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Full Length Research Paper

Study of genetic variation in population of *Bipolaris victoriae,* the causal agent of rice brown spot disease, in Guilan Province of Iran

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Isolates of *Bipolaris victoriae* were analysed by random amplified polymorphic DNA (RAPD) techniques to determine the amount of intraspecific genetic variability. Seven primers were applied and DNA bands of 200-5000 bp were produced. Cluster analysis using UPGMA method gave five groups. Levels of polymorphism were observed between DNA of different isolates. The pattern of RAPD bands did not show any correlation between polymorphism and climates or geographical areas.

Key words: *Bipolaris victoriae*, rice, genetic variation, RAPD.

INTRODUCTION

Brown spot is one of the most important seed borne diseases of rice in Guilan province of Iran and was reported by Petrak et al. (1957) for the first time (Safari Motlagh et al., 2005). It affects the grain yield, both in wet and dry seasons, with heavy natural infection (Bakoni et al., 1995). It was first associated with nectrotic spots on the leaves of cultivated rice (Ou, 1985). At first, the causal agent of brown spot disease by Breda de Haan was named *Helminthosporium oryzae* (Gangopadhyay and Padmanabhan, 1987). Nowadays, the graminicolous *Helminthosporium* species were divided into three genuses based on colony, conidiophore and conidial morphology, type of condial germination and the type of hilum structure: *Bipolaris*, *Drechslera* and *Exserohilum* (Sivanesan, 1987).

Some authors have used the molecular markers to study of the relationship among these species (Bakonyi et al., 1995; Abadi et al., 1996). Increasingly, molecular biology techniques have been used to explore genetic variability in fungi (Caligiorne et al., 1999). Random Amplified Polymorphic DNA (RAPD) was applied for diagnosis *Rhizoctonia solani* (Nicholson and Parry, 1996), *Microdochium nivale* and some plant pathogenic fungi (Parry and Nicholson, 1996). Isolates of *Bipolaris sorokiniana* were analyzed by RAPD to determine the amount of intraspecific genetic variability and to study host pathogen interactions (Oliveira et al., 2002). Genetic variation has been analysed by PCR and PCR-RFLP among pathogens causing "*Helminthosporium*" diseases in rice, maize and wheat (Weikert-Oliveira et al., 2002). The aim of this investigation was to study the genetic variation of *Bipolaris victoriae* population, the causal agent of rice brown spot disease, prevalent in paddy fields of Iran.

MATERIALS AND METHODS

This investigation was conducted in Rice Research Institute of Iran during 2004-2007. Samples of *B. victoriae* were collected from rice fields of Guilan province of Iran, by using Xia et al. (1993) method. Morphological study was applied in TWA+ wheat straw media (Sivanesan, 1987).

Total DNA was extracted from mycelium according to the procedure of Liu et al. (2000). According to the protocol, samples for DNA extraction were grown on potato-dextrose broth for seven days at 22-25°C. 300 mg of myceli were harvested, frozen in liquid nitrogen. Then 500 µL lysis buffer was added. The tubes were then left at room temperature for 10 min. After adding 150 µL of 5 M potassium acetate, the tubes were vortexed briefly and spun at 8,000 rpm for 1 min. The supernatant was transferred to another

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Table 1. RAPD primers used in this study.

Code	Sequence 5′ to 3′
A01	CCCAAGGTCC
A02	GGTGCGGGAA
A06	GAGTCTCAGG
A08	ACGCACAACC
B06	GTGACATGCC
B07	AGATGCAGCC
OPC13	AAGCCTCGTC

1.5 ml Eppendorf tube, and an equal volume of isopropyl alcohol was added. The tubes were mixed by inversion briefly. The tube was spun at 10000 rpm for 2 min, and the supernatant discarded. The resultant DNA pellet was washed in 300 µL of 70% ethanol. The pellet was spun at 10,000 rpm for 1 min and the supernatant was discarded. The DNA pellet was air dried and dissolved in 50 µL of 1X Tris-EDTA buffer, and 1 µL of the purified DNA was used in 25 to 50 µL of PCR mixture. For determining DNA quantity, the genomic DNA extracted was electrophoresed in 1.4% agarose gel, and detected by staining with ethidium bromide.

The primers used in this study were A01, A02, A06, A08, B06, B08 and OPC13 (Oliveira et al., 2002). For standardization of the amplification conditions and selection of the primers, the genomic DNA extracted from original samples of *B. victoriae* was used as a template for the amplifications (Table 1). All reactions were carried out in a volume of 25 µL with 10 ng DNA, 12 mmol/L of each dNTP, 1.25 µmol of primer, 2.5 µL of 10X reaction buffer (50 mM KCl + 20 mM Tris-HCl, pH 8.4, 5 mM $MgCl_2$), 50 µg/ml bovin serum albumin and 1 unit of Taq DNA polymerase. The reactions were performed on a thermal cycler programmed for one initial cycle with 1 min at 94°C, 5 min at 35°C, 2 min at 72°C and 45 cycles of 1 min at 94°C, 1 min at 35°C and 2 min at 72°C. The assays were repeated at least twice with each primer in different experiments. Amplification products were electrophoresed in 1.4% agarose gel, and detected by staining with ethidium bromide.

The RAPD bands were scored as 0 (fragment absent) and 1 (fragment present) in a data matrix and then, distance values were subject to phenetical and cluster analysis using the softwares package NTSYS-pc-2.02, Popgene and Arlequin 2.00 (Sneath and Sokal, 1973; Rohlf , 1998; Yeh et al., 1999; Schneider et al., 2000).

RESULTS AND DISCUSSION

In this investigation, the genetic variation existing among isolates of *B. victoriae* was studied by analyzing the DNA polymorphisms in isolates recovered from different rice cultivars. From seven primers A01, A02, A06, A08, B06, B08 and OPC13, four primers A01, A08, B06 and B07 revealed the best profiled of strains. The RAPD profiled showed a high level of genetic variability among the *B. victoriae* isolates. The most generated and polymorphic bands were amplified with primer B07 and the least generated and polymorphic bands were amplified with A01 and B06. 80 polymorphic fragments were detected in the samples, ranging from 200 – 5000 bp. The phenogram from cluster analysis can divide *B. victoriae* into 5 groups with a similarity coefficient of 0.75 (Figure 1). The biggest group consisted of 41 isolates. The softwares package Popgane and Arlequine indicated that average

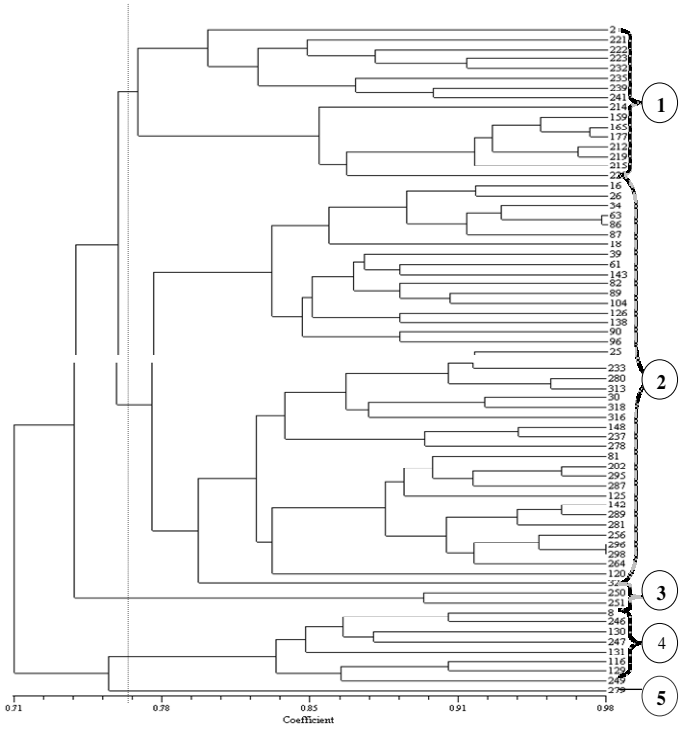

Figure 1. UPGMA-phenogram derived from banding patterns of RAPD analysis in population of *Bipolaris victoriae* in Guilan Province of Iran.

gene diversity of population of *B. victoriae* were 0.8669183 ± 0.471949. The number and percentage of polymorphic loci in this population were 76 and 86.36%, respectively (Table 2).

RAPD profiles did not reveal polymorphism that directly correlated with climatic factors or host origin with geographic source of the isolates of *B. victoriae.* Haplotypes of *B. victoriae* were variated. Host specific pressure had no effect on the genetic variation of this species, because no significant correlation was found between cultivar type and fungus species. But some of the isolates of fungus with high genetic similarity have the same origin (Figure 1).

Weikert et al. (2002) reported that species of *Drechslera*, *Bipolaris* and *Exserohilum* are genetically distinct and demonstrated that the telemorphs of the analyzed species presented high genetic similarity in their electrophoretic profiles when compared to the respective anamorph of each species. According to Burdon and Silk (1997), plant pathogenic fungi most commonly rely on mutation and recombination as the main source of genetically based variation. Within a species, the gene flow between populations supplements these processes as propagules spread from one epidemiological area to another and from one deme to the next.

Gene flow, along with other evolutionary forces, can result in the spread of single genes (or DNA sequences), genotypes and even in the establishment of whole popu-

Table 2. Summary of genic variation statistics for all loci (Nei, 1978) in population of *Bipolaris victoriae* in Guilan Province of Iran.

locus	Sample size	na*	ne*	h*	I*
A011	68	2	1.0921	0.0843	0.1808
A012	68	2	1.0606	0.0571	0.1427
A013	68	2	1.2620	0.2076	0.3622
A014	68	2	1.5241	0.2493	0.5277
A015	68	2	1.7101	0.4152	0.6058
A016	68	2	1.7785	0.4277	0.6295
A017	68	2	1.2265	0.1847	0.3315
A018	68	2	1.0606	0.0571	0.1327
A019	68	1	1	0.0000	0.0000
B061	57	1	1	0.0000	0.0000
B062	57	2	1.0726	0.0677	0.1520
B063	57	1	1	0.0000	0.0000
B064	57	2	1.5888	0.2706	0.5575
B065	57	2	1.0357	0.0345	0.0884
B066	57	2	1.9945	0.4986	0.6918
B067	57	2	1.1905	0.1600	0.2972
B068	57	2	1.0357	0.0345	0.0884
B069	57	2	1.0726	0.0677	0.1520
B0610	57	1	1	0.0000	0.0000
B0611	57	1	1	0.0000	0.0000
locus	**Sample size**	**na***	**ne***	**h***	**I***
A081	67	2	1.3030	0.2326	0.3945
A082	67	2	1.2402	0.1871	0.3348
A083	67	2	1.0615	0.0579	0.1342
A084	67	2	1.9045	0.4749	0.6679
A085	67	2	1.9045	0.4749	0.6679
A086	67	2	1.9889	0.4972	0.6904
A087	67	2	1.0935	0.0855	0.1828
A088	67	2	1.1265	0.1123	0.2261
A089	67	2	1.0303	0.0294	0.0776
A0810	67	2	1.4165	0.2941	0.4700
A0811	67	2	1.6844	0.4063	0.5963
B071	68	1	1	0.0000	0.0000
B072	68	2	1.0298	0.0290	0.0766
B073	68	2	1.6060	0.0571	0.1327
B074	68	1	11	0.000	0.0000
B075	68	2	1.2620	0.2076	0.3612
B076	68	2	1.2981	0.2297	0.3908
B077	68	2	1.9396	0.4888	0.6775
B078	68	2	1.4859	0.3270	0.5084
B079	68	2	1.8105	0.4477	0.6499
B0710	68	2	1.5241	0.3439	0.5277
B0711	68	2	1.5622	0.3599	0.5456
B0712	68	2	1.5241	0.3493	0.5277
B0713	68	2	1.0921	0.0843	0.1808
B0714	68	1	1	0.000	0.0000
B0715	68	2	1.6474	0.3894	0.5779
B0716	68	2	1.1245	0.1107	0.2237
B0717	68	1	1	0.000	0.0000
B0718	68	2	1.0921	0.0843	0.1808
B0719	68	1	1	0.000	0.0000
average	57	1.8636	1.3968	0.2413	0.3736

na = Observed number of alleles; ne = Effective number of alleles; h = Nei's [1978] gene diversity; I = Shanon's information index.

lation in different regions (McDermott and McDonald, 1993). Based on the high degree of genetic variation found among *B. victoriae* isolates from Guilan province, it is suggested that there is considerable gene flow among this population. In conclusion, application of RAPD-PCR is useful for identification of the fungi at the species level and can provide good results for further studies including introduction of resistant varieties.

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