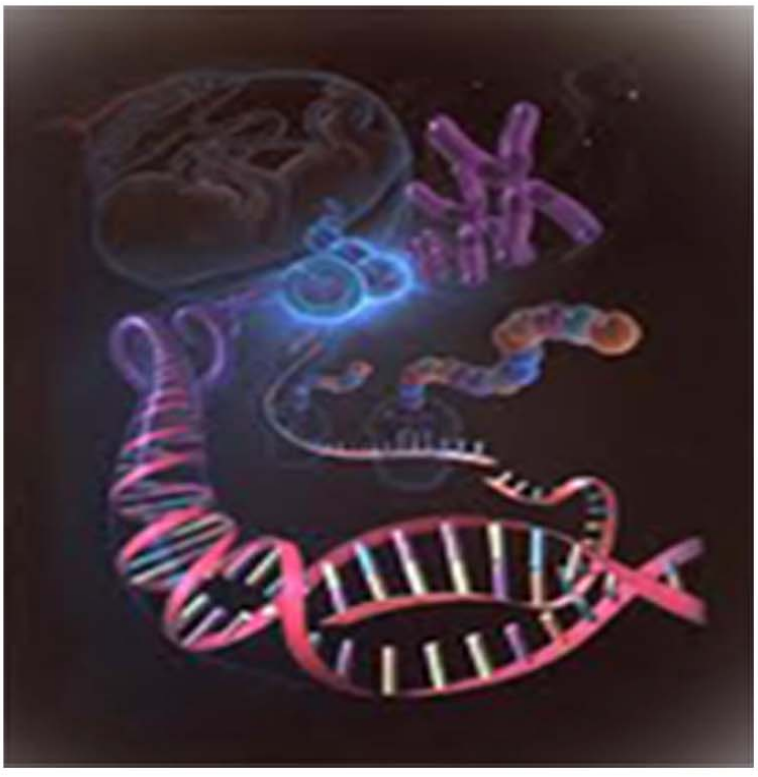
IJLBPR

ISSN 2250 - 3137
Vol1. 3, No. 1, January 2014

International Journal of

Life Sciences Biotechnology and Pharma Research



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

www.ijlbpr.com

Email : editorijlbpr@gmail.com or editor@ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 3, No. 1, January 2014

Research Paper

STUDY OF GENOMIC FINGERPRINTS PROFILE OF INDIAN BIPOLARIS ORYZAE FROM RICE (*ORYZA SATIVA*) BY RAPD-PCR

Basavaraju Archana[1]*, Kukandhuru Ramachandra Kini[1] and Harishchandra Sripathy Prakash[1]

*Corresponding Author:**Basavaraju Archana,** ✉ archanadinesh13@gmail.com

Rice (*Oryza sativa* L.) is a major food for India as well as the world population. Brown spot caused by heterothallic ascomycete *Cochliobolus miyabeanus* (Anamorph: *Bipolaris oryzae* (Breda de Haan). Shoemaker is an important constraint to rice production in most rice growing environments. The pathogen also causes leaf spot diseases on several graminaceous hosts, but is considered host specific. *Bipolaris oryzae* is notorious for its diversity and genetic variability. Understanding the variability of the fungal population associated with brown spot could improve disease control strategies. This study was done to generate genomic finger prints using RAPD markers as well as to find out genetic diversity in *B. oryzae* isolates collected from 12 different geographical regions of India. A total of 12 isolates and 40 RAPD primers were used to generate genomic fingerprint profile for *B. oryzae*. Cluster analysis using UPGMA method revealed polymorphism ranging between 50 to 91.66%. The number of loci produced from each marker ranged from 2-10 with a total of 212 loci from all isolates. The molecular weight of scorable loci ranged from 150 to 2500 bp. The pattern of RAPD bands could not show direct correlation between polymorphism and climatic or geographical areas.

Keywords: Bipolaris oryzae, Rice brown spot, RAPD, Genetic linkage, Dendrogram, Polymorphism

INTRODUCTION

Bipolaris oryzae (Breda de Haan) Shoem. (teleomorph *Cochliobolus miyabeanus* (Ito and Kurib) Drechsler ex Dastur), a heterothallic ascomycetes fungus, parasitizes over 23 species of grass genera and 20 species of *Oryza*. But the pathogen is best known as the causal agent of the rice brown spot disease. Rice is the most cultivated cereal crop worldwide and it has been estimated that half the world's population subsists wholly or partially on this crop (Van Nguyen and Ferrero, 2006). Brown spot is one of the constraints in rice production, first reported in West Bengal in 1943 and also reported in many countries around the world, especially under semi-dry conditions (Ou, 1985). An epidemic of

[1] Department of Studies in Biotechnology, Asian Seed Health Centre, University of Mysore, Manasagangotri, Mysore, 570006, Karnataka, India.

this disease lead to the 'Great Bengal Famine' and killed nearly two million people due to starvation (Padmanabhan, 1973). The disease affects the number of grains per panicle and reduces the weight of kernels. In Asia, brown spot has been noted to reduce yield from 6-90% (Mew and Gonzales, 2002). Growers and experts estimate that typical losses are 5-30%, but without control losses may reach 75- 100% (Percich and Nyvall, 1995). The relative importance of brown spot on rice has increased, in part due to the growing population which has resulted in an increased demand for rice with high quality appearance and free of seed-borne disease. Surveys confirmed that brown spot remains among the most serious constraints to yield in South Asia (Widawsky and O'Toole, 1990). Host plant resistance is the most promising method to brown spot disease control (Bonman *et al.*, 1992). Varietal resistance to brown spot occurs in rice but quantitative and clear physiological races of the pathogen that express cultivar specificity is not evident (Malavolta *et al.,* 2002; Thuy, 2002). There is little information on the aggressive patterns among different strains of *B. oryzae* (Thuy, 2002; Kamal, 2006).

PCR based molecular markers successfully developed during the last two decades have been widely employed as useful tools in detecting genetic variation, characterization and relatedness with population of plant pathogens (Vakalounakis and Fragkiadakis, 1999; Kolmer and Liu, 2000). RAPD markers have been extensively used to estimate genetic diversity in natural populations (Annamalai *et al*., 1995) mainly because the technique does not need previous molecular genetic information and it increases marker density for evaluating genetic relationship.

The analysis of genetic variation in plant pathogen populations is an important prerequisite for understanding co-evolution in the plant pathosystem (McDonald *et al*., 1989). Populations of rice brown spot pathogen throughout the world have been studied for the phenotypic and genetic variation (Ouedraogo *et al.*, 2004; Motlagh and Kaviani, 2008; Kamal and Mia, 2009; Motlagh and Anvari, 2010). The objective of the present study was to study the genetic variability among isolates of *Bipolaris oryzae* from different geographical regions of India.

MATERIALS AND METHODS

Collection of Seed Samples

The brown spot disease samples of rice were collected from 12 different rice growing states of India (Karnataka, Kerala, Assam, Andhra Pradesh, Manipur, Maharashtra, Chattisgarh, Tamil Nadu, West Bengal, Arunachal Pradesh, Delhi and Ranchi) (Table 1). The rice seed samples were obtained from rice research institutes, agricultural research stations, plant breeding stations and farmer's holdings. Two hundred seeds per sample were surface disinfected in 1% freshly prepared sodium hypochlorite solution for 2 min. Seeds were rinsed with sterilized distilled water, air dried and then plated onto 3 layers of moistened filter paper (Whatman No. 2) in Petri dishes and incubated at 25°C for 7 days under alternating near ultraviolet light (nuv) and dark conditions (nuv/dark, 12h/ 12h). Seed samples were screened for the presence of *B. oryzae*. All isolates were propagated on PDA slants, covered with parafilm at 25±1 °C and maintained in a collection at Asian Seed Health Centre, Department of Biotechnology, University of Mysore, Karnataka, India.

Table 1: Bipolaris Oryzae Isolates Used in the Present Study

S. No.	Isolate Name	Rice variety	Origin*
1	Bo*AP*	*Phalgun*	Andhra Pradesh, India
2	Bo*WB*	*Swarna*	West Bengal, India
3	Bo*Maha*	*Ratna*	Maharashtra, India
4	Bo*TN*	*ADT 36*	Tamil Nadu, India
5	Bo*Chatt*	*Mahamaya*	Chattisgarh, India
6	Bo*Ker*	*Waynaad*	Kerala, India
7	Bo*Kar*	*IR 64*	Karnataka, India
8	Bo*Ran*	*Piska Nagri*	Ranchi, India
9	Bo*As*	*Ketki Joha*	Assam, India
10	Bo*Mani*	*Mantriphukri*	Manipur, India
11	Bo*ArunPra*	*Ziro*	Arunachal Pradesh, India
12	Bo*Del*	*KRH2*	Delhi, India

Note: Origin*, Place of collection of rice seed samples

GENOMIC DNA ISOLATION

Mycelium of each isolate of *B. oryzae* was obtained by growing each isolate in potato dextrose broth (Himedia, Mumbai, India). For DNA extraction, 150 ml of potato dextrose broth was inoculated with two colonized agar plugs of 5 mm diameter for each isolate, and cultures were incubated at 22 ± 2°C for 8 days under alternating 12 h durations of darkness and near ultra-violet light. After incubation, the mycelial mat was harvested under aseptic conditions, and genomic DNA was extracted using a DNeasy Plant Mini kit (Qiagen, France) following instructions of the manufacturer. The purity of isolated DNA was analyzed spectrophotometrically.

RAPD-PCR AMPLIFICATION

Forty random primers including 20 RAPD OPA and 20 RAPD OPB series (Operon Technologies) (Table 2) were used in the present study. RAPD-PCR amplification was carried out in 20 µl reaction mixture in 200 µl PCR tubes. Each reaction mixture contained 25 ng genomic DNA, 200 µM of each dNTPs, 0.5 unit of Taq DNA polymerase, 1 X Taq polymerase buffer solution and 0.2 µM of primer. The reaction mixture was overlaid by one drop of mineral oil. Amplifications were performed in a thermal cycler (Mastercycler® Eppendorf®, Germany) programmed for an initial denaturation of 4 min at 94 °C, 35 cycles of denaturation at 94 °C for 1 min, annealing at 36 °C for 1 min and extension at 72 °C for 2 min followed by a final extension at 72 °C for 5 min.

AGAROSE GEL ELECTROPHORESIS

Horizontal submerged gel electrophoresis unit was used for separating RAPD fragments on agarose gel. After amplification, 10 µl of each

amplified product was electrophoresed in a 1.5% agarose gel prepared in 1X Tris-EDTA buffer, 6X DNA loading dye was mixed in the ratio of 5 : 1 v/v to amplified product. Ethidium bromide was used in gel to stain DNA bands. Electrophoresis was performed at 80 V for 4 h in 1X Tris-EDTA buffer. DNA ladder (100 bp) was also loaded in the same gel to estimate the molecular weight of the amplified product.

DATA ANALYSIS

DNA banding pattern generated by RAPD primers (Figure 1) were scored as "1" for presence of an amplified band and "0" for its absence. All gels were scored twice manually and independently. Presence or absence of unique and shared polymorphic bands was used to generate "Squared Euclidean Distances". The linkage distances were then used to construct a dendrogram using "Biostastica" and "Unweighted pair group averages".

RESULTS AND DISCUSSION

In this investigation, the genetic variation existing among 12 isolates of *B. oryzae* was studied by analyzing the DNA polymorphisms in isolates recovered from 12 different Indian rice cultivars (Table 1). The PCR amplifications were performed with 40 RAPD (OPA and OPB series) primers to access the level of polymorphism in 12 isolates of *B. oryzae*. Polymorphism range shown by RAPD primers was 50% (OPB 17) to 91.66% (OPA 18) (Table 2). The range of total loci scored ranged from 2 (OPB 5) to 12 (OPA 18). Molecular weight of loci ranged from 150 bp to 2500 bp, the lowest range was recorded for OPB 5 primer (200-600 bp) and highest for OPB 18 (400-2500 bp).

The genetic diversity of *B. oryzae* has been widely studied in Vietnam (Thuy, 2002), Brazil (Weikert-Oliveira *et al.*, 2002), Bangladesh (Kamal and Mia, 2009), India (Kumar *et al.*, 2010) and Iran (Motlagh and Anvari, 2010). Thuy (2002) found a low correlation between pathogenecity and genetic variation in *B. oryzae*. VNTR analysis grouped *B. oryzae* into two groups based on their aggressive nature (Kamal and Mia, 2002). Telomorphic forms had high genetic similarity with their respective anamorph of each fungal species tested based on RAPD and PCR-RFLP profiles (Weikert-Oliviera *et al.,* 2002). RAPD pattern generated from *B. oryzae* isolates revealed no direct correlation between polymorphism and climatic or geographical areas (Motlagh and Anvari, 2010).

The UPGMA dendrogram in the present study showed the 12 isolates divided into 2 major groups, with 3 isolates in one group and the rest in the other (Figure 4). In the second group, 2 subgroups were evident consisting of four isolates each. The results indicate that the geographic origin of *B. oryzae* isolates does not play crucial role in group formation, as in each group, there were mixed populations of the 12 geographical regions. Our results are consistent with previous reports (Kumar *et al.*, 2010; Motlagh and Anvari, 2010; Weikert-Oliviera *et al*., 2002). The phylogenetic grouping based on our RAPD data did not appear to be harmonious with geographical locations. The topology of the dendrogram suggests that most isolates are about 25-40% different from each other, indicating that both local and geographical polymorphisms exist. Overall a high genetic diversity was obtained in Indian rice brown spot fungus. The significant amount of diversity among

Table 2: Details of Number of Loci Detected With 40 Rapd Primers Used in the Present Study

S. No.	Primer Code	Sequence	Total loci	Polymorphism	
				No. of loci	%
1.	RAPD OPB 1	5' GTTTCGCTCC 3'	10	09	90.00
2.	RAPD OPB 2	5' TGATCCCTGG 3'	04	03	75.00
3.	RAPD OPB 3	5' CATCCCCCTG 3'	09	07	77.77
4.	RAPD OPB 4	5' GGACTGGAGT 3'	08	07	87.50
5.	RAPD OPB 5	5' TGCGCCCTTC 3'	02	01	50.00
6.	RAPD OPB 6	5' TGCTCTGCCC 3'	09	06	66.66
7.	RAPD OPB 7	5' GGTGACGCAG 3'	07	06	85.71
8.	RAPD OPB 8	5' GTCCACACGG 3'	06	05	83.33
9.	RAPD OPB 9	5' TGGGGGACTC 3'	07	06	85.71
10.	RAPD OPB 10	5' CTGCTGGGAC 3'	03	02	66.66
11.	RAPD OPB 11	5' GTAGACCCGT 3'	05	03	60.00
12.	RAPD OPB 12	5' CCTTGACGCA 3'	09	08	88.88
13.	RAPD OPB 13	5' TTCCCCCGCT 3'	07	06	85.71
14.	RAPD OPB 14	5' TCCGCTCTGG 3'	08	07	87.50
15.	RAPD OPB 15	5' GGAGGGTGTT 3'	02	01	50.00
16.	RAPD OPB 16	5' TTTGCCCGGA 3'	10	08	80.00
17.	RAPD OPB 17	5' AGGGAACGAG 3'	02	01	50.00
18.	RAPD OPB 18	5' CCACAGCAGT 3'	05	04	80.00
19.	RAPD OPB 19	5' ACCCCCGAAG 3'	03	02	66.66
20.	RAPD OPB 20	5' GGACCCTTAC 3'	04	03	75.00
21.	RAPD OPA 1	5' CAGGCCCTTC 3'	06	05	83.33
22.	RAPD OPA 2	5' TGCCGAGCTG 3'	07	06	85.71
23.	RAPD OPA 3	5' AGTCAGCCAC 3'	08	07	87.50
24.	RAPD OPA 4	5' AATCGGGCTG 3'	08	07	87.50
25.	RAPD OPA 5	5' AGGGGTCTTG 3'	09	08	88.88
26.	RAPD OPA 6	5' GGTCCCTGAC 3'	08	07	87.50
27.	RAPD OPA 7	5' GAAACGGGTG 3'	05	04	80.00
28.	RAPD OPA 8	5' GTGACGTAGG 3'	05	04	80.00
29.	RAPD OPA 9	5' GGGTAACGCC 3'	08	07	87.50

Table 2 (Cont.)

S. No.	Primer Code	Sequence	Total loci	Polymorphism	
				No. of loci	%
30.	RAPD OPA 10	5' GTGATCGCAG 3'	08	07	87.50
31.	RAPD OPA 11	5' CAATCGCCGT 3'	09	08	88.88
32.	RAPD OPA 12	5' TCGGCGATAG 3'	04	03	75.00
33.	RAPD OPA 13	5' CAGCACCCAC 3'	08	07	87.50
34.	RAPD OPA 14	5' TCTGTGCTGG 3'	04	03	75.00
35.	RAPD OPA 15	5' TTCCGAACCC 3'	09	08	88.88
36.	RAPD OPA 16	5' AGCCAGCGAA 3'	08	07	87.50
37.	RAPD OPA 17	5' GACCGCTTGT 3'	06	05	83.33
38.	RAPD OPA 18	5' AGGTGACCGT 3'	12	11	91.66
39.	RAPD OPA 19	5' CAAACGTCGG 3'	07	06	85.71
40.	RAPD OPA 20	5' GTTGCGATCC 3'	08	07	87.50

Note: %, Percentage of polymorphism.

Figure 1: Genomic Finger Print of 12 Isolates With RAPD Primer Opb 01

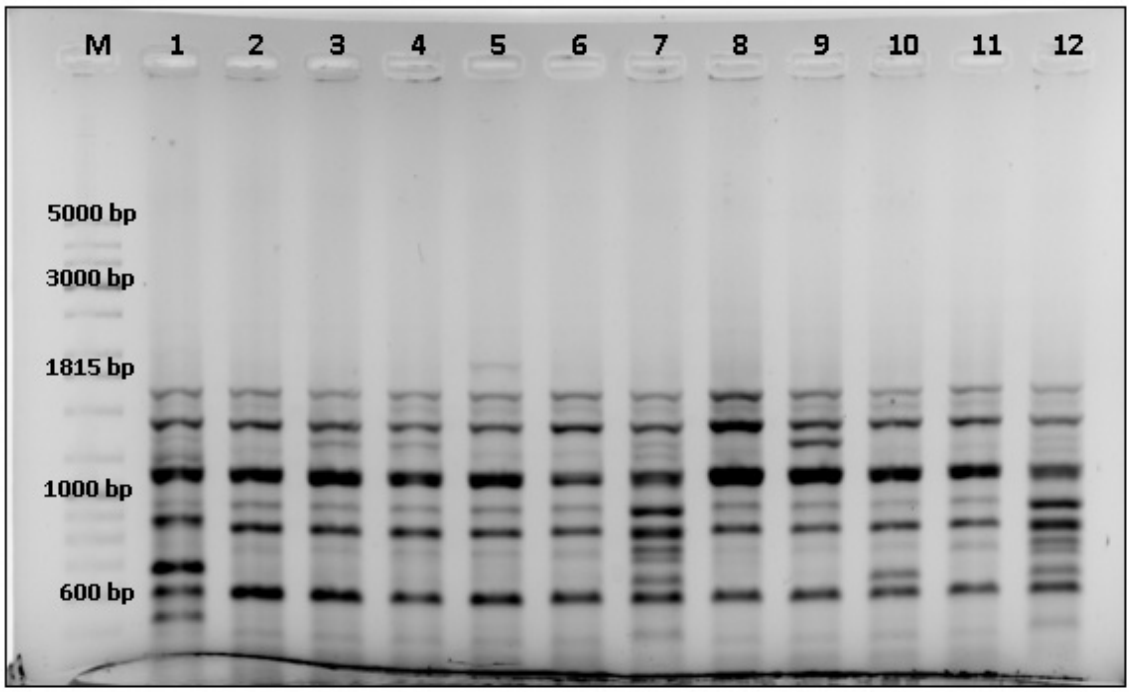


Figure 2: Genomic Finger Print of 12 Isolates With RAPD Primer Opa 19

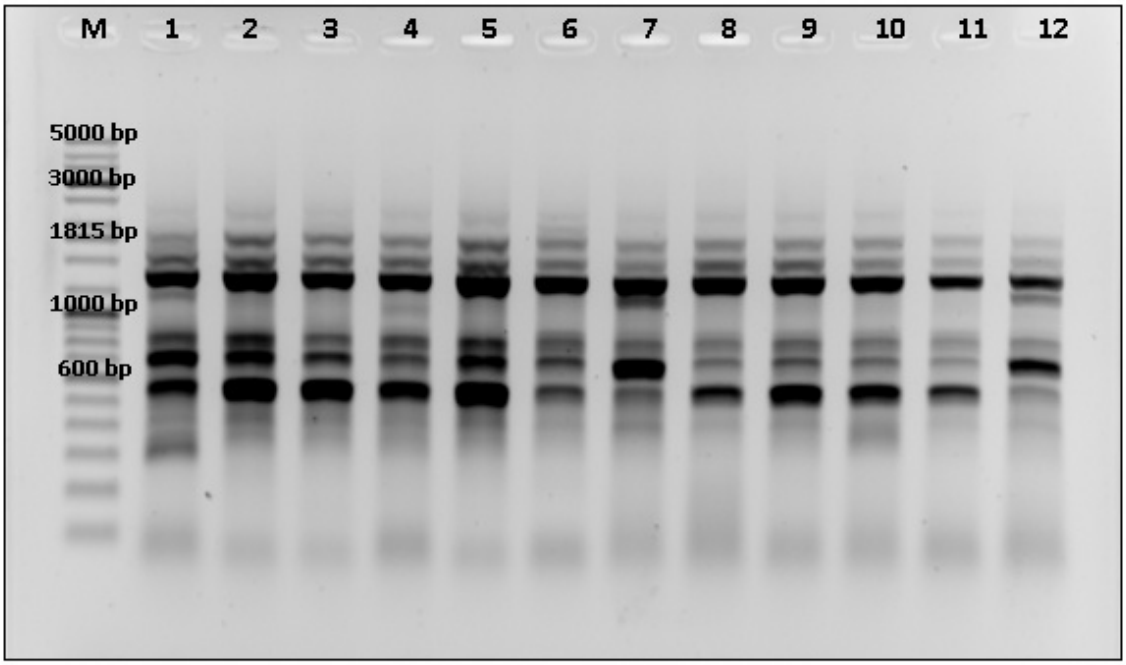


Figure 3: Genomic Finger Print of 12 Isolates With RAPD Primer Opa 05

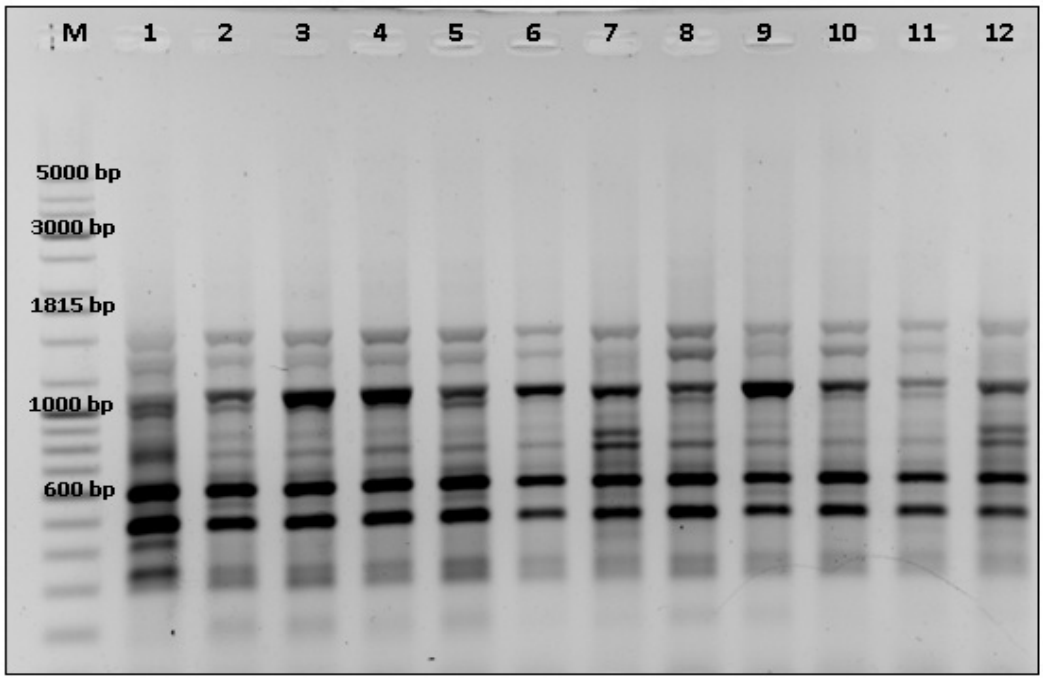
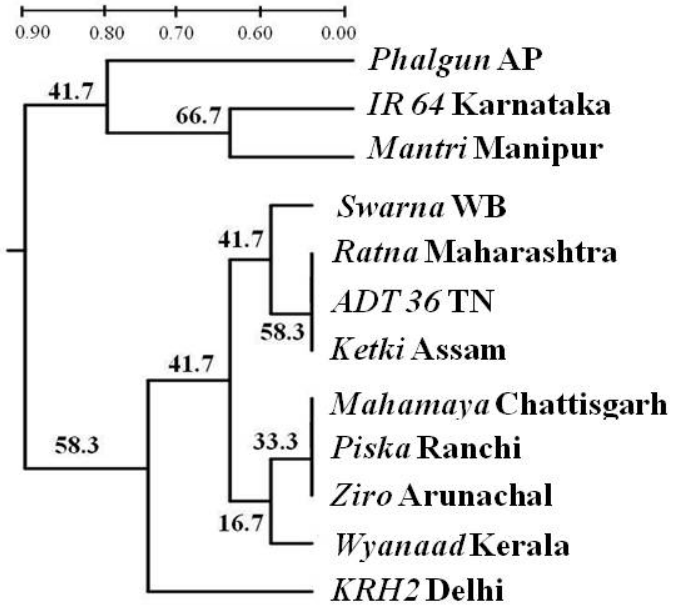


Figure 4: Tree Diagram For 12 Isolates Of B. Oryzae Unweighted Pair Group Average and Squared Euclidean Distances



Indian isolates of *B. oryzae* may be due to natural and stress-induced transposition (Ikeda *et al.*, 2001).

Plant pathogenic fungi most commonly rely on mutation, meiotic recombination and mitotic (parasexual) recombination as the main source of genetically based variation (Motlagh and Anvari, 2010). Gene flow, along with other evolutionary forces, can result in the spread of single genes, genotypes and even in the establishment of whole population in different regions (McDermott and McDonald, 1993).

Bipolaris oryzae isolates genetically distinct from those of indigenous types were introduced to Bangladesh from India through flood water carrying floating propagules of *B. oryzae* infected plant parts. Contaminated seeds are the other possible source for germplasm exchange (Ali, 2002; Ali *et al.,* 2004). On the basis of the present study and in comparison with the previous investigations, it is concluded that the Indian population of the rice brown spot fungus may be genetically heterogeneous and the interrelationships amongst the different isolates can be easily, precisely and reliably explained by RAPD-PCR technology.

ACKNOWLEDGMENT

This research was supported by a grant from the UPG Fellowship. The first author would like to thank the Department of Biotechnology, University of Mysore, India for providing excellent laboratory facilities to carry out this research. We also thank all providers of rice seed samples from different states of India.

REFERENCES

1. Ali M A (2002), "Biological variation and chemical control of *Rhizoctonia solani* causing rice sheath blight diseases in Bangladesh", Ph.D. Thesis, University of London, Imperial College, London, pp. 202.
2. Ali M A, Kamal M M, Archer S A, Buddie A and Rutherford M (2004), "Anastomosis and DNA fingerprinting the rice isolates of *Rhizoctonia solani* Kuhn using AFLP markers", *Bangl. J. Plant Pathol.,* Vol. 20, pp. 1-8.
3. Annamalai P, Ishii H, Lalithakumari D and Revathi R (1995), "Polymerase Chain Reaction and its applications found in fungal disease diagnosis", *J. Plant Dis. Protec.,* Vol. 102, pp. 91-104.
4. Bonman J M, Khush G S and Nelson R J (1992), "Breeding Rice for Resistance to Pests", *Ann. Rev. Phytopathol*., Vol. 30, pp. 507-528.
5. Ikeda K, Nakayasiiki H, Takagi M, Tosa Y and Mayama S (2001), "Heat shock, copper sulphate and oxidative stress activate the retrotransposon MAGGY resident in the plant pathogenic fungus *Magnaporthe grisea"*, *Mol. Gen. Genom.,* Vol. 266, pp. 318-325.
6. Kamal M M (2006), "Characterization of seed borne *Bipolaris oryzae* and quantification of its transmission", Ph.D. Thesis, Department of Plant Pathology, Agricultural University, Mymensingh, pp. 158.
7. Kamal M M and Mia M A T (2009), "Diversity and pathogeneicity of the rice brown spot pathogen, *Bipolaris oryzae* (Breda de Haan) Shoem. in Bangladesh assessed by genetic fingerprinting analysis", *Bangl. J. Bot.* Vol. 38, pp. 119-125.
8. Kolmer J A and Liu J Q (2000), "Virulence and molecular polymorphisms in international collections of the wheat leaf rust fungus *Puccinia triticina*", *Phytopathol*., Vol. 90, pp. 427-436.
9. Kumar P, Anshu V and Kumar S (2011), "Morphopathological and molecular characterization of *Bipolaris oryzae* in Rice (*Oryza sativa*)", *J. Phytopathol.,* Vol. 159, pp. 51-56.
10. Malavolta U M A, Parisi J J D, Takada H M and Martins M C (2002), "Effect of different incidence levels of *Bipolaris oryzae* in rice seeds on physiological aspects: seed-seedling transmission and productions", *Summa Phytopathol*., Vol. 28, pp. 336-340.
11. McDermott J M and McDonald B A (1993), "Gene flow in plant pathosystems", *Ann. Rev. Phytopathol.,* Vol. 31, pp. 353-373.
12. McDonald B A, McDermott J M and Goodwin S B (1989), "The population biology of host pathogen interactions", *Ann. Rev. Phytopathol.,* Vol. 27, pp. 77-94.
13. Mew T W and Gonzales P (2002), *A Handbook of Rice Seedborne Fungi*, Laguna, Philippines/Enfield, NH, USA: International Rice Research Institute/ Science Publishers, Inc., pp. 83.
14. Motlagh M R S and Anvari M (2010), "Genetic variation in a population of *Bipolaris oryzae* based on RAPD PCR in North Iran", *Afr. J. Biotech.,* Vol. 9, pp. 5800-5804.

15. Motlagh M R S and Kaviani B (2008), "Characterization of new *Bipolaris* species: the causal agent of rice brown spot disease in the North of Iran", *Internat. J. Agri. Biol.,* Vol. 10, pp. 638-642.

16. Ou S H (1985), "Rice Diseases. Commonwealth Mycological Institute", 2nd ed. Kew, Surrey, UK, pp. 380.

17. Ouedraogo I, Correll J C, Boza E J, Cartwright R D, Lee F N and Sankara P (2004), "Pathogenic, molecular and genetic diversity among *Bipolaris*, *Drechslera* and *Exserohilum* species on rice", In: Norman, R J, Meullenet J F, Moldenhauer K A K (Eds.) , *BR Wells Rice Research Studies*. Fayetteville, A R, USA, AAES Research Series 529, pp. 111-119.

18. Padmanabhan S Y (1973), "The Great Bengal famine", *Annu. Rev. Phytopathol*., Vol. 11, pp. 11-26.

19. Percich J A and Nyvall R F (1995), "Diseases of cultivated wild rice in Minnesota: A Pictorial Guide", University of Minnesota.

20. Thuy T T T (2002), "Infection biology of *Bipolaris oryzae* in rice and its pathogenic variation in the McKong Delta, Vietnam", Ph.D. Thesis. Department of Plant Biology, The Royal Veterinary and Agricultural University Copenhagen, pp. 308.

21. Vakalounakis D J and Fragkiadakis G A (1999), "Genetic diversity of *Fusarium oxysporum* isolates from cucumber: differentiation by pathogenicity, vegetative compatibility, and RAPD fingerprinting", *Phytopathol.,* Vol. 89, pp. 161-168.

22. Nguyen N V and Ferrero A (2006), "Meeting the challenges of global rice production", *Paddy, Water and Environ.,* Vol. 4, pp. 1-9.

23. Weikert-Oliverira R C B, Resende M A, Valerio H M, Caligiorne R B and Paiva E (2002), "Genetic variation among pathogens causing 'Helminthosporium' diseases of rice, maize and wheat", *Fitopathol. Brasil.* Vol. 27, pp. 238-246.

24. Widawsky D A and O'Toole J C (1990), "Prioritizing the rice biotechnology research agenda for eastern India", The Rockefeller Foundation, New York, pp. 86.

International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA. Ph: +91-09441351700, 09059645577
E-mail: editorijlbpr@gmail.com or editor@ijlbpr.com
Website: www.ijlbpr.com

ISSN 2250-3137