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ISSN: 2157-7471
Journal of Plant Pathology & Microbiology

Review Article

An Overview of Genetic Variability and Population Structure of Rice Brown Spot (*Bipolaris oryzae*)

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ABSTRACT

Rice is the vital staple food crop globally in which an ever-increasing population earns an economic return. However, the rice brown spot reduces its productivity and quality which in turn hurdles the producers' income. An information gap on the genetic diversity and population structure of *Bipolaris oryzae* worldwide and poor design of control strategies have led to its ineffective management. Thus, this review paper is written to grasp information based on the genetic variability of rice *Bipolaris oryzae* and to know its population structures across locations. Molecular markers are used as valuable tools for the characterization of the pathogen loci number and size, cluster of band polymorphism, gene sequence and sexual state over different locations. Random amplified polymorphic DNA, variable number of tandem repeats, inter-simple sequence repeats, universal rice primers and restriction fragment length polymorphisms have been developed and used previously for determining the genetic variation of *B. oryzae* globally. The results of all studies showed that all markers have been provided different band sizes (100-3000 bp) and percent of polymorphism (19.3-100) from place to place. Both mating-type idiomorphs, MAT1-1 and MAT1-2, were found in isolates in all locations. *B. oryzae* isolates are clustered into three to four distinct clusters with several sub-groups *via* internal transcribed spacer sequencing between 0.0 and 0.5% isolate's divergence range. Therefore, from many studies, it is implied that the use of combined markers is highly reliable in Bipolaris oryzae genetic diversity and population structure studies.

Keywords: *Bipolaris oryzae*; Genetic diversity; Molecular markers; Primer; Polymorphism; Population structure

INTRODUCTION

Rice (*Oryzae sativa* L.) is the most popular cereal crop worldwide and over half of the planet's population is dependent on it for food consumption [1]. An increase in population causes it to enhance its production globally [2,3]. Rice provides up to 50% of the dietary caloric supply and a substantial part of the protein intake for about 520 million people living in poverty in Asia. It is a major ingredient in cuisines the world over, in the form of breakfast cereals, staple carbohydrates, snacks, alcoholic beverages and desserts [4].

Brown spot can cause a yield loss from 6% to 90% in Asia and was a cause of the Bengal famine in India. A yield loss of 50%-90% was recorded in Bengal (India) and the outbreak of the disease in 1942 is reported to have resulted in the death of two million people [5]. It also accounts for a yield loss of 5% in all lowland rice production in South and Southeast Asia [6]. Poor germination of infected seeds since the pathogen, B*ipolaris oryzae* may be seed borne, soil and airborne and infection of the leaves reportedly results in the reduction of the effective leaf area for photosynthesis cause losses in rice crop [7]. For this reason, an attack on the grain accounts for a loss in the weight of grains ranging from 4.6%-29.0%.

The high phenotypic and genotypic variability of the pathogen makes it break its susceptibility to certain fungicides, with the development of either quantitative or qualitative resistance against them. One of the most positive features of genetic diversity studies is pathogen tracking, by using the genetic

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Received: 23-Jul-2024, Manuscript No. JPPM-24-26556; **Editor assigned:** 25-Jul-2024, PreQC No. JPPM-24-26556 (PQ); **Reviewed:** 08-Aug-2024, QC No. JPPM-24-26556; **Revised:** 13-Jun-2025, Manuscript No. JPPM-24-26556 (R); **Published:** 20-Jun-2025, DOI: 10.35248/2157-7471.25.16.761

Citation: Ejigu MB (2025) An Overview of Genetic Variability and Population Structure of Rice Brown Spot (*Bipolaris oryzae*). J Plant Pathol Microbiol. 16:761.

pattern of fungus isolates. Knowledge of the pathogen population variability is necessary for effective host breeding for resistance. Variability in morphology and pathology of *B. oryzae* isolates has been reported from various rice-growing countries, but except for one report which was restricted to only one rice-growing state of India [8].

Populations of rice *Bipolaris oryzae* for its phenotypic and genetic variation throughout the world have been studied [9]. The analysis of variation in plant pathogen populations is an important prerequisite for understanding coevolution in the plant pathosystem and in identifying as well as characterizing resistant rice germplasm [10]. To predict the tolerance or resistance stability of rice varieties against *B. oryzae*, it is important to know the pathogen's genetic structure. Therefore, understanding variation and diversity in a population of the pathogen and mechanisms that further influence the genotypic changes in the pathogen population, is an important step in developing disease management strategies.

Molecular markers are used as valuable tools for the characterization of pathogenic genetic diversity where morphological characteristics are either missing or unable to properly distinguish strains [11]. Therefore, the use of molecular marker techniques has solved problems associated with studying various levels of genetic variability in *B. oryzae*. However, the molecular diversity of *B. oryzae* strains is not well known in India and information is not available in Africa, Asia and America [12].

Molecular markers such as Random Amplified Polymorphic DNA (RAPD), Variable Number of Tandem Repeats (VNTRs) and Restriction Fragment Length Polymorphisms (RFLPs) have been developed and used previously for determining the genetic diversity variation of *B. oryzae* although Universal Rice Primers (URPs) have been originally used for fingerprinting of diverse genomes of microbes [13]. The molecular analysis of a few fungi has been made by URP-PCR [14]. Moreover, rice isolates have been characterized using Inter-Simple Sequence Repeats (ISSR) molecular markers. Therefore, this paper aims to review the molecular diversity of *B. oryzae* and its population structure that has been conducted in different locations.

MATERIALS AND METHODS

Genetic variability of *Bipolaris oryzae* and its population structure

Genetic diversity of *Bipolaris oryzae* isolates by inter-simple sequence repeats: Different environmental and cultural conditions have an impact on fungal genetic characteristics. The geographic location is another important aspect influencing the genetic makeup of *B. oryzae*. This could be partially explained by the fact that various types of soil and certain weed hosts serve as important reservoirs for *B. oryzae* inoculum [15]. The knowledge of the fungal pathogen's genetic diversity, population composition and sexual recombination is of paramount importance to implement effective disease control methods [16]. The use of molecular variability characterization facilitates the crop improvement of *B. oryzae* resistance in varietal deployment strategies and a better understanding of the genetic variability of *B. oryzae* strains.

The *B. oryzae* isolates in each collection site were genetically diverse, although clonality was present in some fields. High diversity was observed in *B. oryzae* isolates obtained from infected seeds sourced from sixteen rice-growing regions in India, as determined by the polymorphism of Inter-Simple Sequence Repeat (ISSR) markers. According to a hierarchical clustering approach, there was no relationship between the collection sites and the isolate groups [17]. Further studies of Asian *B. oryzae* strains from Bangladesh, Iran and India found genetic heterogeneity inferred by cluster analysis of molecular fingerprinting patterns [18].

The genetic diversity of *B. oryzae* isolated from various geographic locations can be studied with the help of ISSR markers. They help to understand the dynamics of pathogen populations, which can contribute to the creation of efficient management measures. They are also quick, repeatable and yield a lot of polymorphic bands. According to Archana et al., ISSR markers can therefore be employed as reliable molecular markers for population genetics, epidemiology and ecological research of *B. oryzae* that will help in developing better techniques for managing the rice brown spot disease. By utilizing ISSR to examine the genetic diversity among the Indian isolates of the brown spot pathogen, brown spot samples have demonstrated intraspecific variability.

Using 69 isolates and an average of 13.8 markers per primer, Archana et al. amplified 87 highly reproducible fragments. ISSR primers revealed a polymorphism percentage range of 83.33 to 95.45% and the total loci score fell between 6 and 22. Scorable loci had molecular weights between 150 and 2600 bp. When compared to URP and RAPD, the ISSR marker genetic similarity coefficient was comparatively higher, ranging from 22.1% to 95.5% across all isolates.

All ISSR markers showed high polymorphism (83.33 to 95.45%), but found that it ranged from 81.8 to 100%. With varying primers, distinct polymorphism levels and band counts were achieved for every isolate in India within the 100-3000 bp range (Table 1). DNA bands of about 800, 1500 and 2500 bp were more noticeable among the several DNA bands that were specifically amplified in all *B. oryzae* isolates. All *B. oryzae* isolates had these bands, regardless of where they came from or what kind of rice they were isolated from.

Rice genotypes have high genetic variability with varied numbers of alleles from location to location that was from 113 to 154 and average Polymorphism Information Content (PIC) value from 0.5745/marker to 0.8005/marker, respectively. The high gene diversity could suggest that mutations have accumulated in the populations over a while and/or that populations are large [19]. The genotypic diversity was due to both richness and evenness. The diversity of *B. oryzae* isolates collected and studied previously from Brazil, Bangladesh, India, the United States and the Philippines is also similar to the recent Iraq, Japan and Philippines ones as characterized by different molecular markers (Table 1) [20].

Table 1: Genetic diversity analysis of ISSR primers.

S. no.	Number of ISSR primer	Number of isolates	Range of loci number	Range of loci scored (bp)	Polymorphism % range
1	12	293	6-14	201-336	
2	7	69	5-14	150-2600	83.33-95.45
3	8	36	9-23		81.80-100.00

A precise pattern accounting for genetic variation could be obtained using nucleotide sequence analysis and multiple sequence alignment. The geographic origin of isolates does not play a crucial role in the grouping of isolates in India and both local and geographic polymorphism exists. Similar results have been shown by Motlagh and Anvari in their study on isolates of *B. oryzae* from different regions of the Guilan Province of North Iran.

RESULTS AND DISCUSSION

Allele contents of *Bipolaris oryzae* and induction of sexual state

High genotype numbers were seen in the estimated genotypic diversity as indicated by richness (eMLG). Although the lowest values were almost at the maximum predicted value, the highest eMLG was observed in the isolates from the Philippines and the lowest in the Iranian isolates from Gorgan (eMLG). Each Japanese isolate was distinct even though they were excluded and could not be directly compared to the other groups. Evenness (E5) showed that the MLG distribution in the populations was balanced and either approached or reached the maximum value of 1. Bagh-e-Malek isolates had the greatest number of private alleles, with three, followed by isolates from Japan with two and Amol with one.

High genotypic diversity, low clonal fractions and alleles in linkage equilibrium are predicted characteristics of sexually reproducing heterothallic fungi. The multilocus linkage disequilibrium estimators IA and rd, however, showed no evidence of random mating within the clone-censored *B. oryzae* populations, despite the rd estimator values being near zero. For *B. oryzae* populations gathered from wild rice in the US, there was no evidence to suggest linkage equilibrium. Genetic drift, selection, migration and linkage can all lead to linkage disequilibrium. Admixture can occur as a result of migration events when Asian farmers in nearby regions commercialize rice and hay seeds and the isolates have different alleles than those in the recipient population.

In small populations, random genetic drift or stochastic variations in allele frequencies, can cause a decline in genetic diversity over successive generations. Nonetheless, *B. oryzae* populations are high during the cropping season and are assumed to overwinter in grass leftovers, meaning that there are no appreciable size reductions during the off-season. The lack of genetic resistance in rice cultivars prevents directional selection from acting on fungal populations, but it is still possible that other cultivar features could have an impact on fungal fitness. Each location had isolates containing both mating type idiomorphs, MAT1-1 and MAT1-2; ratios varied from equality within Bagh-eMalek, Astara, the Philippines and Japan populations, and fitted a 1:1 segregation ratio in those of Rasht, Amol and Gorgan.

The genetic structure of the fungal population may be significantly impacted by sporadic, even small-scale sexual recombination if it increases genotypic diversity. More thorough investigations are necessary to verify that random mating happens naturally and adds to the fungus's genetic variety. To identify individuals who have undergone sexual recombination and/or to determine the reduction in linkage disequilibrium if sexual recombination takes place during the season, genetically known (marked) isolates could be released at the start of the season and recaptured at its conclusion. Indeed, other processes can account for high genetic variation, including DNA mutations and parasexual recombination. Hyphal anastomosis and heterokaryosis have been demonstrated for Bipolaris species including *B. oryzae* isolates collected from common rice.

Genetic diversity of *Bipolaris oryzae* using RAPD marker

RAPD has been successfully used to distinguish pathogenic and non-pathogenic isolates of *Leptosphareia maculans* and to differentiate pathotypes of *Peronospora parasitica*. RAPD primers were used to observe polymorphism within isolates. Thus, molecular markers were able to distinguish between the isolates within the same group. Cluster analysis revealed a complex pattern of association among the isolates. UPGMA cluster analysis of RAPD data showing association among isolates revealed huge diversity within and between groups and clustered into three groups, which was different from the four groups obtained based on morphological pattern.

Similar results were obtained by Chen et al. with *Puccinia striiformis*, which showed a high degree of polymorphism with individuals of different species, although the RAPD groups generally formed were not associated with the geographic regions. By contrast, isolates of *Fusarium oxysporum* f. sp. vasinfectum were differentiated into three main groups directly related to both virulence and geographical origin. Genetic similarity classification based on the morpho-pathological level was reflected at the molecular level. Uniformity could also be generated by reproduction through selfing because the progeny of a haploid organism is identical to the parental strain.

The use of Inter-Simple Sequence Repeats (ISSR) and Random Amplified Polymorphic DNA (RAPD) for molecular characterization of 20 isolates demonstrated diversity within the isolates. The results of cluster analysis likewise revealed these differences. Five RAPD and twenty ISSR primers each produced extremely good, repeatable banding patterns out of twenty RAPD and twenty ISSR primers. In RAPD markers, polymorphism ranged from 54.0 to 100%, but in ISSR markers, it varied from 49.2 to 100%.

Genetic diversity of *Bipolaris oryzae* using URP marker

Previous researchers have documented the use of URP markers in the molecular study of a few other fungi, including species of *Colletotrichum*, *Pleurosis* and *Chaetomium*. Nevertheless, there hasn't been any literature-reported application of URP and ISSR marker system to *B. oryzae* molecular study so far. The applications of the URP marker system findings for the genetic diversity analysis of fungi are likewise consistent with those of previous researchers. URP-PCR may identify polymorphic DNA, which is produced by nucleotide changes, insertions, and deletions at fungal start sites.

In comparison to other URP primers, the isolates with URP 30F showed moderate bootstrap confidence values (0.26) and high arithmetic expected heterozygosity values (0.26), indicating that three major genetic groups were present in the UPGMA cluster analysis based on Jaccard similarity coefficients of the URP marker. A small number of isolates that were grouped based on the essential identity of their geographic populations suggests that host selection and gene flow between populations have homogenized the populations, as evidenced by the lack of population differentiation in the cluster analysis. Aggarwal et al., Jana et al., and Kumar et al. have documented the use of URP markers in the molecular study of a few other fungi, namely *Pleurosis, Rhizoctonia, Macrophomina, Chaetomium* and *Collectotrichum*.

Universal Rice Primers (URPs) were used for molecular characterization, which allowed for the discovery of variations and geographic origin clustering across different *B. oryzae* strains that would not have been possible to identify using conventional characterization. The strain level variations shown by the URP marker results are not apparent when using conventional categorization techniques. Regarding the use of the URP marker system for the genetic diversity analysis of fungi, the findings of Kandan, et al. are also consistent with those of previous researchers. These findings include the possibility that insertions and deletions at the initiation sites of fungi may result in polymorphic DNA, which can be detected by the URP-PCR.

Population structure and molecular variability of *Bipolaris oryzae*

With minor adjustments, the CTAB (CetylTrimethyl Ammonium Bromide) technique can be used to extract the whole genomic DNA from pure cultures of the various isolates of *Bipolaris oryzae*. The ribosomal DNA (rDNA) unit includes spacer or non-genetic regions in PCR amplification. Every repeat unit is made up of an Internal Transcribed Spacer (ITS) and copies of the rDNA's 18S, 5.83S and 28S sequences. Since rDNA is largely conserved, it has been used to evaluate evolutionary events; in contrast, ITS rDNA is more variable and so was the subject of research. Ultimately, sequencing was done on the amplified products of the representative samples.

The Basic Local Alignment Search Tool (BLAST) technique has been used to examine the generated sequence findings. *B. oryzae* isolates then have been identified by utilizing the NCBI BLAST tool, representative sample sequencing, and verification of the amplified products on 1.2% agarose gel electrophoresis. Full-length ITS1 rDNA region is amplified for *B. oryzae* isolates using the primers ITS-1 (5'-TCCTAGGTGAACCTGCG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3'). Nayak and Hiremath clustered the isolates of *B. oryzae* into two phylogenetic analyses through UPGMA cluster analysis based on genetic distance coefficients.

The ITS region of fungi is particularly helpful in the identification and evaluation of genetic links among fungi since it displays considerable variation even within species in cluster analysis using the UPGMA method, with polymorphism ranging from 50 to 91.6%. According to Nayak and Hiremath, ITS sequencing the *B. oryzae* isolates to be clustered into 2 distinct clusters with several sub-groups within each genetic group, indicating a low level of genetic diversity within it. On the other hand, Kamal et al. reported the variable number tandem repeat analysis showed considerable diversity among the isolates of *Bipolaris oryzae*, being separated into 12 Fingerprint Types (FPTs) with 75% similarity level. While the majority of samples possessed one FPT and others derived from this by band change.

Ahmadpour, et al. grouped 283 genotypes which were collected from the Philippines, Japan, and Iran into three clusters based on the *Bipolaris oryzae* population structure, while the study of Das et al. showed that the pathogen population structure was clustered into four sub-populations for 91 germplasm of rice. A lack of support for linkage equilibrium was reported for *B. oryzae* populations collected from wild rice in the US. Linkage disequilibrium can be caused by linkage, migration, selection and genetic drift.

CONCLUSION

The genetic variation of *B. oryzae* has been previously determined using a variety of methods, including Random Amplified Polymorphic DNA (RAPD), Variable Number of Tandem Repeats (VNTRs), Inter-Simple Sequence Repeats (ISSR), Universal Rice Primers (URPs) and Restriction Fragment Length Polymorphisms (RFLPs). All of the studies' findings demonstrated that RAPD-based genetic similarity coefficients were generally lower and ranged from 19.3% to 77.1% although each marker had a variable band size (100-3000 bp) and percent polymorphism (19.3-100) from location to place. Isolates exhibiting both mating type idiomorphs (MAT1-1 and MAT1-2) were detected across all sites. The isolates of *B. oryzae* were able to be grouped into three to four different clusters, each with many sub-groups, using ITS sequencing data.

The genetic characteristics of *B. oryzae* isolates from different regions have varied depending on the type of molecular marker that was employed. Therefore, the combined use of three marker systems should be used in defining the genetic variability of *B. oryzae* strains to manage the disease properly.

ACKNOWLEDGMENT

The article was written to complete a seminar that was presented at Bahir Dar University and evaluated by Abaynew Jemal.

AUTHOR'S CONTRIBUTION

The corresponding author contributed to the conception and design of this study, the interpretation of the information and the draft of the manuscript. The author is also responsible for the accuracy, integrity and originality of the article.

CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

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