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Full Length Research Paper

Assessment of genetic diversity among different indigenous *Xanthomonas* isolates via randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR)

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The genetic diversity among seven *Xanthomonas* isolates representing four species was assessed using randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) PCR-based techniques. Both techniques revealed high degrees of polymorphisms among the studied isolates. Cluster dendrogram based on combined data of RAPD and ISSR showed that genetic diversity exists in local isolates of *Xanthomonas*. In terms of percentage similarity values, the genomic variation was found to be in the range of 29.29 to 100% among the isolates. *X. campestris* (*Mangifera indica*) remain unclustered in cluster dendrogram and showed a unique genomic profile as compared to other isolates used in this study.

Key words: *Xanthomonas*, genetic diversity, randomly amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR).

INTRODUCTION

The genus *Xanthomonas* in the gamma subdivision of the Proteobacteria consists of 27 plant-associated species, many of which cause important diseases of crops and ornamentals (Abdo–Hasan et al., 2008). Cells are straight rods usually within the range of 0.4 to 0.7 in width x 0.7 to 1.8 um in length. They are gram negative, aerobic and motile by a single polar flagellum (Bradbury, 1984). Collectively, members of the genus cause disease on at least 124 monocot species and 268 dicot species, including fruit and nut trees, solanaceous and brassicaceous plants, and cereals (Hayward, 1993). Variability within species has been determined by means of genetic techniques such as random amplified polymorphic deoxyribonucleic acid (DNA) (Pooler et al., 1996), restriction fragment length polymorphism (Roberts et al., 1998) and repetitive-sequence polymerase chain reaction (PCR) (rep-PCR) (Gillings et al., 1998). In recent years, randomly amplified polymorphic DNA- polymerase chain reaction (RAPD-PCR) approach has been shown to be useful in classifying a number of microbial strains and species (Williams et al., 1990) including various *Xanthomonas* spp. such as *Xanthomonas albilineas* (Permaul et al., 1996), *Xanthomonas fragariae* (Pooler et al., 1996), *Xanthomonas maltophilia* (Yao et al., 1995) and *Xanthomonas campestris* pv. *pelargonii* (Manulis et al., 1994). Inter simple sequence repeats (ISSRs) are semi-arbitrary markers amplified by PCR in the presence of one primer complementary to a target microsatellite. Such amplification does not require genome sequence information and leads to multilocus and highly polymorphous patterns (Nagaoka and Ogihara, 1997). Each band corresponds to a DNA sequence delimited by two inverted microsatellites. Like RAPDs, ISSRs markers are quick and easy to handle, but they seem to have the reproducibility of SSR markers because of the longer length of their primers. Therefore analysis of genomic DNA using RAPD and ISSR is a suitable typing method

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Table 1. List of isolates of *Xanthomonas* species used for molecular characterization.

FCBP Accession #	Strain	Substrate
1	*X. axonopodis* (I)	Citrus fruit
186	*X.axonopodis* (II)	Rhizospheric soil
8	*X. campestris* (III)	*Mangifera indica*
118	*X. maltophilia* (IV)	*Brassica campestris*
132	*X.maltophilia* (V)	*Pisum sativum*
98	*X. nematophilus* (VI)	Root nodules
115	*X. nematophilus* (VII)	*Brassica campestris*

since both techniques have proved to be fast, sensitive, and reliable for determining genetic relationships among *Xanthomonas* isolates (Abdo-Hasan et al., 2008).

Results are discussed in the light of previous findings regarding their genetic diversity attributed generally to geographical distribution and or pathogenecity of various *Xanthomonas* species or pathovars. The present research sheds light on the characterization of some indigenous *Xanthomonas* species and inter or intra specific variations among them.

MATERIALS AND METHODS

Procurement of bacterial cultures

Seven isolates of *Xanthomonas* representing four different species including *X. campestris*, *X. maltophilia, Xanthomonas axonopodis* and *Xanthomonas nematophilus* were procured from First Fungal Culture Bank of Pakistan (FCBP), Institute of Plant Pathology, University of the Punjab, Lahore. List of these isolates along with their substrate sources is given in Table 1.

Isolation of genomic DNA

DNA was extracted from bacterial cells following the method described by Herrick et al. (1996) with slight modifications. A single colony from a fresh Luria-Bertani agar (LBA) plate was used to inoculate 10 ml LB broth which was grown to saturation. An aliquot of 1.5 ml of the culture was centrifuged at maximum speed (14000 rpm for 5 min), the supernatant was discarded and pellets were re-suspended by vigorous vortexing in 500 µl TE buffer to which 60 µl 10% SDS and 60 µl proteinase K (20 mg/ml) were added. After 1 h of incubation at 37ºC, DNA was extracted twice with phenol or chloroform (1:1) and twice with chloroform. Nucleic acids were precipitated using absolute ethanol and sodium chloride. The resulting pellet was re-suspended in TE buffer containing RNase A and incubated at 37ºC for 30 min. DNA was finally precipitated with 5 M ammonium acetate and isopropanol. After centrifugation, the pellet was washed twice with 70% ethanol, air dried and dissolved in TE buffer.

Randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSRs) analyses.

Seven selected isolates of *Xanthomonas* were tested against twenty eight primers from Gene Link™ while two primers from Operon Technologies (USA) for RAPD analysis (Table 2) according to the method described by Williams et al. (1990). Three ISSR primers (Table 3) were used for the analysis according to Bornet and Branchard (2001).

All PCR reactions were performed in a total volume of 50 µl containing 10 × PCR buffer + 25 mM Mg, 1 mM dNTPs, 10 p mole primer, 2.5 unit of *Taq* polymerase (Eppendorf, USA) and 1 µg genomic DNA. Using a master cycler gradient PCR (TECHNE TC-412), these reactions were subjected to initial denaturation at 94°C for 5 min; followed by 40 cycles of denaturation at 94°C for 45 s, annealing at 33°C for 1.30 min and primer extension at 72°C for 1.30 min. Final extension was set at 72°C for 7 min. The reaction was terminated at 4°C in 2 to 3 h. Amplification products were stored at 4°C until use. Amplified products were run on 2% agarose gel and stained in ethidium bromide. Electrophoresis was carried out at 100 V for 45 min at room temperature. A 100bp ladder (Fermentas, USA) was used to estimate the approximate molecular weight of amplification products. The bands were visualized through UV transilluminator (WiseDoc MUV-M20) and photographed on the gel documentation system.

RESULTS

Genomic DNA extraction of *Xanthomonas* isolates

Total of seven isolates of *Xanthomonas* representing four species were subjected to DNA extraction and the samples were run on 0.8% agarose gel. Results of all these isolates are shown in Figure 1.

RAPD analysis

Primer screening for RAPD analysis

Thirty different available decamers were screened out as described in Table 2. After examining their nanomole values (nm) these were diluted up to 10 pMole concentrations. RAPD results were achieved only with two primers from Operon series which were specifically designed for *Xanthomonas* that is OPR-02 and OPR-20. Different melting temperature conditions were checked across the set of thirty decamers used to screen the genome of different *Xanthomonas* species or isolates that is 20, 25, 28, 30, 33, 34, 35 and 38°C, the melting temperature was optimized at 33°C for the decamers used in the study.

Table 2. List of random primers and their sequences used for RAPD analysis.

S #	Primer	Sequence	Tm = 2(AT)+(GC)/2	nm
1	A-01	5’GGGTAACGCC3’	33.6	11.1
2	A-02	5’GTTGCGATCC3’	29.5	12.2
3	A-03	5’AGTCAGCCAC3’	29.5	11.2
4	A-04	5’AATCGGGCTG3’	29.5	11
5	A-05	5’AGGGGTCTTG3’	29.5	11.3
6	A-06	5’GGTCCCTGAC3’	33.6	12.4
7	A-07	5’GAAACGGGTG3’	29.5	10
8	A-08	5’GTGACGTAGG3’	29.5	10.6
9	A-11	5’CAATCGCCGT3’	29.5	11.9
10	A-12	5’TCGGCGATAG3’	29.5	11
11	A-13	5’CAGCACCCAC3’	33.6	11.8
12	A-14	5’TCTGTGCTGG3’	29.5	12.6
13	A-15	5’TTCCGAACCC3’	21.5	12.4
14	A-16	5’AGCCAGCGAA3’	21.5	10.9
15	A-17	5’GACCGCTTGT3’	29.5	12.2
16	A-18	5’AGGTGACCGT3’	29.5	11
17	A-19	5’CAAACGTCGG3’	29.5	10.8
18	B-01	5’GTTTCGCTCC3’	13.7	29.5
19	B-04	5’GGACTGGAGT3’	10.6	29.5
20	B-05	5’TGCGCCCTTC3	33.6	13.9
21	B-11	5’GTAGACCCGT3’	11.4	29.5
22	B-12	5’CCTTGACGCA3’	11.9	29.5
23	B-13	5’TTCCCCCGCT3’	33.6	14.6
24	B-14	5’TCCGCTCTGG3’	33.6	13.3
25	B-16	5’TTTGCCCGGA3’	29.5	12.2
26	B-17	5’AGGGAACGAG3’	21.5	9.5
27	B-18	5’CCACAGCAGT3’	29.5	11.2
28	B-19	5'ACCCCCGAAG3'	33.6	11.3
29	OPR-20	5'ACGGCAAGGA3'	22	77.7
30	OPR-02	5'CACAGCTGCC3'	26	164.8

Table 3. List of primers and their sequences used in ISSR analysis.

S. #	Primer	Sequence	Tm = 2(AT)+4(GC)/2	nm
1	A-16	5’CACACACACACAR3’	29	55.8
2	A-31	5’AGCAGCAGCAGC3’	35	118.0
3	D-3	5’GACAGACAGACAGACA3’	43	80.7

Analysis of amplified DNA fragments with different Decamers

Seven isolates of *Xanthomonas* species were subjected to RAPD analysis along with negative control and DNA marker (100 bp) by using two primers from Operon series. In negative control sterile distilled water was replaced by the template DNA so that no amplification was observed indicating the validity of RAPD assay. The dendrograms (gene tree) were constructed from amplification patterns of each primer by using the software MINITAB.

Amplification and dendrogram observed by Decamer OPR-02

Genetic variation among different isolates of *Xanthomonas* species was assessed through RAPD analysis by using decamer OPR-02 (Figure 2). The result showed that all the isolates exhibited a common band at 450 bp. Both *X. maltophilia* (V) and *X. nematophilus* (VII)

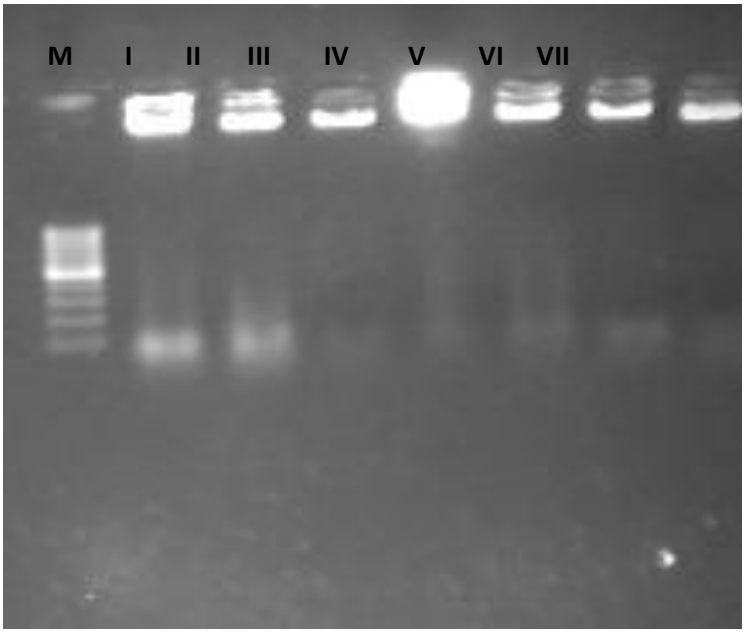

Figure 1. Genomic DNA isolation from different isolates of *Xanthomonas* species. M: marker (100 bp) I: *X. axonopodis* (citrus fruit) II: *X. axonopodis* (Rhizospheric soil) III: *X. campestris* (*Mangifera indica*) IV: *X. maltophilia* (*Brassica campestris*), V: *X. maltophilia* (*Pisum sativum*) VI: *X. nematophilus* (Root nodules) VII: *X. nematophilus* (*Brassica campestris*).

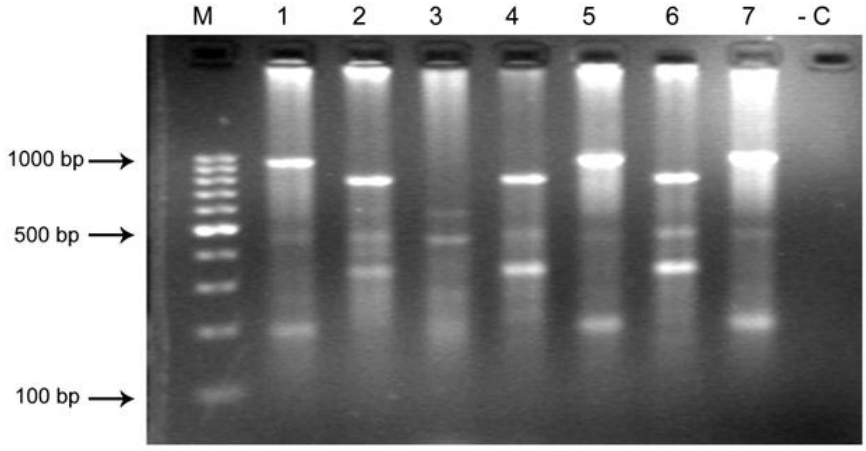

Figure 2. RAPD analysis of different isolates of *Xanthomonas* by using primer OPR-02. M**:** DNA marker (100bp) 1: *X. axonopodis* (citrus fruit) 2: *X. axonopodis* (Rhizospheric soil) 3: *X. campestris* (*Mangifera indica)* 4: *X. maltophilia* (*Brassica campestris*) 5: *X. maltophilia* (*Pisum sativum*) 6: *X. nematophilus* (Root nodules) 7: *X. nematophilus* (*Brassica campestris*) -C: Negative control.

shared two common bands at 200 and 1000 bp. Isolates of *X. axonopodis* (I and II) showed two similar bands at 300 and 450 bp, whereas polymorphic band produced by *X. axonopodis* (I) was at 950bp and by *X. axonopodis* (II) was at 800bp. *X. campestris* (III) exhibited four bands at 200, 250, 450 and 550 bp, respectively, whereas five bands were produced by *X. maltophilia* (IV) at 200, 300, 450, 550 and 800 bp, respectively. However two bands were shared by both isolates at 200 and 550 bp.

The dendrogram constructed on the basis of amplification by using primer OPR-02 comprised of two main clusters (Figure 3). Strains falling in cluster one includes *X. axonopodis* (II), *X. maltophilia* (IV) and *X. nematophilus* (VI). Genomic homology of 59.18% was

Figure 3. Dendrogram obtained from amplification by primer OPR-02. 1: *X. axonopodis* (citrus fruit) 2: *X. axonopodis* (Rhizospheric soil) 3: *X. campestris* (*Mangifera indica*) 4: *X. maltophilia* (*Brassica campestris*) 5: *X. maltophilia* (*Pisum sativum*) 6: *X. nematophilus* (Root nodules) 7: *X. nematophilus* (*Brassica campestris*).

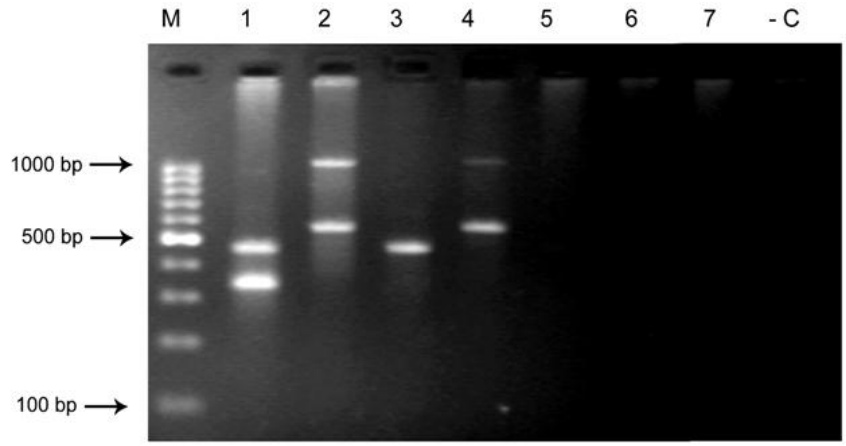

Figure 4. RAPD analysis of different isolates of *Xanthomonas* by using primer OPR-20. M: DNA marker (100bp) 1: *X. axonopodis* (citrus fruit) 2: *X. axonopodis* (Rhizospheric soil) 3: *X. campestris* (*Mangifera indica)* 4: *X. maltophilia* (*Brassica campestris*) 5: *X. maltophilia* (*Pisum sativum*) 6: *X. nematophilus* (Root nodules) 7: *X. nematophilus* (*Brassica campestris*) **-**C: Negative control.

observed among *X. axonopodis* (II) and *X. nematophilus* (VI) whereas *X. maltophilia* (IV) showed 42.26% similarity with other members representing cluster one. Cluster two was composed of *X. maltophilia* (V) and *X. nematophilus* (VII) strains representing 100% genetic similarity. *X. axonopodis* (I) and *X. campestris* (III) showed unique behaviour in terms of genetic profile variation, indicating 29.29% genetic similarity with other isolates.

Amplification and dendrogram observed by OPR-20

The amplification results obtained from primer OPR-20 showed that two bands were shared by *X. axonopodis* (II) and *X. maltophilia* (III) strains at 550 and 1000 bp (Figure 4). *X. axonopodis* (I) exhibited two bands of size 350 and 450 bp, respectively. *X. campestris* (III) represented a single band at 450bp whereas rest of the isolates displayed no amplification and revealed that they are genomically diverse than the other isolates.

The dendrogram constructed on amplifications observed by decamer OPR-20 showed two main clusters (Figure 5). First cluster displayed 50% similarities between *X. axonopodis* (I) and *X. campestris* (III) whereas second cluster indicated maximum similarity 100% among *X. axonopodis* (II) and *X. maltophilia* (IV)

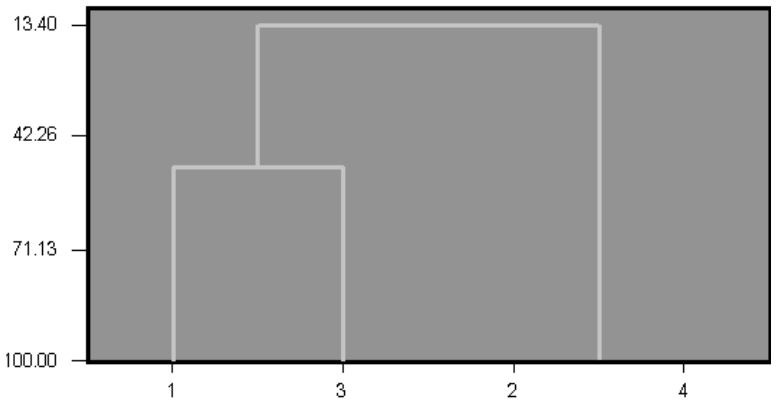

Figure 5. Dendrogram obtained from amplification by primer OPR-20. 1: *X. axonopodis* (citrus fruit) 2: *X. axonopodis* (Rhizospheric soil) 3: *X. campestris* (*Mangifera indica*) 4: *X. maltophilia* (*Brassica campestris*).

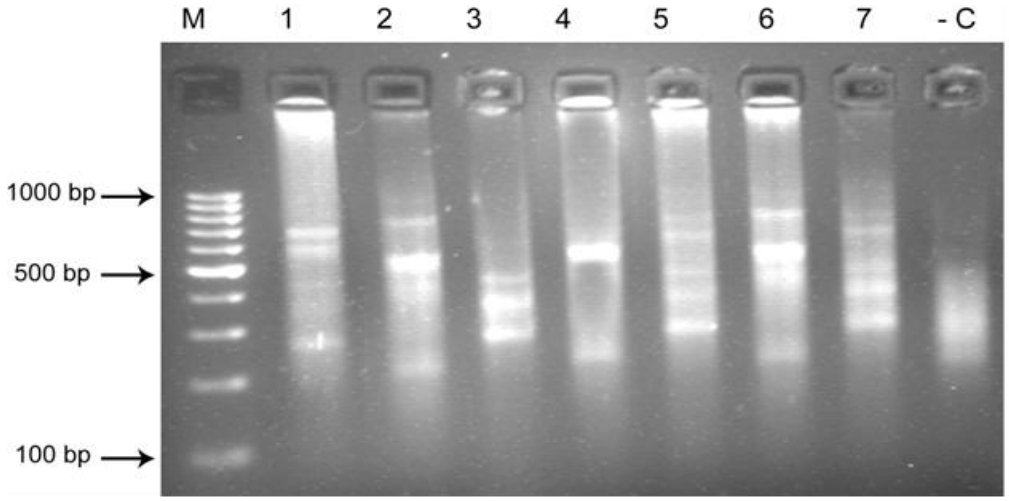

Figure 6. ISSR analysis of different isolates of *Xanthomonas* by using primer A-31. M: DNA marker (100bp) 1: *X. axonopodis* (citrus fruit) 2: *X. axonopodis* (Rhizospheric soil) 3: *X. campestris (Mangifera indica*) 4: *X. maltophilia* (*Brassica campestris*) 5: *X. maltophilia* (*Pisum sativum*) 6: *X. nematophilus* (Root nodules) 7: *X. nematophilus* (*Brassica campestris*) -C: Negative control.

isolates. Both clusters showed 13.40% similarity with each other.

ISSR analysis

Amplification and dendrogram observed by ISSR primer A31

Isolates of *Xanthomonas* species subjected to ISSR analysis showed thirty two polymorphic bands by using primer A-31 (Figure 6). Six similar bands were observed in case of *X. maltophilia* (V) and *X. nematophilus* (VII) at 250, 350, 450, 600, 700 and 1000 bp showing homogeneity in their genetic profile.

Isolates of *X. axonopodis* exhibited intraspecific variation as one of the isolate *X. axonopodis* (I) showed amplification (distinct bands) at 250 bp and 600 bp whereas the other isolate *X. axonopodis* (II) revealed bands at 200, 500 and 900 bp. This result showed a marked discrimination in genetic profiles of pathogenic and non pathogenic isolates. Four bands were observed in case of *X. campestris* (III) at 250, 350, 450 and 600 bp. *X. maltophilia* (IV) displayed only two bands at 200 and 500 bp whereas amplification of sizes 200, 400, 500, 700 and 900 bp was observed in case of *X. nematophilus* (VI). The dendrogram comprised of two main clusters exhibiting 25.46% similarity with each other (Figure 7). Cluster one is further divided into two subclusters. *X. axonopodis* (II) and *X. nematophilus* (VI) fell under subcluster one showing 52.86% genetic homology with each other whereas *X. maltophilia* (IV) was seen in

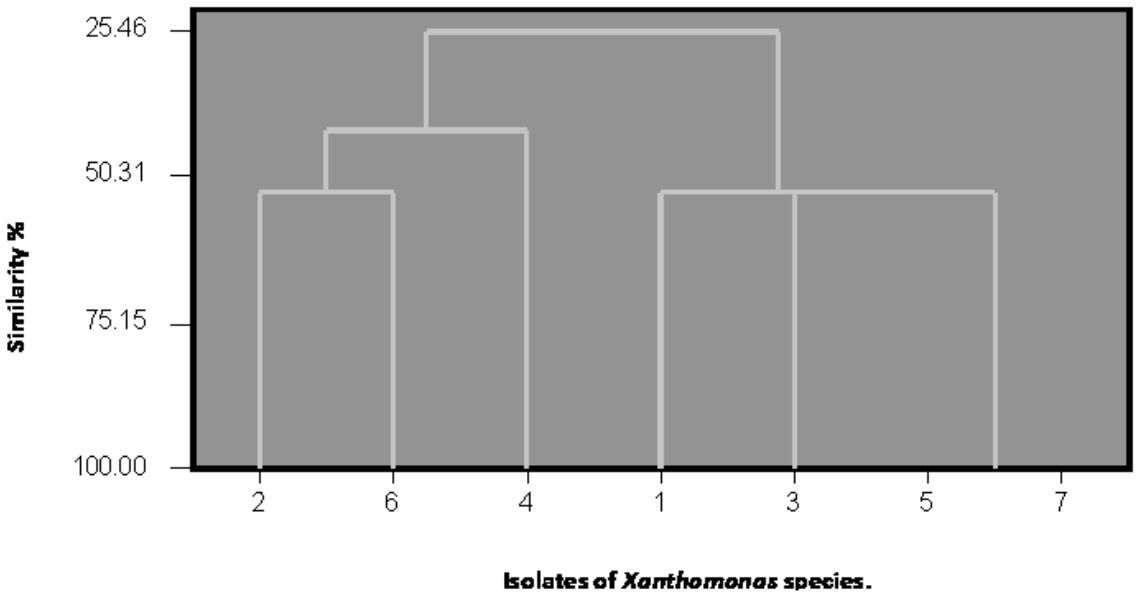

Figure 7. Dendrogram obtained from amplification by primer A-31. 1: *X. axonopodis* (citrus fruit) 2: *X. axonopodis* (Rhizospheric soil) 3: X. campestris (*Mangifera indica*) 4: *X. maltophilia* (*Brassica campestris*) 5: *X. maltophilia* (*Pisum sativum*) 6: *X. nematophilus* (Root nodules), 7: *X. nematophilus* (*Brassica campestris*)

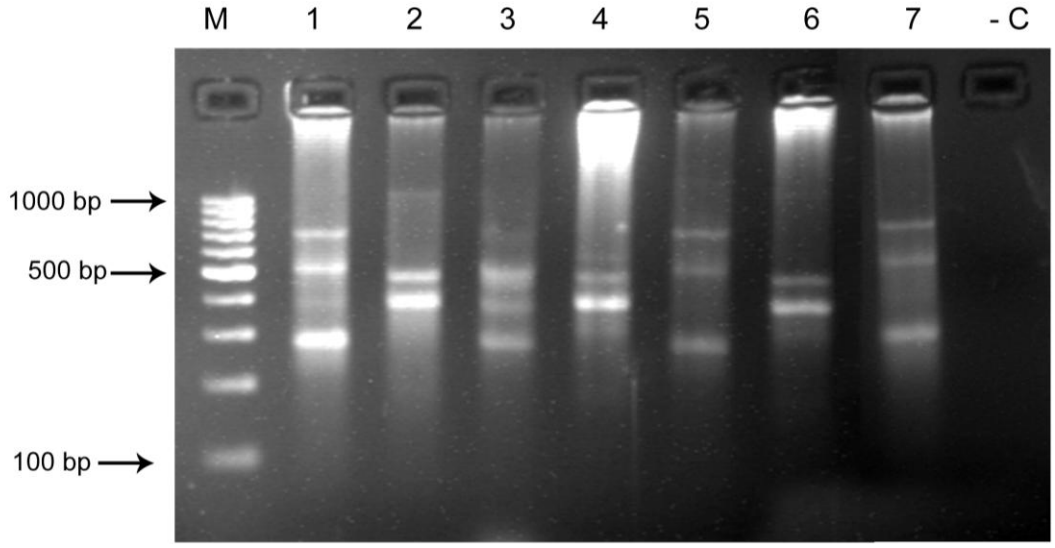

Figure 8. SR analysis of different isolates of *Xanthomonas* by using primer D-3. M: DNA marker (100bp) 1: *X. axonopodis* (citrus fruit) 2: *X. axonopodis* (Rhizospheric soil) 3: *X. campestris (Mangifera indica*) 4: *X. maltophilia* (*Brassica campestris*) 5: *X. maltophilia (Pisum sativum*) 6: *X. nematophilus* (Root nodules) 7: *X. nematophilus* (*Brassica campestris*) -C: Negative control.

second subcluster showing 40% similarity with other members of cluster one. *X. axonopodis* (I), *X. campestris* (III), *X. maltophilia* (V) and *X. nematophilus* (VII) formed cluster two. Maximum genetic similarity 100% was quite evident among isolates of *X. maltophilia* (V) and *X. nematophilus* (VII). *X. axonopodis* (I) and *X. campestris* (III) exhibited 52.86% homology with each other. Least genomic similarity 25.46% was found among two isolates of *X. axonopodis* from different substrates i.e. citrus fruit and rhizospheric soil.

Amplification and dendrogram observed by ISSR primer D-3

The results obtained from primer D-3 showed that genetic variation exists among different isolates of *Xanthomonas* (Figure 8). Similar amplifications at 300, 500, and 700 bp

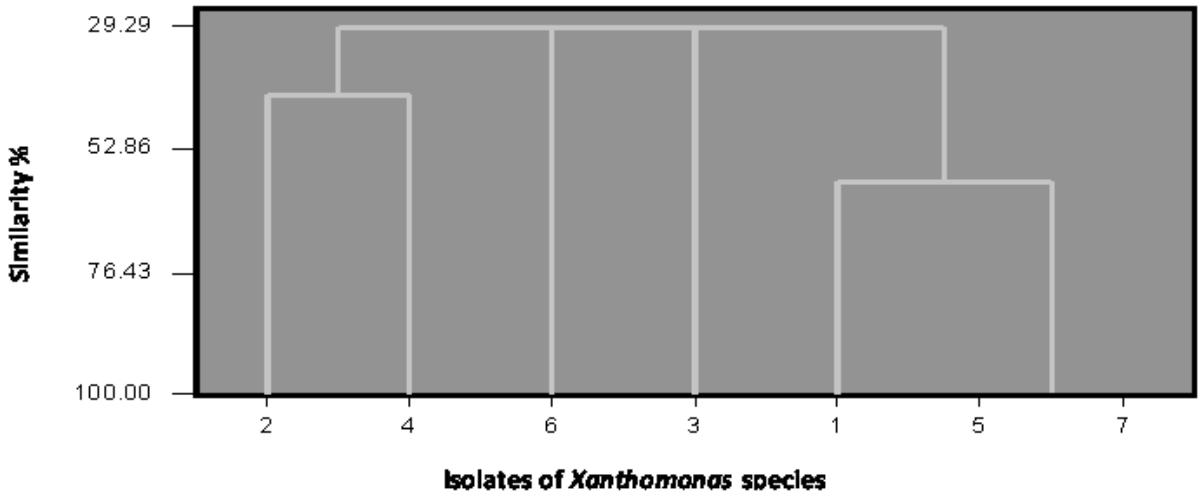

Figure 9. Dendrogram obtained from amplification by primer D-3. 1: *X. axonopodis* (citrus fruit) 2: *X. axonopodis* (Rhizospheric soil), 3: *X*. *campestris* (*Mangifera indica*) 4: *X. maltophilia* (*Brassica campestris*) 5: *X. maltophilia* (*Pisum sativum*) 6: *X. nematophilus* (Root nodules) 7: *X. nematophilus* (*Brassica campestris*).

were observed among *X. maltophilia* (V) and *X. nematophilus* (VII). *X. axonopodis* (I) represented amplification with four bands at 300, 350, 500 and 700 whereas three bands at 350, 450 and 1000 bp were observed in case of *X. axonopodis* (II). This showed that isolates of *X. axonopodis* from different substrates were genetically diverse from each other. *X. campestris* (III) exhibited amplifications at 300, 350, 450 and 600 bp. *X. maltophilia* (IV) revealed three bands at 350, 450 and 500bp however *X. nematophilus* (VI) showed two bands at 350 and 400 bp. Amplification patterns obtained through primer D-3 exhibited genetic diversity among isolates.

The dendrogram (Figure 9) based on ISSR analysis using primer D-3 exhibited two main clusters showing 29.29% similarity with each other. *X. campestris* (III) and *X. nematophilus* (VI) exhibited unique genetic profiles. Cluster one consisted of two strains that is *X. axonopodis* (II) and *X. maltophilia* (IV) indicating 42.26% genetic similarity among them. Second cluster was comprised of three strains from which *X. maltophilia* (V) and *X. nematophilus* (VII) showing maximum genomic homology 100% with each other whereas *X. axonopodis* (I) showed 59.18% similarity with both of them.

Amplification observed by ISSR primer A-16

The results obtained from ISSR primer A-16 showed amplification with only two isolates that is *X. axonopodis* (I) and *X. maltophilia* (V). Strains exhibited no amplification at all, indicating that the primer sequence was incompatible with their genomes. *X. axonopodis* (I) represented three bands of size 350, 500 and 700 bp whereas *X. maltophilia* (V) displayed a single band at 700 bp (Figure 10).

Cluster dendrogram

The amplification data of all the primers (OPR-02, OPR-20, A-31 and D-3) were collectively used for the dendrogram construction as described in Figure 11. The cluster dendrogram comprised of two main clusters representing 29.29% similarity with each other. Cluster one consisted of two subclusters having *X. axonopodis* (II) and *X. maltophilia* (IV) in subcluster one with 50% similarity however *X. nematophilus* (VI) comprising second sub cluster showed 38.76% genomic similarity with subcluster one. Cluster two displayed 100% similarity among *X. maltophilia* (V) and *X. nematophilus* (VII) whereas *X. axonopodis* (I) showed 38.76 similarity with other strains in the same cluster. *X. campestris* (III) remained unclustered in cluster dendrogram. Genetic homology in different isolates of *Xanthomonas* species by RAPD and ISSR analyses using cluster dendrogram is shown in Table 4.

DISCUSSION

Genetic diversity was assessed among *Xanthomonas* isolates through RAPD and ISSR. In case of RAPD analysis amplification in size range of 200 to 1000 bp were observed by using decamer OPR-20. Random primer, OPR-02, only amplified four strains in the range of 350 to 1000 bp. High rate of amplification were observed by using ISSR primers. The amplification pattern in terms of size range by using primer A-31 was 200 to 1000 bp. In case of primer D-3 amplification was

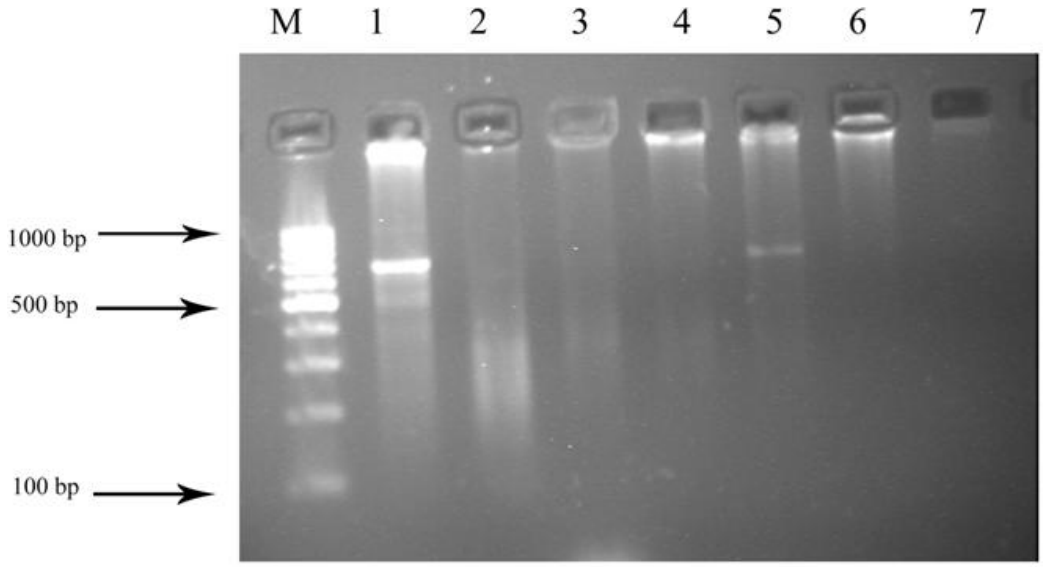

Figure 10. ISSR analysis of different isolates of *Xanthomonas* by using primer A-16. M: DNA marker (100bp) 1: *X. axonopodis* (citrus fruit) 2: *X. axonopodis* (Rhizospheric soil) 3: *X. campestris (Mangifera indica*) 4: *X. maltophilia* (*Brassica campestris*) 5: *X. maltophilia (Pisum sativum*) 6: *X. nematophilus* (Root nodules) 7: *X. nematophilus* (*Brassica campestris*).

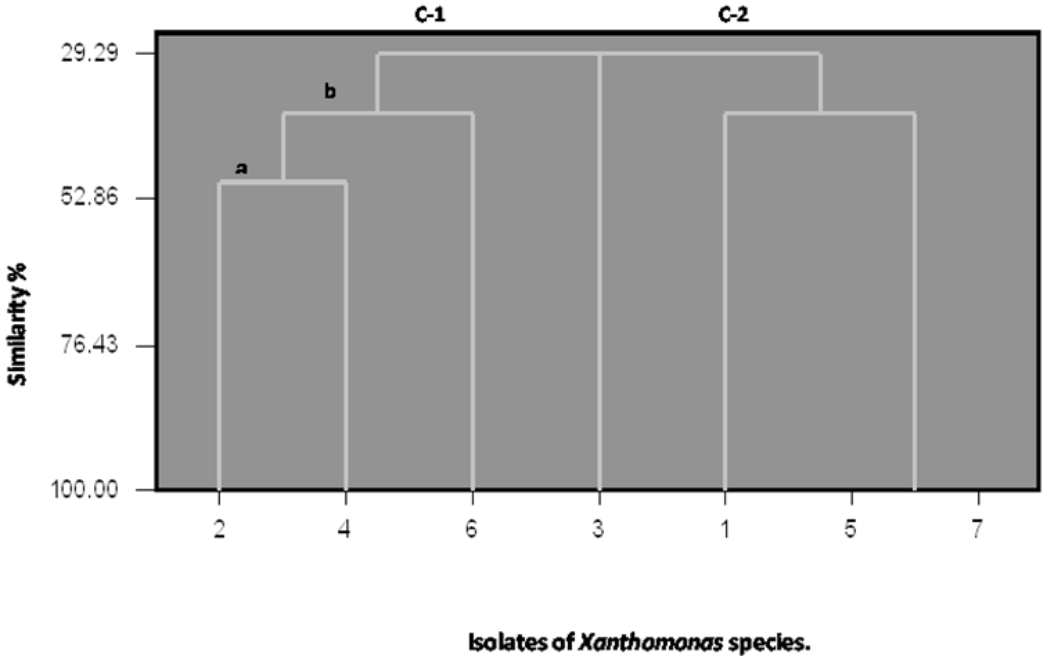

Figure 11. Dendrogram obtained from amplification by RAPD & ISSR primers. 1: *X. axonopodis* (citrus fruit) 2: *X. axonopodis* (Rhizospheric soil), 3: *X. campestris (Mangifera indica*), 4: *X. maltophilia* (*Brassica campestris*), 5: *X. maltophilia* (*Pisum sativum*), 6: *X. nematophilus* (Root nodules), 7: *X. nematophilus* (*Brassica campestris*).

observed in the range of 300 to 1000 bp.

In this study, through combined analyses of RAPD and ISSR it was found that genetic variability exists among seven *Xanthomonas* isolates in the range of 29.29 to 100% (in terms of similarity values). Maximum genomic homology was represented by two isolates that is *X. maltophilia* (V) and *X. nematophilus* (VII) from different localities. This similarity might be due to the fact that both

Table 4. Genetic homology in different isolates of *Xanthomonas* species by RAPD and ISSR analysis using cluster dendrogram.

Acc. #	Species	Substrate	Genomic similarity (%)	Cluster
01	*X. axonopodis* (I)	Citrus fruit	38.76	C-2
186	*X. axonopodis* (II)	Rhizospheric soil	50	C-1a
008	*X. campestris* (III)	*Mangifera indica*	29.29	Unique isolate
118	*X. maltophilia* (IV)	*Brassica campestris*	50	C-1a
132	*X. maltophilia* (V)	*Pisum sativum*	100	C-2
98	*X. nematophilus* (VI)	Root nodules	38.76	C-1b
115	*X. nematophilus* (VII)	*Brassica campestris*	100	C-2

strains are pathogenic. Isolates belonging to different species may exhibit similar genomic similarity on the basis of their pathogenic behaviour. Similar to our findings, Pooler et al. (1996) used three PCR–based techniques that rely on different amplification priming strategies, that is RAPD, repetitive extragenic palindromic (REP), and enterobacterial repetitive intergenic consensus (ERIC) to study the genetic relationships among 25 isolates of *X. fragariae* from diverse geographic regions. The three methods gave consistent results, indicating that pathogenic strains are very closely related to each other.

Two isolates of *X. axonopodis* from different substrates that is, citrus fruit and rhizospheric soil displayed high intraspecific variation (29.29% similarity value). In the cluster dendrogram, cluster one contained non pathogenic isolate whereas the pathogenic one was included in second cluster. Similarly Abdo-Hasan et al. (2008) detected genetic diversity among forty Syrian isolates of *X. axonopodis* pv. *malvacearum (Xam),* representing nine defined races through RAPD and ISSR PCR-based techniques. Both techniques revealed high degrees of polymorphisms among the studied races. Combined data showed that percent disagreement values (PDV) ranged between 0.13 and 0.37. The combined dendrogram based on unweighted pair group method with arithmetic averaging (UPGMA) analysis contained two main clusters. The first cluster contained four isolates (races), three of which were the lowest in virulence, and the second cluster contained three highly virulent isolates (races). So the strains used in our study showed a resemblance with Syrian isolates. It represents that isolates are mainly distinguished on the pathogenecity criteria not by geographical distribution. Variability of strains within the same species in our findings was further supported by Ogunjobi et al. (2007), who characterized the *X. axonopodis* population in Nigeria with RAPD. At a coefficient level of 90% extensive genetic diversity was observed in the 74 strains of the bacteria studied by RAPD analysis, which made it difficult to categorize the strains. Ten clusters and five unclustered strains were identified at 75% similarity coefficient on the dendrogram. The diversities observed were not regionally influence or agroecologically determined. Similar diversity has been described for *X. campestris* pv *passiflorae* studied with the same molecular marker in southern Brazil (Goncalves and Rosato, 2000).

Similar studies of Kishun and Gupta (2008) revealed that *X. campestris* pv. *mangiferaeindicae* (*Xcmi*) population exhibited a significant level of genetic diversity as it formed 2 clusters in phylogenetic tree. There was 7.66% polymorphism in individual isolates which indicate significant polymorphism among the evaluated strains, with mean difference of 0.33 (*Xcmi* 2 *vs*. *Xcmi* 8) and 0.29 (*Xcmi* 10 *vs*. *Xcmi*12). This result is contrary to work of Assigbetse et al. (1998), as they reported that RAPD analysis did not show high level of polymorphism within *Xanthomonas* strains used in their study. Kaur et al. (2005) results showed that RAPD markers revealed a high level of genetic diversity across the isolates of *X. axonopodis* pv. *cyamopsidis* in comparison with the other molecular markers employed.

Our findings contradict with those by Odipio et al. (2009), who found very low genetic diversity among Ugandan isolates of *X. campestris* pv. *musacearum* (Xcm) by RAPD. Regardless of the source and geographical origin, similar fingerprints were generated from the tested isolates. Using a similarity coefficient of 58%, the UPGMA analysis did not reveal any significant differences in clustering, with exception of a single isolate (Wkk) that had unique fingerprints.

The cluster dendrogram based on the collective amplification results of all the primers indicated that isolates of *Xanthomonas* were divided into two main clusters. Cluster 1 subdivided into two groups (a and b) exhibited approximately 50% genetic homology. Cluster 2 comprised three isolates of which *X. maltophilia* (V) and *X. nematophilus* (VII) showed 100% similarity with each other and 38.76% homology with *X. axonopodis* (I). *X. campestris* (III) remained unclustered in collective dendrogram analyses. It represented unique genetic profile showing 29.29% genetic homology with other isolates used in the study. Both RAPD and ISSR techniques showed similar results. Support for this type of techniques comes from Pooler and Hartung (1995) who showed that estimations of genetic distances within bacterial species based on RAPD analysis using carefully

selected primers is consistent with RFLP data.

In the absence of complete sequence information about genome of the pathogen, RAPD is the ideal technique since it scans for sequence variation throughout the whole genome. Recently, inter-simple sequence repeat (ISSR) markers have emerged as an alternative system with reliability and advantages of microsatellites (SSR). ISSR analyses are more specific than RAPD analyses, due to the longer SSR-based primers with higher primer annealing temperature, which enable higher-stringency and greater band reproducibility (Wolfe et al., 1998). Coupled with the separation of amplification products on agarose gel, ISSR amplification can reveal a much larger number of fragments per primer than RAPD. Techniques are cost effective, sequence information of template DNA is not required and markers can be generated in the regions containing repetitive sequences.

The study concludes that biochemical and molecular techniques employed presently give strong evidence to the existence of high level of variability among indigenous *Xanthomonas* isolates.

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