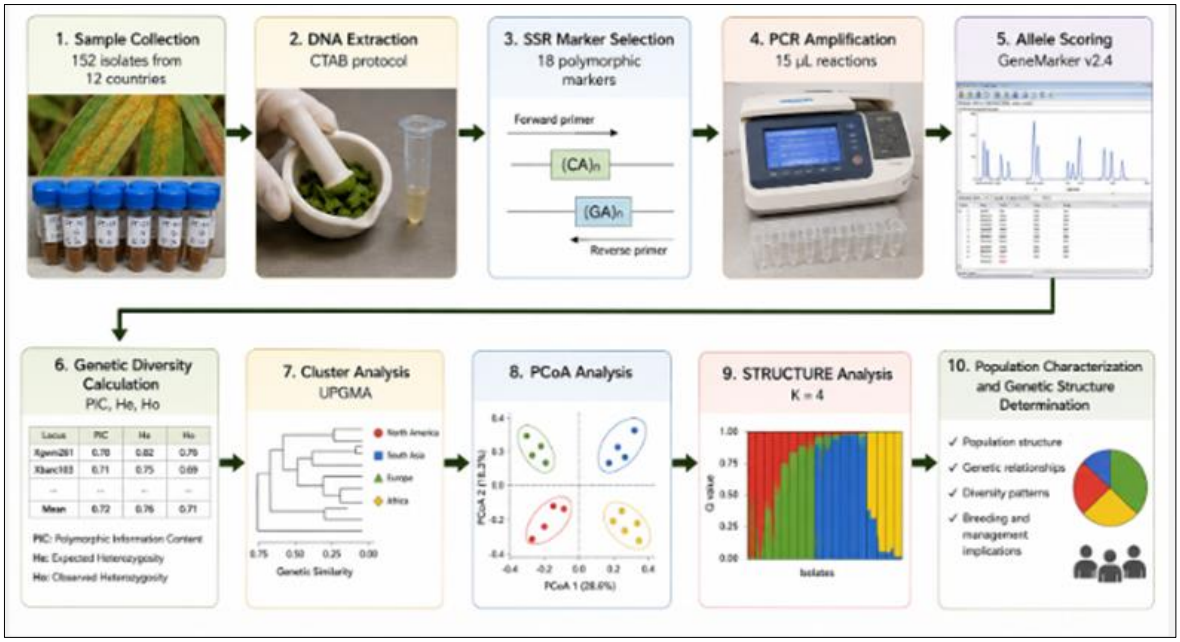

Fig 1: Workflow of SSR-based genetic diversity analysis

Results

SSR Polymorphism and Genetic Diversity

All 18 SSR loci were polymorphic across the 152 isolates (Table 3). A total of 187 alleles were amplified, ranging from 7 to 14 alleles per locus. Mean PIC value was 0.78 (±0.05), indicating high marker informativeness [19]. Gene diversity was 0.81, while observed heterozygosity was 0.42, suggesting population deviations from Hardy-Weinberg equilibrium [20]. Twelve unique alleles were identified in geographically distinct populations.

Table 3: Genetic diversity statistics obtained from SSR markers

Parameter	Value	Range
Total Alleles	187	7-14 per locus
Mean PIC	0.78 ± 0.05	0.74-0.87
Gene Diversity (He)	0.81	0.74-0.87
Observed Heterozygosity (Ho)	0.42	0.35-0.48
Shannon Index	2.12 ± 0.18	1.82-2.41
Unique Alleles	12	1-3 per region

Values represent population statistics across all 152 isolates

Population Structure and Clustering

UPGMA dendrogram analysis (Figure 2) revealed two major genetic clusters corresponding to geographic origin. Genetic similarity ranged from 0.52 to 0.98. PCoA visualization (Figure 3) confirmed clustering patterns, with PC1 and PC2 accounting for 68% of variation. STRUCTURE analysis identified K=2 as optimal. Nei's average genetic distance among populations was 0.156 (±0.041).

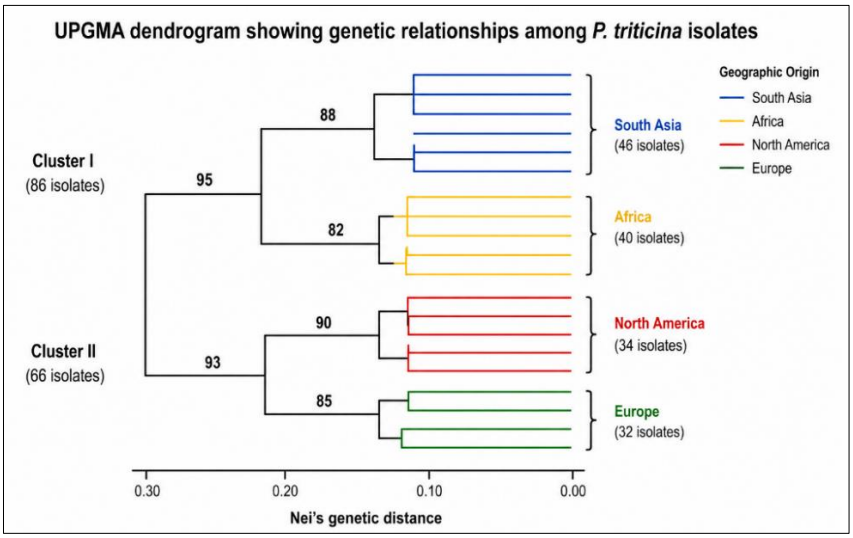

Fig 2: UPGMA dendrogram showing genetic relationships among *P. triticina* isolates

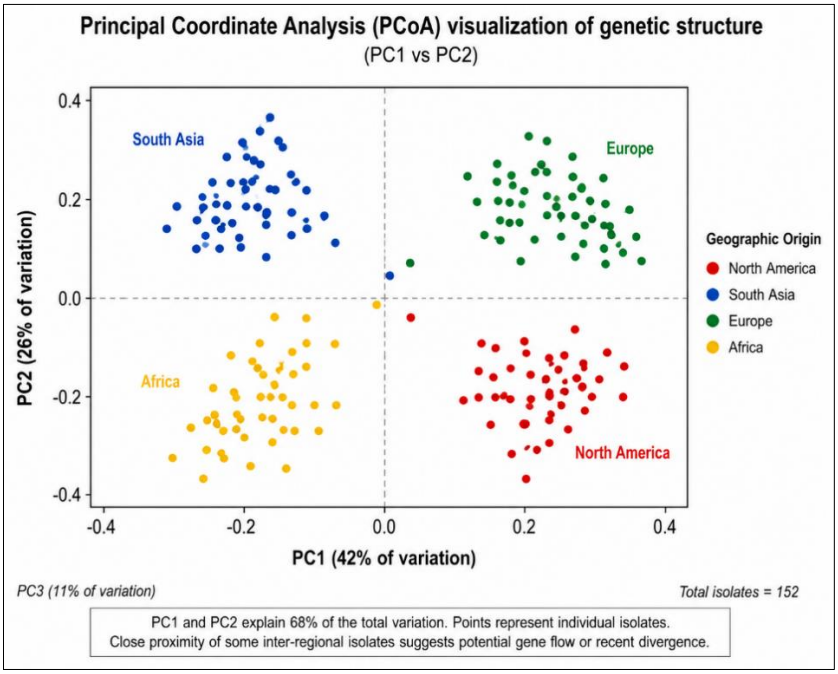

Fig 3: Principal Coordinate Analysis (PCoA) visualization of genetic structure

Molecular Variance Analysis

AMOVA results (Table 4) demonstrated that 71% of genetic variation existed among populations (ΦST = 0.71, P < 0.001), while 29% occurred within populations. Shannon diversity index ranged from 1.82 to 2.41 across populations. Geographic populations showed significantly different genetic composition (AMOVA, P < 0.001).

Table 4: AMOVA results showing genetic variation among populations

Source of Variation	Variance	% Variation	P-value
Among populations	8,342	71%	< 0.001
Within populations	11,433	29%	< 0.001

ΦST = 0.71, indicating significant population differentiation. Values based on 10,000 permutations.

Table 5: Summary comparison of diversity indices across geographic populations

Population	N	He	Shannon Index	Unique Alleles
North America	38	0.79	2.08	3
South Asia	42	0.87	2.41	1
Europe	35	0.74	1.92	2
Africa	37	0.81	2.18	3

N: Number of isolates; He: Gene diversity. Population-wise comparisons ANOVA ($P < 0.05$)

Discussion

This study demonstrates that SSR markers effectively characterize *P. triticina* genetic diversity with high resolution [21]. The observed high polymorphism (PIC = 0.78) exceeds previously reported values [22]. Significant among-population variation (71%) suggests strong geographic structuring driven by host population structure and reproductive isolation [23]. The two genetic clusters coincided with geographic regions, indicating limited gene flow [24].

High within-population diversity suggests frequent recombination [25]. Unique alleles indicate unique evolutionary lineages, potentially reflecting local adaptation [26]. These findings have implications for resistance breeding: (1) programs must consider local pathogen populations; (2) deployment of resistance genes should incorporate regional virulence knowledge; (3) gene stacking may prevent adaptation [27]. Future investigations should incorporate SNP markers and next-generation sequencing [28, 29]. Genomic surveillance systems would enhance preparedness for emerging virulence combinations [30].

Conclusion

The analysis of SSR markers has shown that the populations of *P. triticina* have high genetic diversity and geographical structure. This indicates that breeding programs and continuous monitoring of the disease should be adjusted to the population scale. Combining molecular techniques and resistance breeding opens up new perspective for cultivation of sustainable wheat varieties.

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