JEB™
Website : www.jeb.co.in
E-mail : editor@jeb.co.in
Journal of Environmental Biology
ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Genetic diversity analysis of fungal pathogen *Bipolaris sorghicola* infecting *Sorghum bicolor* in India

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Abstract

Bipolaris sorghicola (Lefebvre and Sherwin) is a well known and economically important seed-borne pathogen with the specific species of sorghum (*Sorghum bicolor* [L] Moench) as host. Thirty-two strains were obtained from different geographical area of sorghum growing places in India. Molecular characterization using three marker systems *i.e.*, universal rice primers (URP), inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) was carried out. Molecular marker work revealed differences along with geographical origin clustering of various *B. sorghicola* strains which could not be revealed through conventional method of characterization. Out of 13 URPs, 20 ISSR and 50 RAPD primers screened, 8 primers each from URP and ISSR, and 10 primers from RAPD marker were found to result in reproducible banding pattern. One hundred per cent of polymorphic bands was recorded in all three molecular markers. Total number of bands was recorded 1986 with average of 248.25 in URP marker, and 2026 bands with average of 253.25 in ISSR marker and 2158 bands with average of 215.80 in RAPD markers. Maximum heterozygosity (*Hn*) was revealed by URP 17R (0.40), ISSR 10 (0.41) and RAPD marker OPC-5 (0.34). The polymorphism information content (PIC) values ranged between 5.89 to 8.28 in URP, 4.57 to 8.79 in ISSR and 4.44 to 9.64 in RAPD marker profiles. Maximum cophenetic correlation was found in URP ($r = 0.910$) followed by ISSR ($r = 0.904$) and RAPD ($r = 0.870$). The combined analysis of all three marker systems showed high cophenetic correlation ($r = 0.911$), which indicated a very good fit of the data for genetic diversity analysis. To best of our knowledge, this is a first report of genetic characterization of *B. sorghicola*. Hence, combined use of three marker systems would be more sensitive and reliable in characterizing genetic variability in *B. sorghicola* strains.

Publication Info

Paper received:
07 July 2015

Revised received:
30 October 2015

Re-revised received:
10 February 2016

Accepted:
14 March 2016

Key words

Bipolaris sorghicola, Genetic diversity, Molecular markers, Random markers

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Introduction

Sorghum (*Sorghum bicolor* L. Moench) is the fifth most commonly grown cereal in the world and is a rich source of sorghum-specific natural products. It is an important forage crop, especially in the semi-arid tropics because of its tolerance of hot and dry environments. Target leaf spot is one of the major foliar diseases of sorghum under conditions of high humidity. The disease is caused by a necrotrophic fungus, *Bipolaris sorghicola* (Zummo and Gourley, 1987; Kawahigashi *et al.,* 2011). Infected leaves of sorghum show orange red spots with straw-colored centers. Severe infection is characterized by complete blighting of leaves followed by smaller ear heads, which substantially reduces the yield and biomass of the plant. This disease has been reported to occur in Argentina, Australia, China, Cyprus, India, Israel, Philippines, Sudan, Taiwan, USA, Venezuela and Yugoslavia (Katewa *et al.,* 2008). The pathogen was also

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found to survive on infested debris in soil for up to 3 months after crop harvesting and significant positive correlation was observed between disease severity and maximum relative humidity (Katewa *et al.,* 2008). High variability within the pathogen leads to a break in the susceptibility to certain fungicides, with the development of either quantitative or qualitative resistance against the fungicides. Tolerance or resistance ability of sorghum varieties against *B. sorghicola* is not predictable without knowing the genetic structure of the pathogen. 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. Very importantly, variability in pathogen population is also useful in identifying and characterizing resistant sorghum germplasm (Kandan, 2013; Kandan *et al.,* 2015).

Extensive knowledge of the pathogen population variability is necessary for effective host breeding for resistance (Savary *et al.,* 2011). Variability in morphology and pathology of *B. sorghicola* isolates has not been reported so far from any sorghum-growing countries. Excepting few epidemiological, biochemical studies and trancriptome analysis of this pathogen, there are no reports on diversity analysis of this pathogen so far (Khan *et al.,* 2001; Katewa *et al.,* 2008; Yazawa *et al.,* 2013).

During the last two decades, several molecular marker techniques such as Random amplified polymorphic DNA (RAPD), Variable number of tandem repeats (VNTRs), and Restriction fragment length polymorphisms (RFLPs), Inter simple sequence repeats (ISSR), Simple Sequence Repeats (SSR) have been developed for assessing the genetic variation of fungal pathogens from different countries (Kumar *et al.,* 2011a; Burgos *et al.,* 2013; Pradeep Kumar *et al.,* 2014; Kandan *et al.,* 2015). Repeat sequences from Korean weedy rice, originally referred to as Universal Rice Primers (URPs), have been used for fingerprinting of diverse genomes of plants, animals, and microbes (Kang *et al.,* 2002). URP-PCR has been used earlier in molecular analyses of only a few fungi (Kang *et al.,* 2002; Aggarwal *et al.,* 2008; Kandan *et al.,* 2015). Hence, the aim of the present study was to characterize the isolates of *B. sorghicola* isolated from S. *bicolor* using three different molecular markers, *viz.,* inter-simple sequence repeats (ISSR), RAPD and URP-PCR.

Materials and Methods

Isolation and maintenance of fungus : Thirty-two isolates of *B. sorghicola* were isolated from infected seeds collected from major sorghum growing regions in India. These seeds were surface sterilized and plated under aseptic conditions. The isolates were purified by modified single spore isolation technique (Akhtar *et al.,* 2014), and the purified cultures maintained on potato dextrose agar (PDA) medium (HiMedia, India) with periodic sub-culturing and stored at 4°C. Isolates were designated as DQDS. Two isolates from Karnataka, four isolates from Madhya Pradesh, seven isolates from Maharashtra, six isolates from West Bengal, five isolates from Uttar Pradesh and Andhra Pradesh each, two isolates from Gujarat and one isolate from Haryana were isolated and purified.

Genomic DNA isolation and PCR amplification : Thirty-two isolates of *B. sorghicola* were grown on potato dextrose broth (HiMedia, India) at 28 ± 1°C for seven days. Fresh mycelial mat were filtered through autoclaved muslin cloth, and about 1 g mycelia were ground in a pre-chilled mortar with liquid nitrogen. Approximately, 200 mg of powdered mycelia were taken in sterile eppendorf tube and HiPurA™ fungal DNA Purification kit (HiMedia, India) was used to extract DNA as per manufacturer's protocol. The purified DNA was quantified by Nanodrop spectrophotometer (Thermo Fisher Scientific, India) and the concentration was adjusted to 25 ng μl^{-1} for use in PCR analysis.

The sequences of URP primers (derived from the repeat sequences of Korean weedy rice) were adapted from Kang *et al.* (2002) and URP, ISSR and RAPD primers were synthesized and obtained from Promega Inc. (Pragati Biomedical, India). For all three molecular marker analysis PCR amplifications were carried out in a reaction volume of 25 µl containing 25 ng of template DNA. The PCR reaction mixture consisted of 10x PCR buffer (100 mM Tris-HCl, 500 mM KCl, 0.8% (v/v) Nonidet P40), 10 mM dNTPs, 1 U *Taq* DNA polymerase, and 0.2 µM primer. All the PCR reaction components were obtained from Fermentas, Thermo Fisher Scientific, India. The amplifications were performed using a Thermal Cycler (GenePro PCR, Bioer, New Hampshire, USA). For URP, ISSR and RAPD markers, PCR temperature profiles are as follows: initial DNA denaturation at 94°C for 4 min, followed by 35 PCR cycles at 94°C for 1 min, 1 min for primer specific annealing temperature (Table 1), 72°C for 2 min and a final cycle at 72°C for 10 min. All the amplified PCR products were resolved by electrophoresis on 1.6% agarose for 2.5 h in 1x TBE buffer at 80 V, stained with ethidium bromide and photographed using the Gel Documentation System (AlphaImager® Corporation, California, USA).

Data analysis : The genetic relationship among the 32 isolates was analyzed from the combined 0/1 matrix data of URP, ISSR and RAPD profiles using the software program NTSYSpc version 2.02e (Exeter Software, New York) (Rohlf 1998) and the Jaccard's similarity coefficient and unweighted pair group method using arithmetic average

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(UPGMA) (Sneath and Sokal, 1973). The resultant matrix data was also subjected to principal component analysis (PCA), correlation analysis and matrix correspondence test (Mantel 1967; Prevost and Wilkinson, 1999; Peakall and Smouse, 2006). The confidence level of clustering in the dendrogram was estimated through bootstrap analysis using the PAST version 2.03 program with 1000 permutations (Hammer *et al.,* 2001).

Results and Discussion

Genetic variation was detected among thirty-two strains of *B. sorghicola* using URP, ISSR and RAPD markers. Of the 13 URPs, 20 ISSR and 50 RAPD primers screened, eight primers of URPs and ISSR each and 10 RAPD markers were found to result in reproducible banding pattern (Fig. 1). The number of amplified products ranged from 16 to 25 in URP, 12 to 22 in ISSR and 15 to 25 in RAPD markers. The average number of amplified products obtained per primer were 19.63, 17.63 and 19.80 in URP, ISSR and RAPD, respectively. Total number of bands was observed 1986 with average of 248.25 in URP, 2026 with average of 253.25 in ISSR and 2158 with average of 215.80 in RAPD markers. One hundred per cent polymorphic bands were recorded in all the three markers.

Maximum heterozygosity (*Hn*) was observed with URP 17R (0.40) and URP 13R (0.33) and the least with URP

Table 1 : Primer names with their sequences and genetic diversity analysis of URP, ISSR and RAPD showing various marker utility parameters

Primer name	Primer Sequence (5'————3')	Annealing temperature (°C)	Average band size	Number of bands	Total number of bands	PM (%)	Hn	EMR	PIC	MI	RP
URP											
URP 2F	GTGTGCGATCAGTTGCTGGG	54	2.5kb-0.10kb	25	226	100.0	0.24	25	8.15	6.00	14.19
URP 6R	GGCAAGCTGGTGGGAGGTAC	54	2.5kb-0.10kb	21	286	100.0	0.32	21	7.85	6.72	17.88
URP 9F	ATGTGTGCGATCAGTTGCTG	49	3.0kb-0.10kb	21	241	100.0	0.30	21	8.17	6.30	15.06
URP 13R	TACATCGCAAGTGACACACC	49	2.5kb-0.20kb	21	280	100.0	0.33	21	8.28	6.93	17.44
URP 17R	AATGTGGGCAAGCTGGTGGT	50	2.0kb-0.15kb	17	304	100.0	0.40	17	6.77	6.80	18.94
URP 25F	GATGTGTTCTTGGAGCCTGT	49	3.0kb-0.30kb	16	267	100.0	0.26	16	5.90	4.16	10.44
URP 30F	GGACAAGAAGAGGATGTGGA	49	3.0kb-0.30kb	16	198	100.0	0.32	16	6.45	5.12	12.38
URP 38F	AAGAGGCATTCTACCACCAC	49	2.5kb-0.20kb	20	184	100.0	0.25	20	5.89	5.00	11.50
	Total			157	1986	-	2.42	157	57.46	47.03	117.83
	Average			19.63	248.25	-	0.30	19.63	7.18	5.88	14.73
ISSR											
ISSR 8	GACACGACACGACACGACAC	53	2.0kb-0.20kb	16	209	100.0	0.30	16	5.20	4.80	13.06
ISSR 10	GAGAGAGAGAGAGAGAYG	46	3.0kb-0.20kb	15	267	100.0	0.41	15	6.24	6.15	16.69
ISSR-11	AGAGAGAGAGAGAGAGT	42	3.0kb-0.20kb	22	286	100.0	0.30	22	7.72	6.60	17.75
ISSR-13	TGTGTGTGTGTGTGTGRG	46	1.5kb-0.30kb	12	151	100.0	0.31	12	4.57	3.72	9.44
ISSR 15	DBDACACACACACACAC	39	2.0kb-0.20kb	22	362	100.0	0.38	22	8.79	8.36	22.63
ISSR 16	AGAGAGAGAGAGAGAGTG	46	3.0kb-0.20kb	19	288	100.0	0.36	19	7.47	6.84	18.06
ISSR 17	AGAGAGAGAGAGAGAGCG	47	3.0kb-0.20kb	17	188	100.0	0.28	17	5.92	4.76	11.75
ISSR 19	GAGCAACAACAACAACAA	40	3.0kb-0.30kb	18	275	100.0	0.35	18	6.66	6.30	17.25
	Total			141	2026	-	2.69	141	52.57	47.53	126.63
	Average			17.63	253.25	-	0.34	17.63	6.57	5.94	15.83
RAPD											
OPA-1	CAGGCCCTTC	34	3.0kb-0.20kb	21	217	100.0	0.28	21	7.74	5.88	13.56
OPA-2	TGCCGAGCTG	34	3.0kb-0.30kb	19	173	100.0	0.24	19	6.45	4.56	10.75
OPA-9	GGGTAAACGCC	34	3.0kb-0.20kb	22	198	100.0	0.25	22	7.78	5.50	12.44
OPA-10	GTGATCGCAG	32	3.0kb-0.30kb	22	256	100.0	0.31	22	8.92	6.82	16.19
OPB-6	TGCTCTGCCC	34	3.0kb-0.20kb	15	178	100.0	0.26	15	4.44	3.90	11.00
OPB-9	TGGGGGACTC	34	3.0kb-0.20kb	16	134	100.0	0.24	16	5.69	3.84	8.44
OPC-2	GTGAGGCGTC	34	3.0kb-0.20kb	20	262	100.0	0.32	20	7.52	6.40	16.38
OPC-5	GATGACCGCC	34	3.0kb-0.50kb	21	296	100.0	0.34	21	8.08	7.14	18.56
OPC-8	TGGACCGGTG	34	3.0kb-0.40kb	25	248	100.0	0.27	25	9.64	6.75	15.50
OPE-4	GTGACATGCC	34	2.5kb-0.40kb	17	196	100.0	0.30	17	6.31	5.10	12.31
	Total			198	2158	-	2.81	198	72.57	55.89	135.13
	Average			19.80	215.80	-	0.28	19.80	7.26	5.59	13.51

PM= Polymorphic, Hn= Expected heterozygosity, EMR= Effective multiplex ratio, PIC= Polymorphism information content, MI= Marker index, RP= Resolving power

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Table 2 : Mantel test and cophenetic correlation (r) for comparison of similarity matrices derived from URP, ISSR and RAPD markers

	URP	ISSR	RAPD	ALL*
URP	0.910			
ISSR	0.687	0.904		
RAPD	0.669	0.544	0.870	
ALL*	0.911	0.845	0.849	0.911

*Combined analysis of all the markers

2F (0.24). In ISSR marker, maximum *Hn* was observed with ISSR 10 (0.41) and ISSR 15 (0.38) and the least with ISSR 17 (0.28). In case of RAPD marker, maximum *Hn* was observed with OPC-5 (0.34) followed by OPC-2 (0.32) and the least with OPA-2 and OPB-9 (0.24) (Table 1). In URP-PCR, effective multiplex ratio (EMR) ranged from 16.0 to 25.0, in which URP 2F scored high EMR (25.0) and least value observed with URP 25F and URP 30F (16.0). In ISSR-PCR, EMR range from 15.0 to 22.0, in which ISSR 11 and ISSR 15 scored high EMR (22.0) and the least value with ISSR 10 (15.0). In RAPD marker, EMR ranged from 15.0 to 25.0, in which OPC-8 scored high EMR (25.0), followed by OPA-9 and OPA-10 (22.0), and the least with OPB-6 (15.0). The polymorphism information content (PIC) values ranged between 5.89 to 8.28 in URP, 4.57 to 8.79 in ISSR and 4.44 to 9.64 in RAPD marker profiles. URP13R scored highest PIC values (8.28) among 8 URPs, whereas ISSR 15 scored highest PIC value (8.79) among 8 ISSR primers and OPC-8 scored highest PIC value (9.64) among 10 RAPD primers on genetic diversity analysis of *B. sorghicola* (Table 1).

Based on banding pattern information, the marker index (MI) values ranged from 4.16 to 6.93 in URP, 3.72 to 8.36 in ISSR and 3.84 to 7.14 in RAPD marker studies. In URP-PCR, URP 13R showed highest MI value (6.93) followed by URP 17R (6.80) and URP 6R (6.72). Among ISSR primers, ISSR 15 showed highest MI value (8.36) followed by ISSR 16 (6.84) and ISSR 11 (6.60). In case of RAPD marker, OPC-5 showed highest MI value (7.14) followed by OPA-10 (6.82) and OPC-8 (6.75). The resolving power (RP) values ranged from 10.44 to 18.94 in URP, 9.44 to 22.63 in ISSR, and 8.44 to 18.56 in RAPD marker profiles. Highest RP was observed with URP 17R (18.94) followed by URP 6R (17.88) and the least with URP 25F (10.44). In case of ISSR markers, the highest RP value was observed in ISSR 15 (22.63) followed by ISSR 16 (18.06) and least with ISSR 13 (9.44). Among RAPD markers, the highest RP value scored in OPC-5 (18.56) followed by OPC-2 (16.38) and least with OPB-9 (8.44) (Table 1).

Analysis of data obtained from individual URP marker showed genetic similarity coefficient as 10.3 to 94.9%. The least similarity difference (10.3%) was observed between DQDS-9 and DQDS-24 strains collected from Maharashtra (Central zone) and Uttar Pradesh (North-eastern plain zone), respectively. Highest similarity (94.9%) with bootstrap value of 100% was observed between DQDS-30 and DQDS-31 strains, both collected from Gujarat state (Central zone). In URP-PCR, strains were classified into three major clusters and sub clusters using UPGMA analysis. Strains from Karnataka, Madhya Pradesh, Maharashtra and West Bengal as first group, Uttar Pradesh, Gujarat and Haryana as second group, Andhra Pradesh as third group (Fig. 2a), respectively.

In case of ISSR marker, genetic similarity coefficient was relatively higher, which ranged from 22.0 to 89.1%, among all the strains and also compared to URP and RAPD. Least similarity difference (22.0%) was observed between DQDS-8 and DQDS-32 strains collected from Maharashtra (Central zone) and Haryana state (North-western plain zone). Highest similarity difference observed between DQDS-28 and DQDS-29 strains (89.1% with bootstrap value 99%) were collected from Andhra Pradesh state (South zone). In ISSR-PCR, strains were classified into five major clusters based on UPGMA analysis (2 strains belonged to Karnataka as first group, 3 strains belonged to Madhya Pradesh and 7 strains belonged to Maharashtra, 6 strains from West Bengal and 2 strains from Uttar Pradesh as second group, 2 strains from Gujarat as third group and 5 strains from Andhra Pradesh, and one strain Haryana, Madhya Pradesh and Uttar Pradesh as fourth group, and 2 strains from Uttar Pradesh as fifth group) (Fig. 2b).

RAPD based genetic similarity coefficient ranged from 13.5 to 86.2% among all the strains and the least similarity difference was observed between DQDS-10 and DQDS-16 strains collected from Maharashtra state (Central zone) and West Bengal state (North-eastern plain zone). Highest similarity difference was observed between DQDS-7 and DQDS-8 strains (86.2%) with bootstrap value of 100%, both collected from Maharashtra state (Central zone). In RAPD-PCR, strains were classified into three major clusters and subclusters with very few strains grouped as separate subclusters *viz.* strains collected from Gujarat, Maharashtra and Karnataka. (Fig. 2c).

Three different aspects of performance of marker systems (URP, ISSR and RAPD) were considered for comparison. Combined data analysis of URP, ISSR and RAPD showed genetic similarity ranging from 22.4 to 86.4%. Least similarity difference (22.4%) was observed between DQDS-13 and DQDS-23 strains collected from Maharashtra (Central zone) and Uttar Pradesh (North-eastern plain zone), respectively. Highest similarity difference (86.4%) was observed between DQDS-30 and DQDS-31 strains with 100% bootstrap value, both collected from

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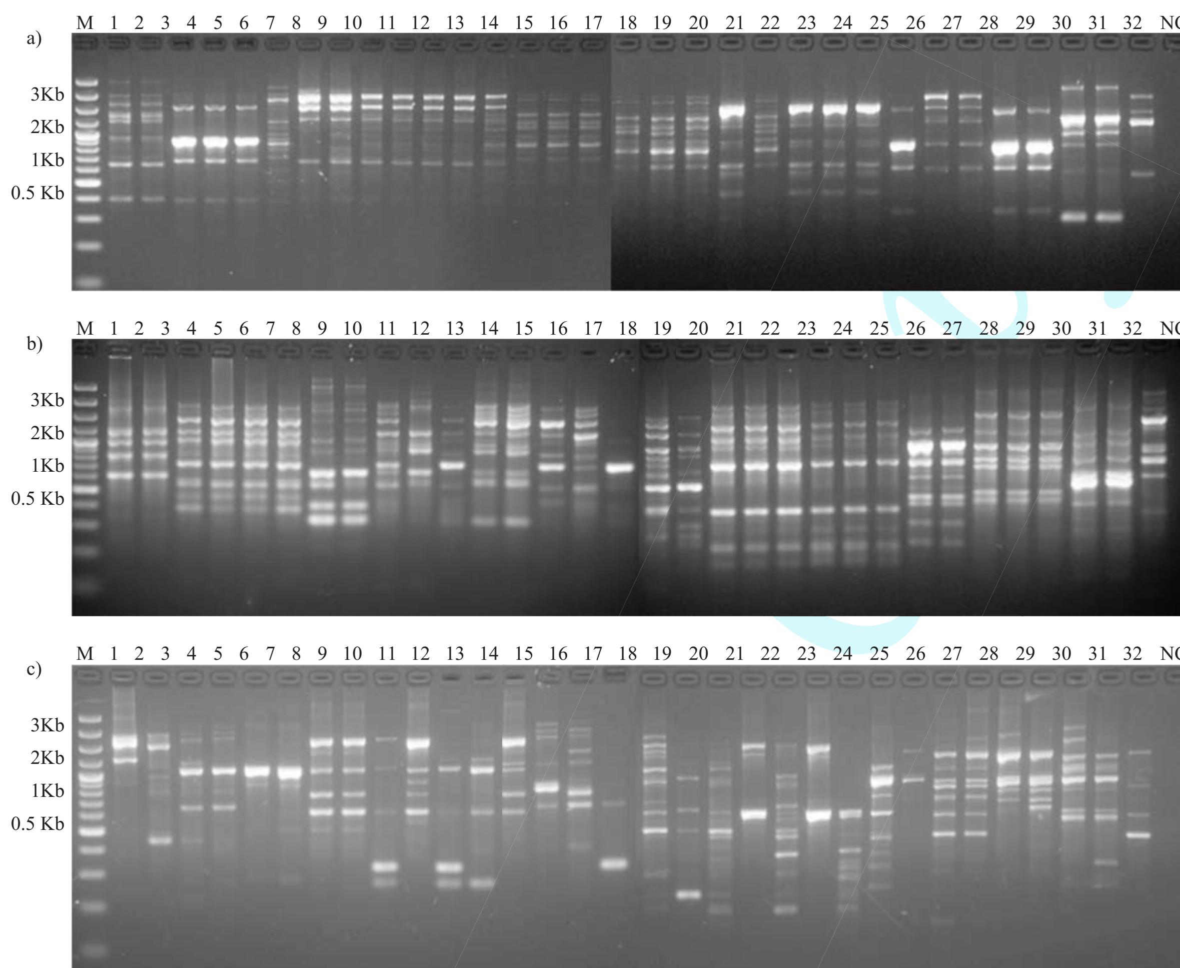

Fig. 1 : DNA fingerprinting profiles of 32 *B. sorghicola* strains obtained with (a) URP 30F, (b) ISSR 16 primers, and (c) RAPD primer OPA-9. Lanes with serial number 1-32 are fingerprinting profile of each *B. sorghicola* strains, NC – Negative control, M – 100 bp plus DNA ladder (Fermentas).

Gujarat state (Central zone). Strains was classified into four major clusters indicating that strains belonged to Karnataka (South zone) and Uttar Pradesh (North eastern plain zone) as individual subcluster and other strains belonged to Maharashtra and Madhya Pradesh state (Central zone), and few strains from West Bengal state (North eastern plain zone) grouped as major cluster. Strains collected from Haryana (North-western plain zone) and Gujarat state (Central zone) were grouped as single subcluster. Few strains collected from same state showed highest genetic similarity with 100% bootstrap value *viz*. Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh and West Bengal (Fig. 2d).

Arithmetic means of expected heterozygosity (Hn), polymorphic information content (PIC), effective multiplex ratio (EMR), marker index (MI) and resolution power (RP) were calculated. Mean number of bands (253.3), arithmetic mean of *Hn* (0.34), MI (5.94) and RP (15.83) were the highest value detected in ISSR followed by URP and RAPD. Highest mean number of DNA markers (19.8), PIC (7.26) and EMR (19.80) was observed in RAPD followed by URP and ISSR markers (Table 1). URP markers are equally efficient to ISSR in diversity analysis of *B. sorghicola* as it consistently holds the second highest position in all the above marker evaluating criteria (Table 1).

Mantel test was carried out on three marker systems to obtain reliable estimates of genetic similarities among the *B. sorghicola* strains tested with their cophenetic correlation values. Maximum cophenetic correlation was found in URP (r = 0.910) followed by ISSR (r = 0.904) and RAPD (r=0.870) (Table 2). The combined analysis of all three marker system showed high cophenetic correlation (r= 0.911), which indicated a "very good fit" of data for genetic

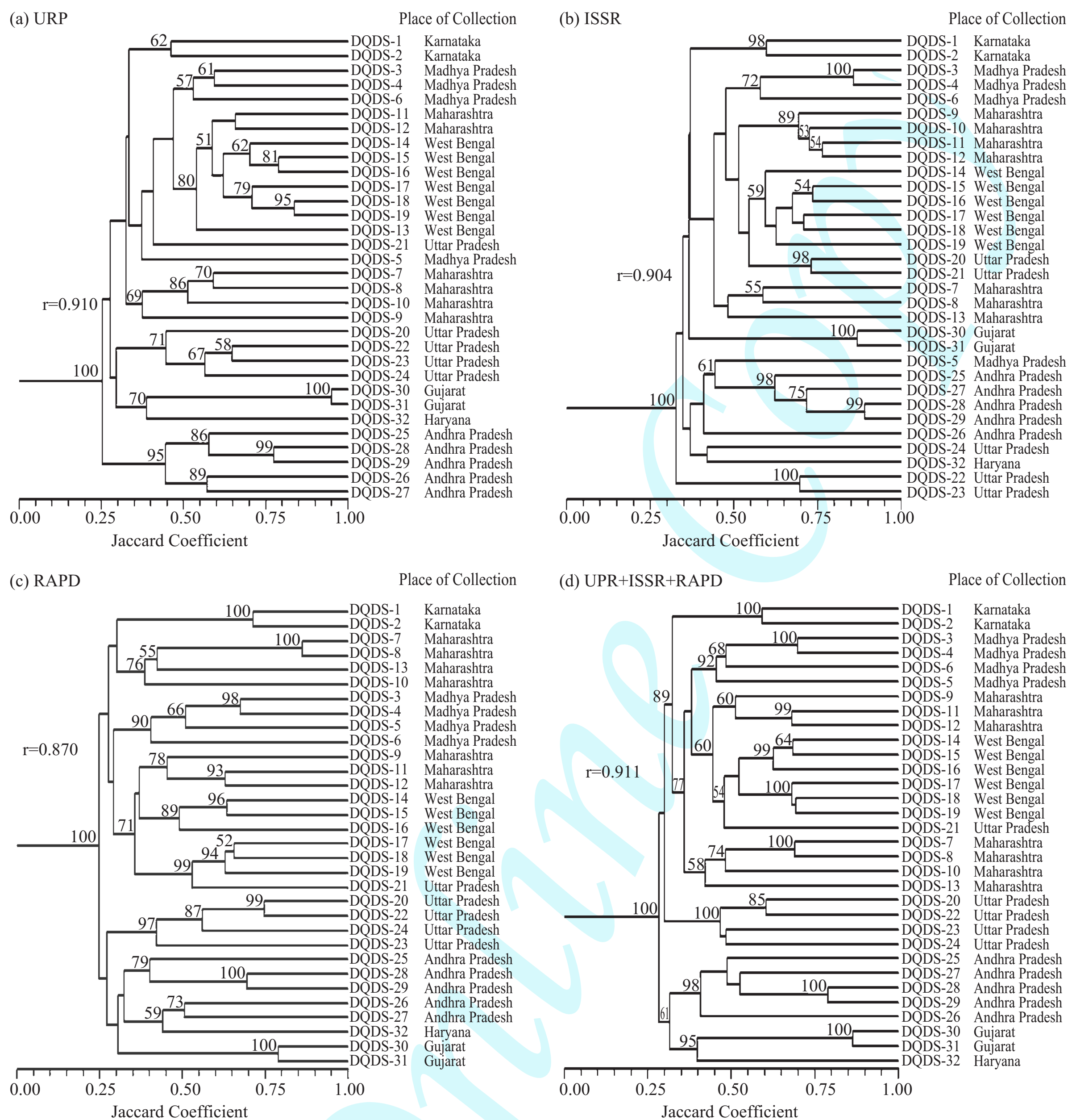

Fig. 2 : Dendrogram based on UPGMA clustering of individual and pooled molecular data obtained from (a) URP, (b) ISSR, (c) RAPD, and (d) URP + ISSR + RAPD markers among 32 *B. sorghicola* strains along with their place of collection. Correlation coefficient r, calculated by the Mantel test represents goodness of fit of the dendrogram. The percent bootstrap values above 50% were indicated in the major forks

diversity analysis. The results of Mantel test showed a fair and poor correlation between any two marker systems (Table 2).

Target leaf spot of sorghum caused by *B. sorghicola* has been reported in all the sorghum-growing countries in Asia, Americas and Africa (Katewa *et al.,* 2008). But so far no scientific reports are available on genetic variation of this pathogen population. For the disease resistance breeder, primary factor in plant protection is sufficient knowledge about genetical structure of pathogen populations (Nagaty and El Assal, 2011).

Molecular markers are used as important tools for the characterization of genetic diversity in the pathogens where

morphological characteristics are either absent or not able to differentiate strains properly (Sharma *et al.,* 1999). Nevertheless, fungal genetic characters are effectively influenced by various environmental and cultural conditions. Geographic region is also one of the major factors influencing the genetic structure of *B. sorghicola* in India (Katewa *et al.,* 2008). Therefore, problems associated with studying different levels of genetic variability of pathogens have been addressed by using molecular marker techniques (Ouedraogo *et al.,* 2004; Kumar *et al.,* 2011a; Burgos *et al.,* 2013; Pradeep Kumar *et al.,* 2014; Kandan *et al.,* 2015).

The UPGMA cluster analysis based on Jaccard similarity coefficients of URP marker, revealed three major cluster and subcluster groups based on geographical regions supported by moderate to high bootstrap confidence values. The critical identity of geographic populations grouped for most of the strains with minor exceptions indicates that the population was not evident in the cluster analysis indicating homogenizing effects of gene flow between population coupled with host selection. Here, the URP marker used in the molecular analysis of few other fungi *viz*. *Pleurosis*, *Chaetomium*, *Collectotrichum, Bipolaris oryzae* species has been reported by earlier workers (Kang *et al.,* 2002; Aggarwal *et al.,* 2008; Kumar *et al.,* 2011b, Kandan *et al.,* 2015). However, the applicability of this URP, ISSR and RAPD marker system in the molecular analysis of *B. sorghicola* has not been reported so far in the literature. The results obtained with URP marker either individual or combined data analysis with ISSR and RAPD marker system, revealed differences at strain level which are not noticeable applying conventional classification methods. Results of the present study, is also in agreement with earlier workers regarding use of URP marker system for genetic diversity analysis of fungi which includes nucleotide alterations, insertions and deletions at initiation sites of fungi may result in polymorphic DNA, which is detectable by the URP-PCR (Kang *et al.,* 2002; Aggarwal *et al.,* 2008; Kumar *et al.,* 2011b).

ISSR markers are useful for studying genetic diversity in *B. sorghicola* isolated from different geographical regions. These are rapid, reproducible and produce a large number of polymorphic bands and aid the understanding of pathogen population dynamics, which can effectively facilitate the development of effective control strategies. ISSR based UPGMA analysis of all the strains were classified into five major clusters with few exceptions supported with high bootstrap confidence values. These results are in concurrence with the previous reports demonstrated ISSR marker showed good polymorphism within and between *Rhizoctonia* spp. (Li *et al.,* 2011), *Fusarium oxysporum* f. sp. *ciceris* (Dubey *et al.,* 2012) and *B. oryzae* (Kandan *et al.,* 2015).

Clustering based on UPGMA for 10 RAPD markers, classified 32 *B. sorghicola* strains into three major clusters with very few strains grouped as separate subclusters viz. strains collected from Gujarat, Maharashtra and Karnataka, supported with high bootstrap confidence values (Fig. 2). Although RAPD PCR analysis is widely used method for discriminating among closely related organisms. In the present study, with few exceptions most of the strains were able to cluster together in the dendrogram, according to their geographical origin, which strengthened the fact that RAPD based variation is related to geographical origin of the strains, whereas earlier reports other than our laboratory report (Kandan *et al.,* 2015) related to molecular diversity studies of *B. oryzae* were partially in concurrence with the current *B. sorghicola* study *i.e*. even RAPD data revealed huge diversity within and between *B. oryzae* groups, there is lack of grouping based on geographical origin of the strains (Weikert-Oliveira *et al.,* 2002; Motlagh and Anvari, 2010; Kumar *et al.,* 2011a).

The present data could be useful in better understanding of genetic variability of *B. sorghicola* strains and might serve as the basis for developing disease management strategies under sorghum breeding programmes for resistance against this pathogen.

Acknowledgment

Authors gratefully acknowledge the Director, NBPGR for support and encouragement and Indian Council of Agricultural Research (ICAR), New Delhi for financial support.

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