academicJournals

Vol. 14(13), pp. 1097-1106, 1 April, 2015
DOI: 10.5897/AJB10.1951
Article Number: CA9A64E52086
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Relative efficiency of RAPD and ISSR markers in assessment of DNA polymorphism and genetic diversity among Pseudomonas strains

Jaipreet Kaur Rayar[1]*, Mohammad Arif[2] and Uma Shankar Singh[2,3]

[1]Department of Molecular Biology and Genetic Engineering, G. B. Pant University of Agriculture & Technology, Pantnagar 263145, Uttarakhand, India.
[2]Centre of Advance Study in Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar, U.S.Nagar, Uttarakhand, India.
[3]International Rice Research Institute, IRRI-India office, 2nd floor, NASC Complex, DPS Marg, Pusa, New Delhi, INDIA-110012.

Received 7 October, 2010; Accepted 9 March, 2011

This study presents the results from comparison of random amplified polymorphic DNA (RAPD), random amplified polymorphic DNA (RAPD) and RAPD-ISSR markers together in determining the genetic diversity and polymorphism among strains of fluorescent *Pseudomonas.* In order to compare the efficiencies of these markers, 17 different isolates were investigated using RAPD and ISSR. In RAPD marker analysis, 147 out of 222 bands (63.85%) were polymorphic and the RAPD based genetic similarity (RAPD-GS) ranged from 0.11 to 0.73. In ISSR analysis, a total of 134 alleles were detected, among which 105 alleles (77.9%) were polymorphic. The ISSR derived genetic similarity (ISSR-GS) ranged from 0.38 to 0.81. Cluster analysis indicated that all the 17 isolates could be distinguished by both RAPD and ISSR markers and worked effectively to determine high level of polymorphism in *Pseudomonas*. But ISSR was found to be slightly better than RAPD in assessing genetic diversity among the isolates while the combination of these techniques will give the comprehensive genetic analysis of *Pseudomonas*. Principal component analysis (PCA) was employed to evaluate the resolving power of the markers to differentiate between them.

Key words: Fluorescent Pseudomonas, Molecular markers, RAPD , ISSR, PCR, Genetic diversity, Phylogeny, Principal component analysis.

INTRODUCTION

Fluorescent *Pseudomonas* are plant, animal and human pathogens; exhibit plant growth-promoting and plant pathogen-suppressing properties, useful in biological control and express metabolic versatilities valued in bio-technology and bioremediation. The genus *Pseudomonas* includes species with functions of ecological, economic, and health-related importance (Siddiqui et al., 2005). This genus encompasses arguably

*Corresponding author. E-mail: jaipreet.rayar26@gmail.com.

Abbreviation: RAPD (DNA), Random amplified polymorphic deoxyribonucleic acid; **ISSR,** inter-simple sequence repeat; **PCA,** principal components analysis; **UPGMA,** unweighted pair groups method using arithmetic averages.

the most diverse and ecologically significant group of bacteria on the planet.

For the establishment of effective identification of potent *Pseudomonas* strain as a bio-control agent, the intra-species relationship within the isolates needs to be study in detail which presumes knowledge of simple and objective means of identification of the genetic diversity. Molecular markers which reveal extensive polymer- phism at the deoxyribonucleic acid (DNA) level are suitable for discriminating closely isolates. Random amplified polymorphic DNA (RAPD) markers (Williams et al., 1990) and ISSR markers (Zietkiewicz et al., 1994) are two molecular typing approaches that have been used to detect genetic variation among plants and micro-organisms. Each method has been used extensively to identify and determine the relationships at the species level (Rajaseger et al., 1997; Raina et al., 2001; Martins et al., 2003). These methods are widely applicable because they are rapid, inexpensive, simple to perform, do not require prior knowledge of DNA sequen and require very little starting DNA template (Esselman et al., 1999). Since RAPD primers are not designed based on a known target sequence, they are considered as arbitrary or random primers.

Inter-simple sequence repeat (ISSR) primers are derived from an arbitrary nucleotide sequence of di and tri-nucleotide repeats with a 5' or 3' anchoring sequence of a few nucleotides to prevent strand-slippage (14-22 bases). These nucleotide repeats are based on the ubiquitous presence of simple sequence repeats (SSRs; aka microsatellites, simple tandem repeats) that are distributed throughout the genomes. Polymerase chain reaction (PCR) reaction amplifies the sequence between two SSRs.

Due to these abundant and rapidly evolving SSR regions, ISSR amplification has the potential to reveal much larger numbers of polymorphic fragments per primer than RAPD. In this investigation, RAPD and ISSR markers were employed (1) to detect the genetic diversity and polymorphism among different isolates of Pseudomonas collected from rhizospheric soil of different locations and (2) to estimate the relative efficiencies of both RAPD and ISSR markers.

MATERIALS AND METHODS

Bacterial isolates and culture conditions

Isolates of fluorescent *Pseudomonas* used in this study were isolated from rhizospheric soils of different infected host plants from different areas of Uttarakhand, India (Table 1). These isolates were cultured and preserved on King's B medium, and stored at 4°C prior to use.

Bacterial genomic DNA extraction

Total genomic DNA was extracted by using ultraclean microbial DNA isolation kit (Mo Bio laboratories, Inc, U.S.A.). Pure cultures of each isolate of fluorescent *Pseudomonas* were first grown in King's B medium (*P*F; Difco catalog no. 0448-17-1), for 2 days at the incubation temperature of 28°C and then were used for the extraction of DNA according to the kit manufacturer's instructions. The concentration of DNA was determined by UV visible spectrophotometer (Biomate, Thermo Spectronic, and Cambridge, UK) and the DNA samples were diluted to 50 ngμl^{-1} for PCR amplification.

PCR amplification

DNA amplification was done with arbitrary decamer oligonucleotide primer (RAPD) or long primers (ISSR) obtained from Life Technologies, India (Table 2). The primers were selected after a preliminary screening for their ability to produce clear and reproducible patterns of multiple bands. PCR amplification was performed in 20 µl reaction volume containing 2 µL dNTPs (250 µM each dNTP), 1 µL primer (30 ngμL^{-1}), 1 µl template DNA (50 ng μL^{-1}), 2.5 µL reaction buffer (10 X), 0.3 µL Taq DNA polymerase (5 UμL^{-1}), 2 µl $MgCl_2$ (25 mM) and 11.2 µL deionized water. PCR reactions were performed in PTC-200 Peltier thermal cycler (MJ Research inc., Watertown, MS, USA). The cycling conditions were: 94°C for 5 min for pre-denaturation, followed by 35 cycles (for RAPD) or 40 cycles (for ISSR) of 94°C for 60 s to denaturation, 42°C (for RAPD) or 51°C (for ISSR) for 60 s to annealing, 72°C for 120 s to extension, with a final extension at 72°C for 7 min. Amplification products were fractionated on 1.4% (RAPD) or 1.8% (ISSR) agarose gel plus 0.5 µg/ml ethidium bromide in 0.5X TBE buffer. The size of the amplified DNA fragments was estimated with 100 bp ladders (Fermentas, USA). The gels were visualized under UV using gel documentation system (BioRad, USA). These gel pictures were used to score the DNA bands for analysis.

Data analysis

DNA fingerprints were scored for the presence (1) or absence (0) of bands of various molecular weight sizes in the form of binary matrix. Data were analyzed to obtain Jaccard's coefficients among the isolates by using NTSYS-pc (version 2.11W; Exeter Biological Software, Setauket, NY, Rohlf, 1993). The SIMQUAL program was used to calculate the Jaccard's coefficients. The common estimator of genetic identity was calculated as follows:

$$\text{Jaccard's coefficient} = N_{AB}/(N_{AB} + N_A + N_B)$$

Where, N_{AB} is the number of bands shared by samples, N_A represents amplified fragments in sample A, and N_B represents fragments in sample B. Similarity matrices based on these indices were calculated. Correlation between the two matrices obtained with the two marker types (ISSR and RAPD) was estimated by means of Mantel matrix correspondence test (Mantel, 1967). Product moment correlation (r) was obtained from this test which provided one measure of relatedness between the two matrices. In this instance, the matrix correlation corresponds to two independently derived dendrograms.

Similarity matrices were utilized to construct the UPGMA (unweighted pair-group method with arithmetic average) dendrograms to study the genetic relationship between different genotypes. In order to estimate the congruence among the dendrograms, cophenetic matrices for each marker and index type were computed and compared using Mantle test. Principal coordinate analysis was performed in order to highlight the resolving power of the ordination. To determine robustness of

Table 1. List of Isolates, their source and place of isolation

Isolate	Crop	Variety	Place of isolate in India
Pf1	Rice	RH-10	Kali Nagar
Pf2	Rice	RH-47	Kali Nagar
Pf3	Rice	Narendra	Kali Nagar
Pf4	Rice	RH-10	Kali Nagar
Pf5	Rice	Pusa Basmati	Benjeer farm
Pf6	Rice	Taravdi	Benjeer farm
Pf7	Rice	Sarvati	Dauha
Pf8	Tomato	Dev	Chorgalia
Pf9	Rice	Mota dhan	Chorgalia
Pf10	Rice	Mota dhan	Naya gaon
Pf11	Tomato	Trishul	Jagatpur
Pf12	Tomato	5005	Chorgalia
Pf13	Rice	Hybrid	Kathgodam
Pf14	Tomato	5005	Chorglia
Pf15	Tomato	5005	Golapar
Pf16	Rice	Taravdi	Benjeer farm
Pf17	Rice	Taravdi	Benjeer farm

Table 2. Primers list of ISSR and RAPD used for the comparative study of genetic variability of *Pseudomonas* isolates.

Primer code RAPD primers	Primer sequence (5' to 3')	Amplified product range (bp)	Total bands	Mono bands	Poly bands	% Poly.	Average Expected gene diversity (H_i)	PIC
P-71	TGCCGAGCTG	210-1450	17	8	9	52.9	0.220	0.751
P-72	AGTCAGCCAG	280-1480	24	4	20	83.3	0.293	0.607
P-73	AATCGGGCTG	480-1640	16	6	10	62.5	0.174	0.802
P-79	CAATCGCCGT	250-1400	18	2	16	88.8	0.186	0.795
P-87	AGGTGACCGT	250-1500	19	4	15	78.9	0.179	0.801
P-89	AGTCAGCCAC	300-900	9	9	0.0	0.00	0.00	0.000
P-94	GTCGCCGTCA	290-2000	19	6	13	68.4	0.798	0.181
P-96	TTGGCACGGG	240-1550	11	5	6	54.5	0.145	0.626
P-97	GTGTGCCCCA	250-1550	17	5	12	70.5	0.199	0.776
P-99	AGCGCCATTG	400-1350	16	8	8	50.0	0.259	0.622
P-100	CACCGTATCC	310-1150	7	1	6	85.7	0.149	0.835
P-104	GAGAGCCAAC	290-1600	12	7	5	41.6	0.226	0.551
P-124	ATCGGGTCCG	280-1530	12	1	11	91.6	0.109	0.885
P-125	ACTGGCCTGA	500-1500	9	3	6	66.6	0.158	0.828
P-136	CCGGCCTTCC	290-1400	16	6	10	62.5	0.215	0.754
	Average	308-1466	14.8	5	9.8	63.85	0.220	0.654
ISSR primers								
P-45	AGGAGGTGATCCAACCGCA	350-1200	20	4	16	80.0	0.159	0.810
P-34	CCTCTCTCTCTCTCTCT	300-1360	24	1	23	95.8	0.285	0.635
P-54	TCTCTCTCTCTCTCTCG	280-1150	8	1	7	87.5	0.251	0.611
P-58	AGAGAGACAGACAGAGYA	340-1200	11	0	11	100.0	0.499	0.500
P-62	HBHAGAGAGAGAGAGAG	280-1400	7	1	6	85.7	0.207	0.511

Table 2. Contd.

Primer code RAPD primers	Primer sequence (5' to 3')	Amplified product range (bp)	Total bands	Mono bands	Poly bands	% Poly.	Average Expected gene diversity (Hi)	PIC
P-63	BHBGAGAGAGAGAGAGA	340-1500	14	2	12	86.0	0.226	0.670
P-64	BHBGTGTGTGTGTGTGT	300-1200	19	8	11	58.0	0.302	0.579
P-65	HVHTGTGTGTGTGTGTG	250-1100	13	5	8	61.5	0.254	0.647
P-67	ATGATGATGATGATGATG	350-1260	12	5	7	58.3	0.336	0.555
P-68	CTCCTCCTCCTCCTCCTC	400-1500	6	2	4	66.6	0.344	0.562
	Average	319-1287	13.4	2.9	10.5	77.9	0.286	0.608

Single letter abbreviation for mixed base positions: Y=(C, T); B=(C, G, T that is, not A); H = (A, C, T that is, not G); V= (A, C, G, that is, not T); poly, polymorphism.

the dendrogram, the data were bootstrapped with 2500 replications along with Jaccard's coefficient by the computer programme WINBOOT (Yap and Nelson, 1996).

Marker index for different ISSR markers was calculated in order to characterize the capacity of each primer to detect polymorphic loci among the genotypes. It is the sum total of the polymorphism information content (PIC) values of all the markers produced by a particular primer. PIC value was calculated using the formula:

$$PIC = 1 - 1/n \sum_{i=1}^{n} Pij$$

Where, Pij, is the frequency of the jth allele in the ith primer (Kumar et al., 2003). Average expected gene diversity was calculated using the formula,

$$H_i = h_1 + h_2 / \text{Total number of loci}$$

Where, h_1 and h_2 [i.e. $h_j = (1-p^2-q^2)$] are intra-locus gene diversity.

RESULTS

RAPD analysis

Polymerase chain reaction (PCR) with 10-mer oligo nucleotide RAPD primers produced a total of 222 loci of which 63.8% were polymorphic with cumulative size range of 308 to 1466 bp (Table 2). The number of bands ranged from seven (P-100) to twenty four (P-72) and varied in sizes from 210 bp (P-71) to 2000 bp (P-94). Average number of bands and polymorphic bands per primer were 14.8 and 9.8, respectively.

Percentage polymorphism ranged between 0% (P-89) to 92% (P-124), with an average of 63.8% across all the genotypes of fluorescent *Pseudomonas.* PIC value varied from 0 (P-89) to 0.885 (P-124), while average expected gene diversity ranged from 0 (P-89) to 0.789 (P-94) across all the genotypes. Jaccard's similarity coefficient matrix (Table not shown) for RAPD data depicted the minimum similarity coefficient between Pf16 and Pf17 (0.112) whereas maximum similarity coefficient was shown between Pf4 and Pf5 (0.736). The cluster analysis for the distribution of 222 RAPD bands is shown as a dendrogram (Figure 1a). The 17 genotypes of fluorescent *Pseudomonas were* grouped into three major clusters. Cluster I comprised the six isolates Pf1, 2, 3, 4, 5 and 6, among which Pf4 and Pf5 showed 73% similarity. Cluster II consisted of five isolates containing Pf7, 8, 9, 10 and 12, among which Pf9 and Pf10 showed 60% similarity. Five isolates formed the cluster III which included Pf11, 13, 14, 15 and 16. One unique strain, Pf17 which showed a slightly different banding pattern from the rest of the *Pseudomonas* strains was not included in any cluster. The result of PCA analysis was comparable to the cluster analysis (Figure 2a). The first five most informative PC components explained 49.51% of the total variation.

ISSR analysis

ISSR primers produced different numbers of DNA fragments, depending upon their simple sequence repeat motifs. The ISSR bands were scored for presence (1) or absence (0) among the genotypes and were used for UPGMA analysis. PCR amplification using 10 ISSR primers produced 134 reproducible bands across the 17 genotypes, out of which 105 were polymorphic, accounting for 78% poly- morphism. The cumulative size ranges for the amplified fragments was 319 to 1287 bp (Table 2). The number of bands ranged from six (P-68) to twenty four (P-34) and varied in size from 250 bp (P-65) to 1500 bp (P-63 and P-68).

Average number of bands and polymorphic bands per primer were 13.4 and 10.5, respectively. Percentage polymorphism ranged between 58% (P-64) to 100% (P-58), with an average of 77.9% across all the genotypes of fluorescent *Pseudomonas*. PIC value varied from 0.5 (P-58) to 0.67 (P-63), while average expected gene diversity ranged from 0.159 (P-49) to 0.499 (P-58) across all the genotypes. A dendrogram based on UPGMA analysis with ISSR data is shown in Figure1b. At 60% similarity

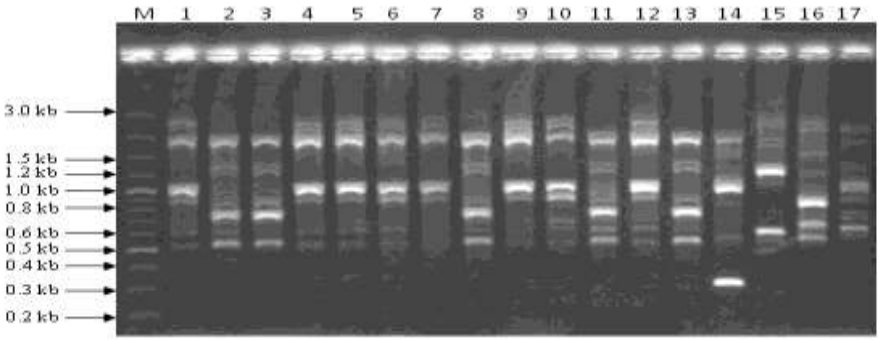


Figure 1a. RAPD profile of different isolates of fluorescent Pseudomonas obtained with primer LC-72. Lane M is 100bp ladder and Lanes 1 to 17 represent different isolates of Pseudomonas as listed in table 1

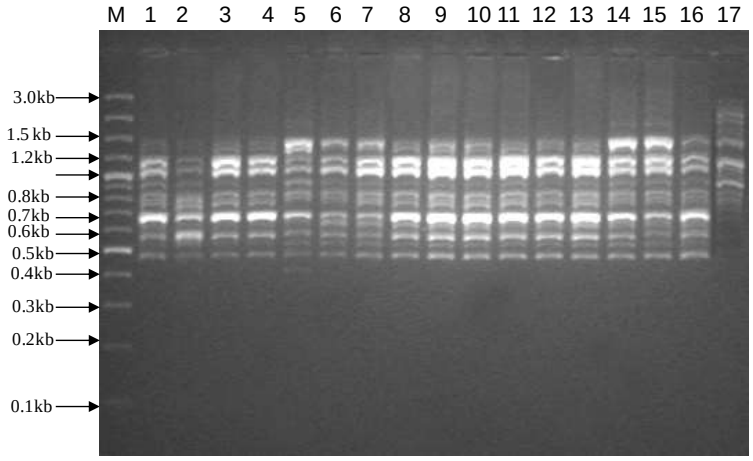


Figure 1b. ISSR profile of different isolates of fluorescent *Pseudomonas* obtained with primer LC-455. Lane M is 100bp ladder and Lanes 1 to 17 represent different isolates of fluorescent *Pseudomonas* as listed in table 1.

level, the 17 genotypes of fluorescent Pseudomonas were clustered into four clusters. Cluster I comprised Pf1, Pf8 and 15. Cluster II comprised seven isolates which were Pf2, 3, 4, 5, 6, 7 and 17, among which Pf6 and Pf7 showed 81.7% similarity between them. Cluster III comprised the two isolates, Pf9 and 10 whereas Pf16 was a distinct genotype among all the isolates. With reference to Jaccard's similarity coefficient matrix, Pf10 had the minimum similarity coefficient (0.383) with Pf2 (Table not shown) whereas Pf6 showed the maximum similarity coefficient with Pf7 (0.817). The result of PCA analysis was comparable to the cluster analysis (Figure 2b). The first five most informative PC components explained 54.62% of the total variation.

Combine data

The ISSR and RAPD data were combined for UPGMA cluster analysis. The Jaccard's similarity coefficient ranged from 0.258 to 0.711 for the combine data (Table not shown). The dendrogram based on the combined (RAPD and ISSR) data is shown in Figure 1c. The clustering pattern of the genotypes remained more or less the same in the RAPD and combined (ISSR and RAPD) data, while ISSR based dendrogram showed some variation in the clustering of *Pseudomonas* genotypes.

The matrices for the two markers, ISSR and RAPD were also compared by using Mantel's test (Mantel, 1967) for matrix correspondence. The correlation between the matrices of cophenetic correlation values for the dendrogram based on the combine data (ISSR and RAPD) was high (r = 0.875), which indicated good fit among RAPD and ISSR markers system. The results of PCA analysis were comparable to the cluster analysis (Figure 2c). The first five most informative PC components explained 48.46% of the total variation.

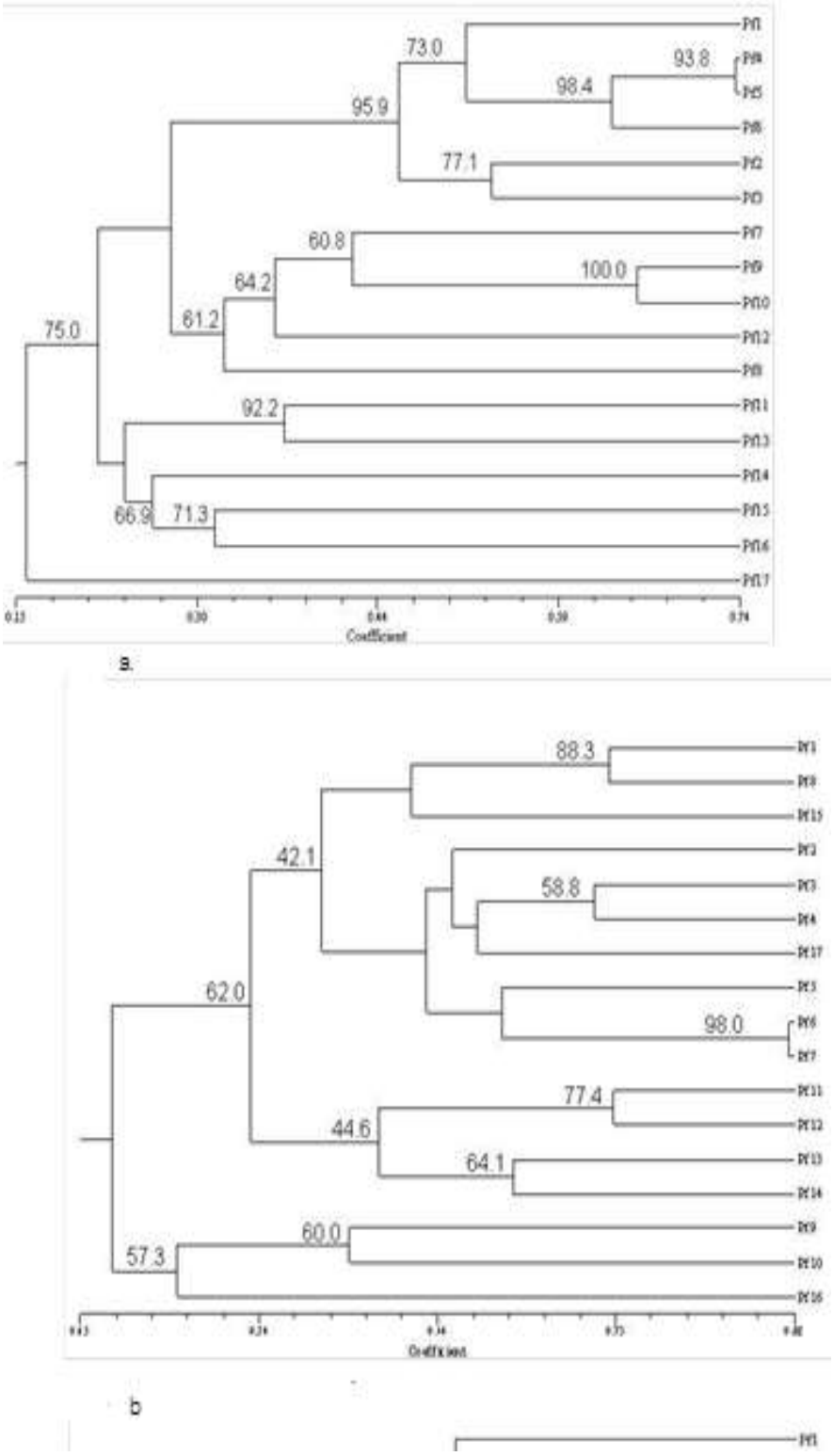


Figure 2. Dendrograms obtained from the 17 genotypes of fluorescent *Pseudomonas* with UPGMA based on Jaccards coefficient. The numbers at the forks indicate the confidence limits for the grouping. Genotypes in a branch occurred, based on 2500 cycles in bootstrap analysis using the winboot program. The scale bar indicates the similarity index. **a.** RAPD data based dendrogram; **b.** ISSR data based dendrogram; **c.** Combined (ISSR and RAPD) data based dendrogram.

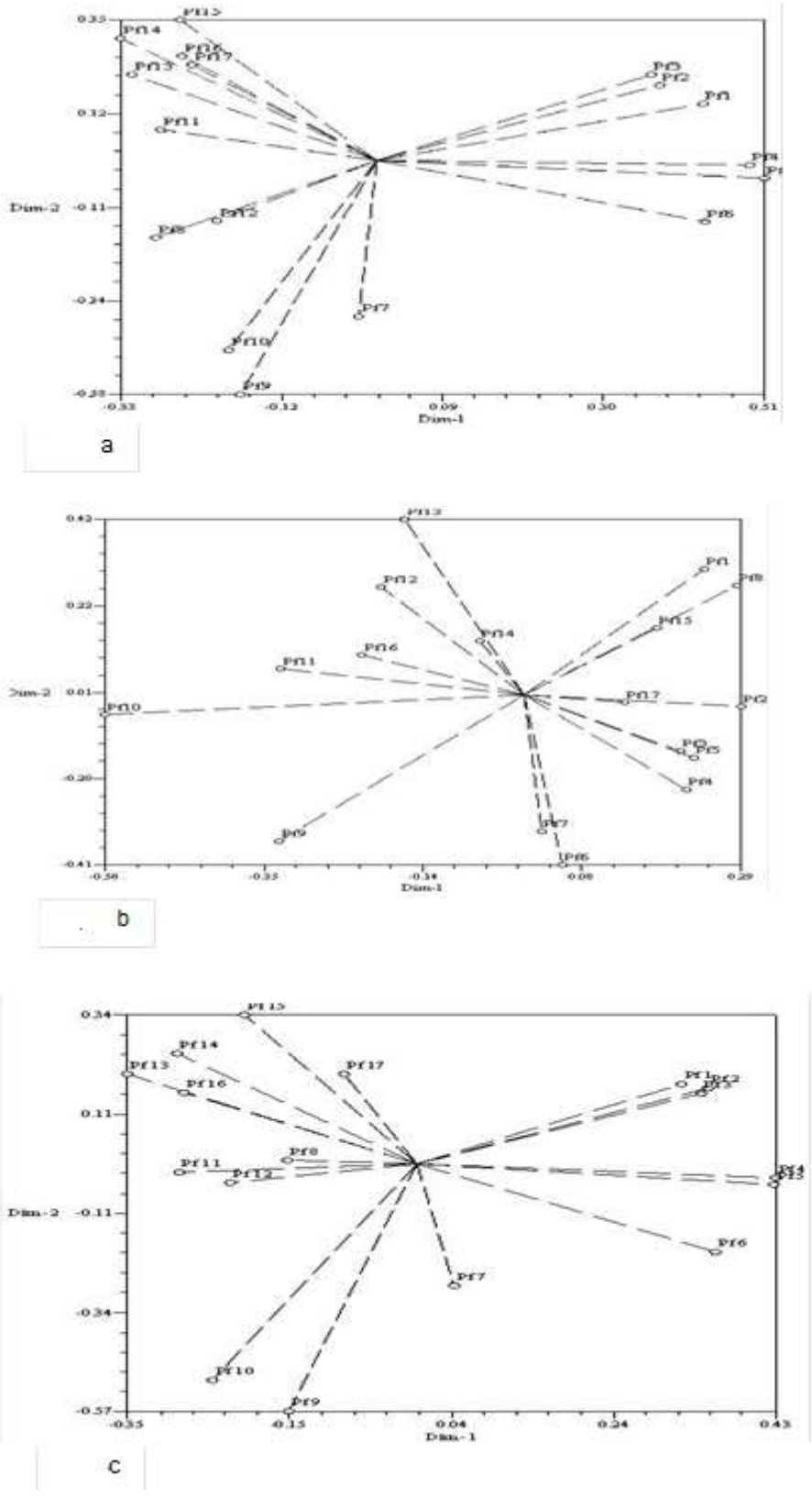
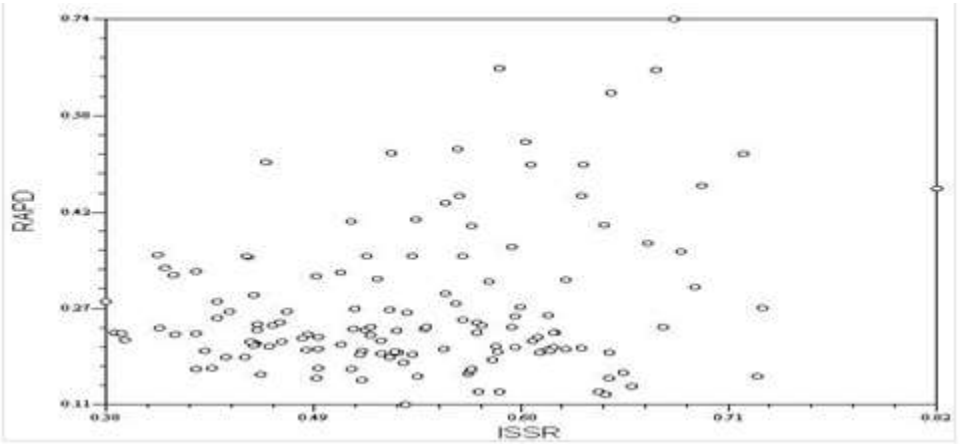
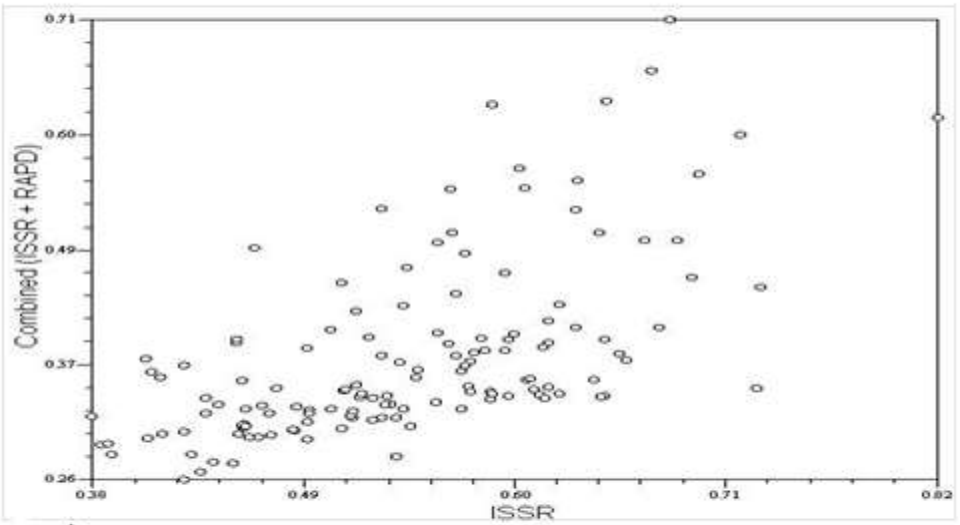


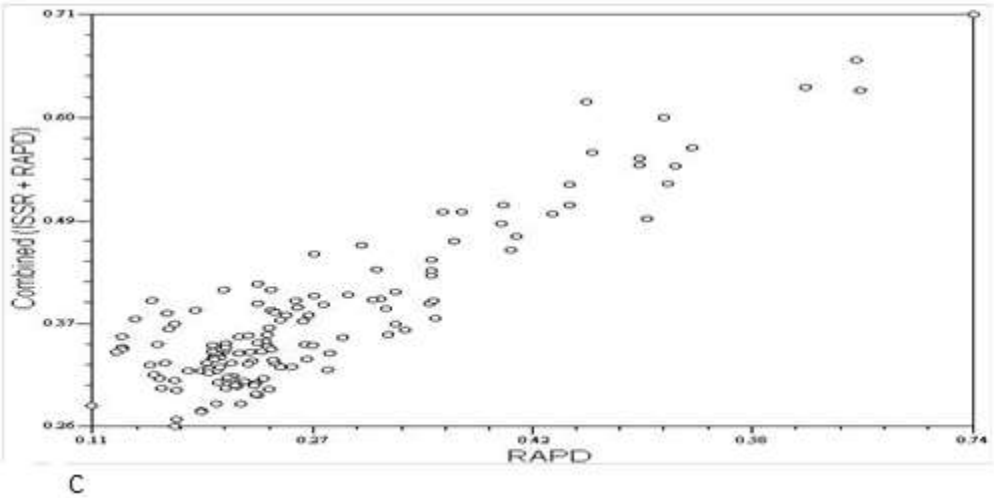
Figure 3. Two-dimensional plot (with vectors) of the 17 fluorescent *Pseudomonas* genotypes obtained using principal coordinate analysis. 3a, RAPD data based 3D plot; 3b, ISSR data based 3D plot; 3c; combined (ISSR and RAPD) data based on 3D plot.



a



b



c

Figure 4. Pearson correlation coefficient (r) estimates between genetic distances (GD) obtained using random amplified polymorphic (RAPD) DNA and Inter simple sequence repeats markers. 4a; RAPD Vs. ISSR plot; 4b, ISSR Vs. combined (ISSR and RAPD) plot; 4c, RAPD Vs. combined (ISSR and RAPD) plot.

Correlation between genetic distances measured with RAPD and ISSR

The lowest Pearson correlation value was between the ISSR and RAPD genetic distances (r =0.257) and it seemed that these two markers were the distinct type of markers in terms of the magnitude of the genetic distances produced. The RAPD and combined markers produced the highest correlation value (r = 0.91), followed by ISSR and the combined markers (r =0.67), which indicated that the RAPD markers contributed more in the combined data in comparison to ISSR (Figures 3 and 4).

DISCUSSION

In this study, the potential ability of two molecular markers that is, ISSR and RAPD were tested to evaluate the genetic diversity of the most commonly used bio-control agent, fluorescent *Pseudomonas*. This assessment showed that the percent polymorphism, primer information content (PIC) and average expected gene diversity (Hi) values were slightly higher in case of ISSR marker system as compared to RAPD marker. It was reported by Nagoaka and Ogihara (1997) that ISSR primers are more informative than RAPD because of higher percentage of polymorphic bands. They also found that RAPD produced less reliable bands than ISSR.

The ISSR method has been reported to produce more complex marker patterns than the RAPD approach (Parsons et al., 1997; Chowdhury et al., 2002), which is advantageous when differentiating closely related species. In addition, ISSR markers are more reproducible than RAPD markers (Goula˜o and Oliveira, 2001), because ISSR primers, designed to anneal to a microsatellite sequence, are longer than RAPD primers, allowing higher annealing temperatures to be used, which leads to greater consistency. Longer primers, ISSR's 14-22 bases vs. RAPD's 10 bases, can have higher annealing temperatures.

It was found that few RAPD primer which were used in this investigation could not produced the reproducible results. In one study, Qian et al. (2001) also found that RAPD bands were less reproducible. In other study, Skroch and Nienhuis (1995) tested the reproducibility of RAPD replicates and found that nearly a quarter of RAPD bands were dropped from statistical analysis because of inconsistency. They proposed that the amplification differences could be due to subtle variation in the reaction environment, band intensity, and concentration of ethidium bromide staining.

Clustering of genotypes within groups was not similar when RAPD and ISSR derived dendrograms were compared. These differences may be attributed to marker sampling errors and or the level of polymorphism detected, reinforcing again the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among isolates. The putatively similar bands originated for RAPDs in the different individuals are not necessarily homologous, although they may share the same size in base pairs (Souframanien and Gopalakrishna, 2004).

The RAPD and ISSR survey between the 17 isolates of fluorescent *Pseudomonas* revealed 63.85 and 77.9% polymorphic bands, respectively. The number of total polymorphic and discriminant fragments was higher for ISSR than for RAPD.

A possible explanation for the difference in resolution of RAPDs and ISSRs is that the two marker techniques targeted different portions of the genome. The ability to resolve genetic variation among different genotypes may be more directly related to the number of polymorphisms detected with each marker technique rather than a function of which technique is employed (Souframanien and Gopalakrishna, 2004).

The results from the comparison of the two techniques indicated that ISSR does slightly better in revealing the genetic diversity among fluorescent *Pseudomonas* without any additional technical problems. But the combination of these two techniques will be better for comprehensive genetic analysis of *Pseudomonas* strains.

Future work will focus on these aspects along with antagonist test against the different pathogen to assess the relative ability of each isolate to infect different pathogen. In addition to understanding the antagonist potential of the bio-control agent, this information will improve the basic understanding of the genetic variability of this fluorescent *Pseudomonas* and will be of importance to many other agriculture systems.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

The authors are thankful to Department of Biotechnology and the Government of India, for providing financial support for the investigation and fellowship to the first and second authors.

REFERENCES

Chowdhury MA, Vandenberg B, Warkentin T (2002). Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.). Euphytica, 127: 317-325.

Esselman EJ, Jianqiang L, Crawford DJ, Windus JL, Wolfe AD (1999). Clonal diversity in the rare Calamagrostis porteri ssp. Insperata (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. Mol. Ecol. 8:443-451.

Goula˜o L, Oliveira CM (2001). Molecular characterization of cultivars of apple (*Malus domestica* Borkh.) using microsatellite (SSR and ISSR) markers. Euphytica, 122:81-89.

Kumar P, Singh K, Vikal Y, Randhawa LS, Chahal GS (2003). Genetic diversity studies of elite cotton germplasm lines using RAPD markers and morphological characteristics. Indian J. Genet. 63(1):5-10.
Mantel NA (1967). The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209-220.
Martins M, Tenreiro R, Oliveira MM (2003). Genetic relatedness of Portuguese almond cultivars assessed by RAPD and ISSR markers. Plant Cell Rep. 22:71-78.
Nagoaka T, Ogihara Y (1997). Application of Inter simple sequence repeat polymorphism in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet. 94:597-602.
Parsons BJ, Newbury HJ, Jackson MT and Ford-Lloyd BV (1997). Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. Mol. Breed. 3:115-125.
Qian W, Ge S, Hong DY (2001). Genetic variation within and among populations of a wild rice *Oryza granulata* from China detected by RAPD and ISSR markers. Theor. Appl. Genet. 102:440-449.
Raina SN, Rani V, Kojima T, Ogihara Y, Singh KP, Devarumath RM (2001). RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietals identification, and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species. Genome, 44: 763-772.
Rajaseger G, Tan HTW, Turner IM, Kumar PP (1997). Analysis of genetic diversity among *Ixora* cultivars (Rubiaceae) using random amplified polymorphic DNA. Ann. Bot. 80:355-361.
Rohlf FJ (1993). NT-SYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 2.11 W. Exteer Software: Setauket, NY.
Siddiqui IA, Dieter H, Stephan H (2005). Extracellular Protease of *Pseudomonas fluorescens* CHA0, a Biocontrol Factor with Activity against the Root-Knot Nematode *Meloidogyne incognita.* Appl. Environ. Microbiol. 71(9):5646-5649.
Skroch P, Nienhuis J (1995). Impact of scoring error and reproducibility of RAPD data on RAPD-based estimates of genetic distance. Theor. Appl. Genet. 91: 1086-1091.
Souframanien J, Gopalakrishna T (2004). A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theor. Appl. Genet. 109: 1687-1693.
Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitary primers are useful as genetic markers. Nucleic Acids Res. 18: p. 6535.
Yap IV, Nelson RJ, (1996). Winboot: A program for performing bootstrap analysis for binary data to determine the confidence limitsof UPGMA-based dendrograms. In International Rice Research Institute. p. 14.
Zietkiewicz E, Rafalski A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176-183.