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Comparative analysis of RAPD and ISSR marker assays for detecting genetic polymorphism in *Tilletia indica*

Shabana Parveen, M. S. Saharan, Ajay Verma and Indu Sharma

Directorate of Wheat Research, Karnal, Haryana, India

ABSTRACT

Karnal bunt of wheat, caused by Tilletia indica Mitra is an important disease prevalent in several countries. The pathogen due to its heterothallic nature shows high variability. In the present study, genetic relationships among ten isolates of T.indica collected from different locations of India and 15 monosporidial lines raised from these isolates were investigated by using 34 RAPD and 28 ISSR primers. RAPD and ISSR primers revealed 92.82 and 98.4 % polymorphism, respectively. Similarity coefficient values ranged from 0.61 to 0.97 for RAPD and 0.61 to 0.96 for ISSR primers. The dendrogram developed by RAPD and ISSR primers based analysis grouped the isolates and monosporidial lines in different clusters. Mental test employed for detection of goodness of fit established cophenatic correlation value for both the primer systems and it was significant at P 0.01. Clustering of isolates within groups was also similar based on RAPD and ISSR derived dendrograms. In our study, both marker systems were similar except for the percentage polymorphism which was found to be greater using ISSR, thus indicating the greater effectiveness of ISSR primers for estimating genetic diversity of Tilletia indica.

Key Words: Karnal bunt, *Tilletia indica*, Wheat, DNA polymorphism, Variability

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INTRODUCTION

Tilletia indica Mitra [synonym *Neovossia indica* (Mitra) Mundkur], the causal agent of Karnal bunt of wheat was first described on wheat near Karnal, India in 1931(13). *Tilletia indica* a floret infecting basidiomycetes causes a partial bunting of wheat seeds and infected seeds have a trimethyleamine odor (5). Karnal bunt is viewed as a serious disease for international trade in wheat because it reduces grain quality. The pathogen has a very restricted distribution, being limited largely to the Indian subcontinent and a small area of Mexico and the south-western United States of America (4). The pathogen is heterothallic and undergoes sexual reproduction after teliospore germination. Primary and secondary sporidia or hyphae as compatible mating types must fuse to form a dikaryon which readily increases the chances of variation due to heterozygosity and plays an important role in the production of new variants. Karnal bunt of wheat became an important disease of wheat after the introduction of dwarf wheat varieties in North West India, where it was of minor importance earlier (17).

Knowledge of diversity of the Karnal bunt pathogen in the form of distinct monoteliosporic and monosporidial cultures is essential for its effective management. The variability in *T. indica* has been documented on the basis of pathogen morphology, cultural characteristics, temperature response and pathogenicity tests (15-21-7). Understanding the genetic variability in the pathogen is extensively achieved through the use of genetic markers (3-10) , but these approaches are not yet very efficient for genetic variability studies in *T. indica*. Hence the present investigation was undertaken to compare RAPD and ISSR assays for their utility in generating polymorphic DNA profiles and genetic variation studies in *T. indica* isolates and monosporidial lines to understand the degree of congruency between the two marker systems.

MATERIALS AND METHODS

Collection, culture and preparation of monosporidial lines of T. indica

Karnal bunt infected grain samples of wheat were collected from Karnal, Sonipat, Bhiwani, Asandh (Haryana), Chaksu-Jaipur, Kotputali (Rajasthan), Dehradoon (Uttrakhand), Dhaulakuan, Sirmour and Tarau (Himachal pradesh) of North Western Plain Zone of India during 2009-2010 (table 1). The monoteliosporic cultures and monosporidial lines (Ms) were raised from teliospores (23). The growing liquid cultures of *T. indica* were harvested at desired intervals. Each mycelial mat of *T. indica* was filtered through a Whatman Blotter Paper No.1 and washed several times with sterilized distilled water. The mycelial mats were dried between tissue paper at room temperature. Dried mycelium was stored at -20^{0}C until use.

PCR amplification

Genomic DNA of *T. indica* was extracted by CTAB method (Murray and Thompson, 1980). A set of 53 oligonucleotide RAPD and 32 ISSR primers synthesized by Sigma Aldrich (Sigma Aldrich Chemicals Pvt. Ltd., India Subsidiary of USA) were used for PCR amplification. PCR amplifications were carried out in a total reaction volume of 25 µl containing 2 µl (50 ng) genomic DNA, 2.5 µl PCR buffer A (10 X) containing $Mgcl_2$ (15m M), 1 µl of 10 m M dNTPs, 0.2µl of Taq DNA polymerase (3U/µl), and 2 µl (15ng) each of oligonucleotide primer using a thermocycler (MJ Res). For RAPD amplification, PCR cycles consisted of DNA denaturation at 94^{0}C for 3 min., 40 cycles of 92^{0}C for 1 min, annealing at 36^{0}C for 1 min and extension at 72^{0}C for 2 min., followed by a final extension at 72^{0}C for 15 min. In order to perform ISSR amplification, denaturation at 94^{0}C for 4 min., 40 cycles of 92^{0}C for 1 min, annealing according to primer for 1 min and extension at 72^{0}C for 2 min., followed by a final extension at 72^{0}C for 10 min. was utilized. Amplified products were stored at 4^{0}C until they were subjected to electrophoresis. PCR products were separated by electrophoresis in 1.5% agarose gels (HiMedia) at 4v/cm in 0.5 X TAE buffer for 2 hrs. Approximate fragment sizes were calculated by interpolation from the migration distance of marker fragments of 100-bp and 1 kb DNA ladder (NEB). DNA banding pattern were visualized with UV light and recorded by an image system (Syngene Synoptics Ltd. USA). The reproducibility of DNA profiles was tested by repeating the PCR amplification with each of the selected primers. Only reproducible bands were considered for analysis. A negative control (without DNA template) was included in all the PCR reactions.

Scoring and data analysis

Data were analyzed to calculate various parameters to compare ISSR and RAPD assays. Polymorphic Information Content (PIC) was calculated (6). PIC = 1 – $p2$ – $q2$, where p is the band frequency and q is no band frequency. Frequency of an allele was obtained by dividing the number of isolates where the band was found by the total number of isolates. Diversity Index (DI) was calculated, which is equivalent to the average PIC value. Marker Index (MI) was calculated (23). NTSYSpc version 2.11a (Exeter Software, Setauket, NY, USA) was used for cluster analysis. The SIMQUAL module was used to calculate similarity coefficients between the 10 isolates and 15 monosporidial lines. The SHAN module was used for cluster analysis with the unweighted pair group method with arithmetic mean (UPGMA). The generated DNA bands were analyzed by scoring as present (1) or absent (0) for each alleles. To estimate the congruence among dendrograms, cophenetic matrices for each marker type were computed and compared using the Mantel matrix correspondence test (12), for such matrix correlation, a correlation value greater than 0.5 will be statistically significant at a 0.01 probability level (8).

RESULTS

RAPD Analysis

Out of 53 RAPD primers screened, 34 primers were selected based on their reproducibility, number of polymorphic fragments per assay and levels of polymorphism detected in a specific population. The individual primers produced bands in a range of four (OPAD 15, OPC 15) to ten (OPAA 7, OPAA 15), with an average of 6.55 bands per primer. Out of 223 bands generated with 34 primers, 16 bands were monomorphic (table 2). Product sizes ranged from 0.1 kb to 3.0 kb. Eight unique bands were obtained by primers OPA 20 (22), OPAA 4 (11), OPAA 13 (20 & 10), OPAA 15 (11), OPAA 17 (6), OPAD 10 (8), and OPD2 (20).Values in parantheses denotes the nuber of isolates/monosporidial lines as sequenced in Table 1. Polymorphism obtained with 34 primers for 10 isolates and 15 monosporidial lines of *T. indica* was 92.82%. PIC values ranged from 0.61 (OPC 15) to 0.97 (OPAA 13). The banding profile of the primer OPAA 1 is shown in fig.1 (A).

A dendrogram based on UPGMA analysis using the RAPD data is shown in fig. 2 (A). The Jaccard similarity coefficient ranged from 0.61 to 0.97 with an average of 0.79. Ten isolates and 15 monosporidial lines of *T. indica* were clustered in two clusters i.e., one minor and one major. The major (I-IV) group clustered with 84% of isolates with their monosporidial lines while the minor (V) cluster group indicated only 16% of the isolates and monosporidial lines. Isolates and monosporidial lines of Chaksu (6 &24) and Tarau (7 & 25) were grouped in minor

cluster at Sm 0.67.The remaining isolates and monosporidial lines were grouped together in the major cluster. The major cluster further subdivided in to four sub clusters. Sub cluster I comprises isolates of Sirmour, Dehradoon, Dhaulakuan, Karnal and Sonipat (1, 4, 5, 2 & 3, respectively). Molecular characterization of monosporidial lines through RAPDs indicated very low genetic variation among each Ms of Dehradoon (21-23), as they all were in sub cluster II. Monosporidial lines of Karnal (12-15) were clustered in sub cluster III while monosporidial lines of Sirmour (16-19) grouped in sub cluster IV. Karnal bunt isolates from Kotputali (9), Bhiwani (8) and Asandh (10) showed very high variations and they did not cluster with other isolates. Isolate of Bhiwani (8) was more closely related to the isolates (6) and Monosporidial lines (24) of Chaksu, and Kotputali isolate (9).

Table 1. Details of isolates and monosporidial lines of *T. indica* used in the present investigation

Sample number given	Isolate/ Monosporidial line	State	Year of Collection
1	Sirmour	Himachal Pradesh	2010
2	Karnal	Haryana	2010
3	Sonipat	Haryana	2009
4	Dehradoon	Uttrakhand	2010
5	Dhaulakuan	Himachal Pradesh	2009
6	Chaksu-Jaipur	Rajasthan	2010
7	Tarau	Himachal Pradesh	2009
8	Bhiwani	Haryana	2009
9	Kotputali	Rajasthan	2010
10	Asandh	Haryana	2010
11	Bhiwani	Haryana	2009
12	Karnal	Haryana	2010
13	Karnal	Haryana	2010
14	Karnal	Haryana	2010
15	Karnal	Haryana	2010
16	Sirmour	Himachal Pradesh	2010
17	Sirmour	Himachal Pradesh	2010
18	Sirmour	Himachal Pradesh	2010
19	Sirmour	Himachal Pradesh	2010
20	Dehradoon	Uttrakhand	2010
21	Dehradoon	Uttrakhand	2010
22	Dehradoon	Uttrakhand	2010
23	Dehradoon	Uttrakhand	2010
24	Chaksu	Rajasthan	2010
25	Tarau	Himachal Pradesh	2010

1 – 10: Isolates; 11 – 25: Monosporidial Lines

ISSR Analysis

Out of 32 ISSR primers, 28 primers gave reproducible bands. A total of 192 bands were obtained using the 28 primers with an average 6.85 bands per primer. Out of 192 bands, three were monomorphic. The number of bands produced per primer ranged from four (ISSR primers 808, 810, 824 and 876) to thirteen (ISSR primer 857). The size of fragments ranged from 0.15 kb to 3.5 kb (table 3). Ten unique bands were obtained with ISSR primers, 810 (11), 812 (5), 820 (16), 845 (10), 857 (3 from7, 8 and 10), 864 (7), 890 (7) and 815 (25). A high level of polymorphism (98.4%) indicated the presence of high variability in fungus genome. PIC values ranged from 0.50 (ISSR primer 809) to 0.90 (ISSR primer 826). The banding profile of ISSR primer 890 is depicted in fig. 1(B)

Jaccard's similarity coefficient was calculated using the 28 ISSR primers data. Similarity coefficient (Sm) values ranged from 0.61 to 0.96 (av. 0.78). A phylogenetic tree generated using SAHN cluster analysis and UPGMA method is shown in fig. 2 B. All the 10 isolates and 15 monosporidial lines were clearly separated. Clustering feature in total ISSR dendrogram is compared to the total RAPD dendrogram . The isolates were clustered into two, major (I-V) and a minor cluster (VI). Isolates of Kotputali (9), Asandh (10), Bhiwani (8) and the monosporidial line of Bhiwani (11) were clustered together in the minor cluster. The major cluster was further subdivided into several mini clusters showing varied degree of similarities. Isolates of Sirmour, Karnal, Sonipat and Dehradoon were more close to each other and clustered in the same group. The monosporidial lines of Sirmour were grouped into a second cluster and they all were together as in the RAPD derived tree. Likewise the monosporidial lines of Karnal formed a third sub cluster. As with the RAPD data isolate (7) and monosporidial line (25) of Tarau clustered with isolate (6) and monosporidial line of Chaksu (24) at Sm 0.71. The genetic makeup of isolates and monosporidial lines significantly varied among the groups as the isolate of Dhaulakuan (5) and monosporidial line (21) did not cluster closely together, demonstrating high level of the variability. The monosporidial lines of Dehradoon (20, 22 and 23) formed sub cluster IV separate from the monosporidial line (21) and isolate (4) of Dehradoon, where as in the RAPD cluster all four monosporidial lines of Dehradoon occupied the same cluster. This indicated that the ISSR analysis showed more variability among *T. indica* isolates.

Table 2. RAPD fingerprints data for the molecular characterization of *T. indica* isolates and monosporidial lines

S. No.	Primer	Primer Sequences 5'-3'	Total band	Polymorphic bands	% poly Morphism	Unique bands	Product size kb	PIC
1	OPA2	TGCCGAGCTG	6	6	100		0.3-1.1	0.72
2	OPA3	AGTCAGCCAC	6	6	100		0.3-1.0	0.75
3	OPA9	GGGTAACGCC	5	5	100		0.5-2.0	0.71
4	OPA13	CAGCACCCAC	5	5	100		0.3-1.25	0.69
5	OPA18	AGGTGACCGT	6	6	100		0.6-3.0	0.86
6	OPA20	GTTGCGATCC	6	6	100	01(22)	0.5-3.0	0.93
7	OPAA1	AGACGGCTCC	7	7	100		0.4-2.0	0.62
8	OPAA16	GGAACCCACA	8	8	100		0.3-3.0	0.89
9	OPAA3	TTAGCGCCCC	5	5	100		0.5-3.5	0.84
10	OPAA4	AGGACTGCTC	9	7	77.77	01(11)	0.2-2.0	0.93
11	OPAA7	CTACGCTCAC	10	10	100		0.1-2.5	0.83
12	OPAA11	ACCCGACCTG	7	5	71.42		0.2-2.0	0.83
13	OPAA13	GAGCGTCGCT	6	5	83.33	2(20,10)	0.5-3.0	0.97
14	OPAA15	ACGGAAGCCC	10	10	100	01(11)	0.2-3.5	0.61
15	OPAA17	GAGCCCGACT	7	5	71.42	01(06)	0.2-2.0	0.83
16	OPAA18	TGGTCCAGCC	6	5	83.33		0.4-2.0	0.56
17	OPAA9	AGATGGGCAG	5	3	60.00		0.3-2.0	0.79
18	OPAA10	TGGTCGGGTG	7	6	85.71		0.2-1.5	0.94
19	OPAD10	AAGAGGCCAG	4	4	100	01(08)	0.5-0.9	0.71
20	OPAD12	AAGAGGGCGT	6	6	100		0.3-1.5	0.83
21	OPAD5	ACCGCATGGG	7	7	100		0.2-2.0	0.64
22	OPAD14	GAACGAGGGT	8	8	100		0.3-2.0	0.75
23	OPC14	TGCGTGCTTG	8	8	100		0.4-3.0	0.70
24	OPC8	TGGACCGGTG	8	8	100		0.7-1.5	0.71
25	OPC15	GACGGATCAG	4	4	100		0.4-3.0	0.61
26	OPB6	TGCTCTGCCC	5	5	100		0.3-3.0	0.88
27	OPB8	GTCCACACGG	6	6	100		0.5-3.0	0.80
28	OPD2	GGACCCAACC	7	7	100	01(20)	0.5-3.0	0.85
29	OPD7	TTGGCACGGG	7	7	100		0.4-3.0	0.85
30	OPD16	AGGGCGTAAG	8	8	100		0.4-3.0	0.87
31	OPAC11	CCTGGGTCAG	6	5	83.33		0.2-2.0	0.90
32	OPAC12	GGCGAGTGTG	8	6	75.00		0.2-2.0	0.91
33	OPAC13	GACCCGATTG	5	3	60.00		0.2-1.1	0.96
34	OPAC15	TGCGTGCTTG	5	5	100		0.3-1.1	0.74
	Total bands/Mean values		223	207	92.82	08	0.1-3.0	0.79

Values in parantheses of unique bands denotes number of isolares and monosporidial lines given in Table 1.

Table 3. ISSR fingerprints data for the molecular characterization of *T. indica* isolates and monosporidial lines

S.No.	ISSR UBC	Primer Sequences 5'-3'	Ta (^{0}c)	Total no. of bands	Polymorphic bands	Unique bands	Polymorphism(%)	Product size Kb	PIC
1	826	(AC)8C	48	5	4		80	0.3-1.2	0.90
2	808	(AG)8C	55	4	4		100	0.3-0.7	0.70
3	811	(GA)8C	52	8	8		100	0.3-1.2	0.71
4	834	(AG)(8)T	48	5	4		80	0.3-0.75	0.67
5	810	(GA)8T	50	5	5	1(11)	100	0.15-1.1	0.68
6	841	(GA)8YC	54	9	8		88.88	0.15-1.1	0.72
7	835	(AG)8YC	50	10	9		90	0.3-0.7	0.62
8	809	(AG)(8)G	50	5	5		100	0.3-1.0	0.50
9	842	(GA)8YG	50	6	6		100	0.2-2.0	0.62
10	818	HBH(AG)7	54	10	10		100	0.35-1.5	0.76
11	817	(CA)8A	50	6	6		100	0.25-1.2	0.69
12	812	(GA)(8)A	50	9	9	1(5)	100	0.5-2.5	0.81
13	820	(GT)8C	44	8	8	1(16)	100	0.7-3.5	0.80
14	824	(TC)8G	50	4	4		100	0.3-2.5	0.90
15	845	(CT)8RG	55	5	5	1(10)	100	0.3-1.1	0.75
16	857	(AC)(8)YG	50	13	13	3(7,8,10)	100	0.5-0.7	0.83
17	864	(ATG)6	50	3	3	1(7)	100	0.3-2.5	0.40
18	873	(GACA)4	52	9	9		100	0.3-0.7	0.69
19	876	(GATA)2(GACA)2	50	4	4		100	0.25-1.0	0.77
20	880	(GGAGA)3	50	6	6		100	0.4-3.0	0.79
21	881	(GGGTG)(3)	55	11	11		100	0.2-0.7	0.85
22	885	BHB(GA)(7)	48	5	5	1(7)	100	0.25-2.7	0.84
23	890	VHV(GT)7	48	8	8		100	0.25-1.5	0.52
24	840	(GA)(8)YT	50	7	7		100	0.3-2.5	0.84
25	815	(CT)8G	50	8	8	1(16)	100	0.3-3.0	0.82
26	847	(CA)8 A/G C	51	5	5		100	0.3-1.0	0.77
27	856	(AC)8YA	54	8	8		100	0.3-3.0	0.89
28	844	(CT)8RC	50	6	6		100	0.3-3.0	0.86
	Mean			192	189	10	98.43	0.15-3.5kb	0.74

Values in parantheses of unique bands denotes number of isolares and monosporidial lines given in Table 1. V= A,C,G; B=G,C,T; H=A,C,T; D=A,G,T; Y=Pyrimidine (C,T); R=Purine (A,G).

Table 4. Analysis of banding pattern generated by the ISSR and RAPD assay for *T. indica* isolates and monosporidial lines

S.No.	Component	RAPD	ISSR
1	No.of primers used	34	28
2	No. of total bands	223	192
3	Polymorphic bands	207	189
4	Percentage polymorphism	92.82	98.43
5	Total unique bands obtained	08	10
6	Size of PCR products	0.1-3.0kb	0.15-3.5 kb
7	Average PIC	0.79	0.74
8	Multiplex ratio	6.55	6.85
9	Effective multiplex ratio (EMR)	3.275	3.425
10	Assay efficiency index (AEI)	6.08	6.75
11	Marker index (MI)	2.6196	2.5499

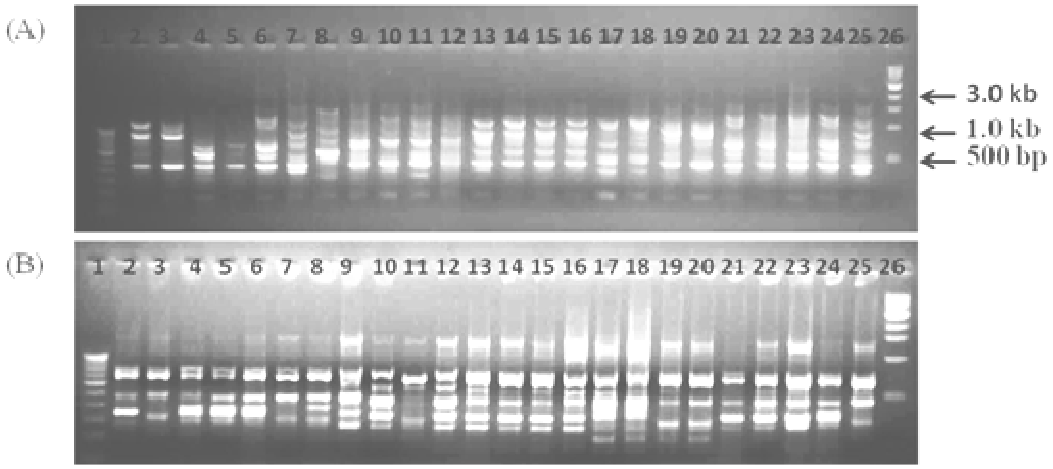
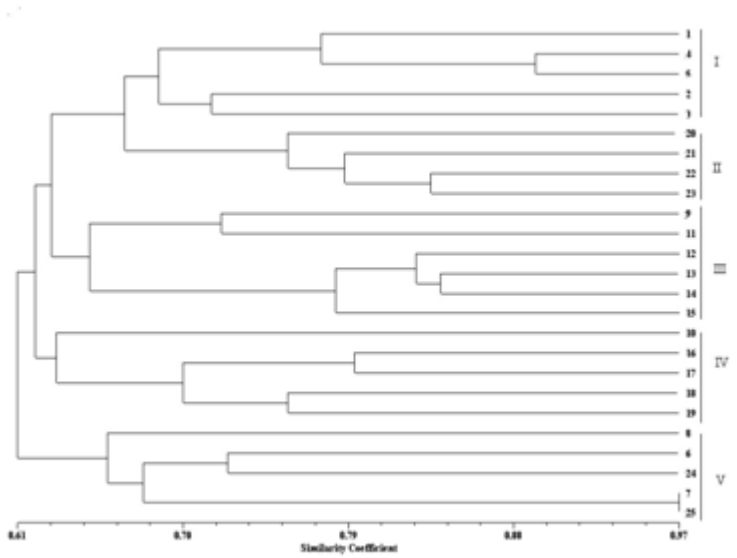


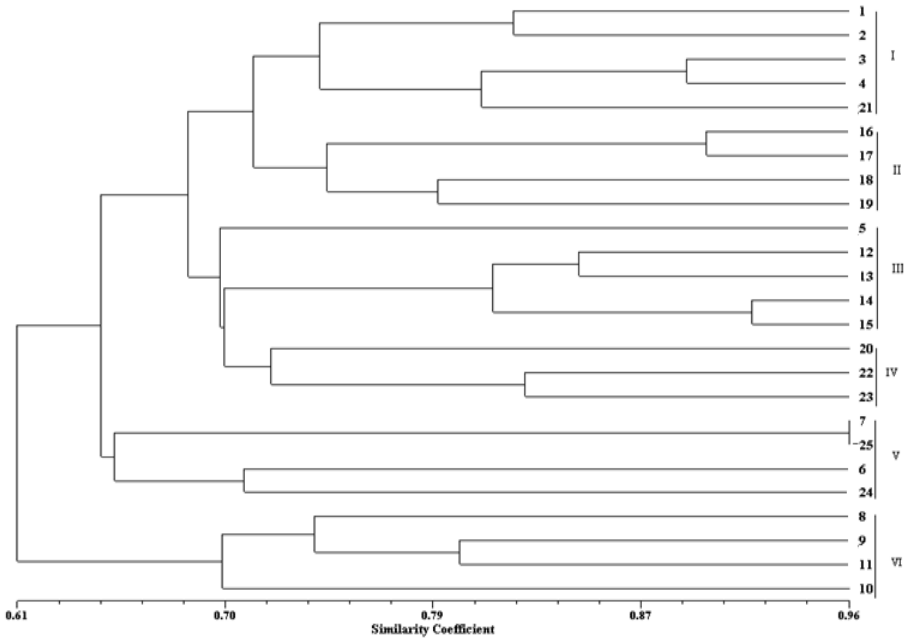
Figure 1. Amplification profile of *T. indica* isolates (2 - 11) and monosporidial lines (12 - 25) with molecular marker RAPD OPAA 1 (A) and ISSR – UBC 890 (B)

1.100 bp DNA ladder, 2. Sirmour, 3. Karnal, 4. Sonipat, 5. Dehradoon, 6. Dhaulakuan, 7. Chaksu, 8. Tarau, 9. Bhiwani, 10. Kotputali, 11. Asandh, 12 – 25 Ms. lines (12. Bhiwani; 13 - 16. Karnal; 17- 20. Sirmour; 21 - 24. Dehradoon; 25. Chaksu) 26. 1 kb DNA ladder

(A)



(B)



(C)

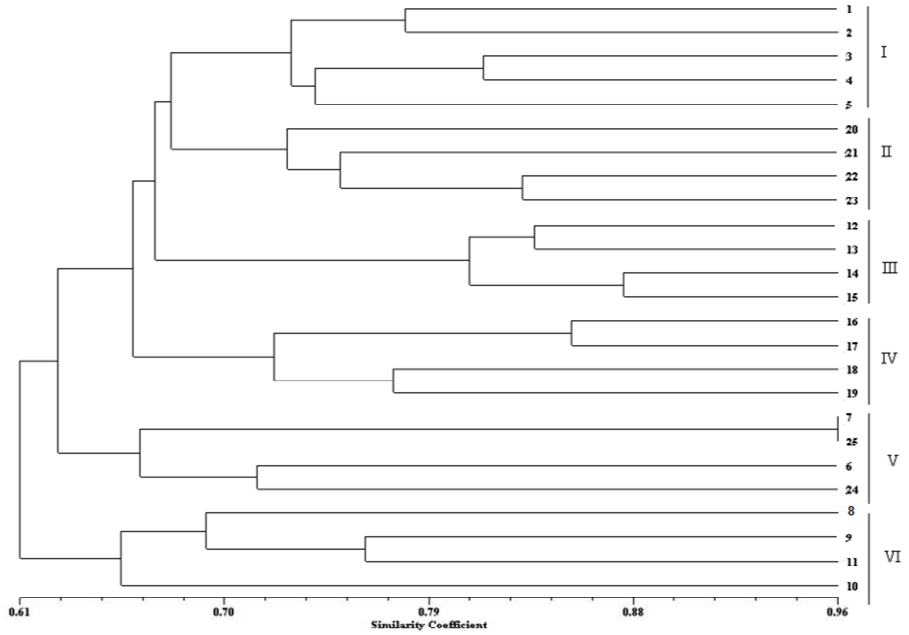


Figure 2. Dendrogram constructed by using UPGMA based on Jaccard's Coefficient of 34 RAPD primers (A), 28 ISSR primers (B) and combined RAPD+ISSR (C) for *T. indica* isolates (1 - 10) and monosporidial lines (11 – 25).

Comparative Analysis

The level of polymorphism revealed by RAPD analysis (92.8%) was lower than the ISSR (98.43%). In the present investigation, the average number of fragments amplified by RAPD and ISSR primers was 6.55 and 6.85, respectively. Such a high variation in the number of fragments produced by these arbitrary primers may be attributed to the differences in the binding sites throughout genome of *T. indica* isolates used. PIC values for the RAPD primers was higher (0.79) than the ISSR primers (0.74). Assay efficiency index (AEI), effective multiplex ratio (EMR), multiplex ratio and percentage polymorphism was higher in the ISSR than RAPD but marker index (MI) and PIC was higher in RAPD (table 4).

The RAPD and ISSR data were combined for UPGMA cluster analysis. Similarity coefficient ranged from 0.61 to 0.96 (fig. 2 C). The combined cluster of RAPD and ISSR was 80-85% similar to each of the separate (RAPD or/ISSR) cluster analysis. In the combined cluster, one major and one minor group were formed. In the minor cluster, isolates of Bhiwani, Kotputali, and Asandh and monosporidial line of Bhiwani were together as similar to the ISSR. The major cluster further subdivided into five sub clusters. Sub cluster I formed by five isolates (1-5) as in the RAPD analysis but in the ISSR analysis isolate number five was absent in this sub cluster. Sub cluster II contained monosporidial line of Dehradoon (20-23). In subcluster III, IV and V the distribution of isolates is similar to the clusters generated by RAPD and ISSR. The cophenetic correlation values for the dendrogram based on RAPD and ISSR data resulted in statistically significant correlation. In the present study, r value (0.60) at P 0.01 was significant.

DISCUSSION

Among several approaches advocated for management of KB, deployment of resistant varieties has been the most effective and economical method. For applying efficient strategies in the breeding process, knowledge about the genetic diversity and structure of naturally occurring pathogen populations is indispensable. Variants have been reported in *T. indica* for the size of teliospore, serology and host reaction, teliospore morphology and germination, isozyme and RAPD patterns (13-15-21-18&3). Also, aggressiveness among variants through pathogenicity tests has been reported (1&20). But, none of these techniques could lead to a practical and widely acceptable procedure to index the variability in *T. indica*. Traditional markers used to study the variability in plant pathogens are based on the differential hosts, cultural characteristics, morphological markers and biochemical tests. These markers distinguish pathogens on the basis of their physiological characters i.e. pathogenicity and growth behaviour. But these markers are influenced by host age, inoculum quality and environmental conditions. Moreover, these techniques are time consuming and laborious, and in some host-pathogen systems, differential hosts are not available. In such cases, molecular markers are used for studying genetic variability in plant pathogens (19).

Our results showed the utility of RAPD and ISSR markers for detecting genetic variation among *T. indica* isolates and monosporidial lines. Efficiency of molecular marker techniques depends on the amount of polymorphism generated by the primers. In the present study, RAPD and ISSR techniques generated numerous polymorphic bands and the presence of a high percentage of polymorphism indicated high variability in this pathogen targated by the primers. The ISSR primers generated greater polymorphism as compared to the RAPD primers. In the present investigation, unique bands were obtained from both primer systems. These unique bands were exclusive to particular isolate and monosporidial line which made them distinct from the other isolates and monosporidial lines. Though both the RAPD and ISSR systems exhibited similar results in our studies, the percentage polymorphism was higher using the ISSR primers. The UPGMA grouping analysis (RAPD + ISSR) allowed us to observe the association between the two types primers evaluated here. The monosporidial lines of different isolates were grouped in the same clusters; this is likely because of their similar genetic makeup. The accessions of three sub clusters of the major group were similar in all the three cluster analysis (RAPD, ISSR, RAPD + ISSR). Similar results for both systems have also been reported (2&11). Mantel matrix was also performed and it was also significant at P 0.01. Hence it is concluded that both the RAPD and ISSR primers used in the present study were found effective in evaluating genetic diversity among different isolates and monosporidial lines of *T. indica* but polymorphism was greater using the ISSR primers, indicating the effectiveness of ISSR primers for estimating genetic diversity of *T. indica*.

CONCLUSION

Knowledge of diversity of the KB pathogen in the form of monosporidial cultures is essential for effective management of the disease. Efficiency of molecular markers depends up on the amount of polymorphism. In the present study both the molecular marker systems exhibited almost similar results but percentage polymorphism was high in ISSR. Based on high percentage polymorphism, it is concluded that ISSR are more effective than RAPD primers for estimating genetic diversity in *T. indica.*

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