JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2012. Vol. **6**(3), p. 1219-1226

Genetic and Pathological Diversity among *Xac* Strains causing Citrus Canker in Maharashtra, India

Minhaj Arshiya, A.R. Suryawanshi*, D.R. More and M.M.V. Baig*

Department of Botany and Biotechnolgy, L.B.S. Mahavidyalaya, Dharmabad, Nanded - 431 602, India
*Department of Botany and Department of Biotechnology,
Yeshwant Mahavidyalaya, Nanded - 431 602, India.

(Received: 26 June 2012; accepted: 04 August 2012)

The present study is based on biochemical characterization, pathogenicity test and DNA based technique. Here apart from biochemical test, RAPD analysis was used to distinguish and differentiate among 24 strains of Xac isolated from Marathwada region in Maharashtra. 200 strains of Xac isolated from Citrus lemona from different location within Marathwada region of Maharashtra and 24 strains representing all the 8 districts were used in this study. All these strains were purified and maintained on YDC (Yeast extract -1gm, D-glucose -2gm, Calcium carbonate -2gm) slants. The presently used primer could resolve genetic diversity and geographical distribution in the study of Xanthomonas axonopodis pv citri and RAPD marker gave reproducible and reliable results.

Key words: *Xac* Strains, DNA based techinques, Citrus Canker.

In Marathwada the common cultivated citrus fruit belongs to *Citrus aurentifolia* (Lime), *Citrus lemona* (Lemon), *Citrus sinensis* (Sweet lime), *Citrus reticulate* (Orange). *Citrus lemona* is cultivated over an area of 87100 hectare in Marathwada region alone, Premalinae, Vikram, Sai sharbati and Local are common varieties cultivated in this region. These varieties are susceptible to citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (Hasse,1915) Vauterin, Hoste, Kersters and Swings, 1995 (*Xac*) affecting plants of all age and location thoughout the year[1].

There are many reports on *Xac* and host pathosystem. In order to device a suitable disease management system, it is essential to understand the population structure of pathogen as well as host[2]. The diversity of pathogen is considered to be one of the important aspects of the host pathogen in interaction, pathogenesis. A variety of techniques are available to study the diversity of the pathogen and molecular methods are nowadays used. Recent study showed differentiation and variability of strain of *Xac* based on DNA based techniques and complete cell protein profiling method[3].

A variety of techniques are available to determine variability within species such as random amplified polymorphic DNA (RAPD).[4] restriction fragment length polymorphism (RFLP)[5] and repetitive-sequence PCR (rep-PCR),[6] Amplified fragment length polymorphism (AFLP)[7]. RAPD is used to reveal DNA-based polymorphism through PCR with random primers[8]. These random primers can amplify randomly distributed loci in the genome of any organism. RAPD has been used for differentiation of variation within pathovar in Iranian *Xanthomonas* strains.[9] The RAPD pattern was able to distinguish the minute difference within the same species and showed a significant level of genetic diversity. RAPD could be used for precise and reliable analysis of genetic variability in *Xanthomonas* strains. RAPD offer fast, sensitive, and reliable for determining genetic relationships

* To whom all correspondence should be addressed.
Mob.:+91-9422170641
E-mail: mmvb@indiatimes.com

among *Xanthomonas* isolates[10]. Therefore, in this study the analysis of genomic DNA by RAPD was carried out.

The present study is based on biochemical characterization, pathogenicity test and DNA based technique. Here apart from biochemical test, RAPD analysis was used to distinguish and differentiate among 24 strains of *Xac* isolated from Marathwada region in Maharashtra.

MATERIALS AND METHODS

Bacterial strains

Two hundreds strains of *Xac* isolated from *Citrus lemona* from different location within Marathwada region of Maharashtra and 24 strains representing all the 8 districts were used in this study. The source and geographical origin of strains are given in Table 1. All the strains were isolated from infected plant parts collected from tree during mid rainy season following standard method. All these strains were purified and maintained on YDC (Yeast extract -1gm, D-glucose -2gm, Calcium carbonate -2gm) slants.

Morphological characteristics

Morphological characteristics were recorded for all these strains include colony characters like Gram staining, Cell morphology, Cell motility [11].

Biochemical test

Bacteriological characteristics of the isolated strains were examined by using the biochemical test described by Goszczynska et al [12] , Aesculin test, Starch hydrolysis, Tween 80 lipolysis, H_2S production, Urease production, Milk proteolysis, Gelatin liquefication, Oxidase test, utilization of sugars like galactose, fructose, arabinose, mannose, cellobiose etc. The results of these entire tests were recorded as either positive or negative were shown in Table- I.

Pathogenicity test

All strains were tested for pathogenicity test and all strains were pathogenic to citrus under field condition[13] (Table-2). The bacterial suspension or the inoculants were prepared by using 48hr old cultures grown on nutrient agar. A loopful of the bacterial colony was suspended in LB broth and photometrically adjusted to an optical density corresponding to 1 to 3X 10^7 CFU/ml. Then the healthy plant were grown in laboratory, after growth the plant leaves, petiole and fruits were punctrured at four to five different location with a sterile needle and approximately 10ìl of the bacterial suspension was spread on each wound. Leaves were inoculated either along with the secondary veins or the laminae. Control plants were inoculated in the same way. Plants were checked for symptoms weekly or after 20-25 days after inoculation[14].

Isolation of Genomic DNA

DNA was extracted from bacterial cells following the method described by Ausbel et al[15] with slight modification in the incubation period and amount of chemicals which was used.

Randomly Amplified Polymorphic DNA

Two primers OPA-2 and OPA-20 were used for PCR Amplification using a Applied Biosys Thermal Cycle 9700, and the reaction was carried out in 25ìl volume containing 10mM Tris- HCL (PH 8.3), 0.15mM of each dNTPs, 60ng primer, 0.5U of Taq polymerase and 20ng genomic DNA[9]. RAPD reactions were performed according to Williams et al [8] to estimate the approximate molecular weight of amplification products. The gel was photographed in Geldoc XR system (Biorad) for viewing the banding patterns.

Phylogenetic Relationship

The strains were compared on the basis of presence or absence of RAPD bands in the gel. The presence or absence of band was used as 1 or 0 for preparing binary matrix and used for analysis using UPGMA. The cluster analysis was performed by similarity index method and the dendrogram was generated with the UPGMA to show the similarity coefficient between the genotype check.[16]

RESULTS

The geographical distribution of the pathogen *Xanthomonas axonopodis* pv *citri* is very broad. The isolated strains were collected from the different area of Marathwada like from Nanded, Parbhani, Hingoli, Aurangabad, Beed, Latur, Jalna and Osmanabad of Maharashtra state in India. In the biochemical characteristic, all strains were positive for aesculin hydrolysis, all strains were negative for oxidase test, strain number *Xac*1, *Xac*2, *Xac*4, *Xac*5, *Xac*7, *Xac*8, *Xac*9, *Xac*10, *Xac*11, *Xac*12, *Xac*13, *Xac*14, *Xac*15, *Xac*17, *Xac*18,

Table 1. Biochemical charactes of *Xanthomonas axonopodis* pv *citri* species used for differentiating various strains :

Biochemical Characteristics	*Xac* 1	*Xac* 2	*Xac* 3	*Xac* 4	*Xac* 5	*Xac* 6	*Xac* 7	*Xac* 8	*Xac* 9	*Xac* 10	*Xac* 11	*Xac* 12	*Xac* 13	*Xac* 14	*Xac* 15	*Xac* 16	*Xac* 17	*Xac* 18	*Xac* 19	*Xac* 20	*Xac* 21	*Xac* 22	*Xac* 23	*Xac* 24
Gram nature	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aesculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
OXidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch Hydrolysis	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tween 80 Lipolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin Liquefaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease Production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Milk Hydrolysis	+	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-	-	+	-	+	-	-
H_2S Production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose Utilization	+	-	-	-	-	+	-	+	-	+	+	-	-	+	-	+	-	+	-	+	-	+	-	+
Mannose Utilization	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose Utilization	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+	+	-	+	-	+	+
Trehalose Utilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cellobiose Utilization	-	+	+	+	+	-	+	-	+	+	+	+	+	-	+	-	+	+	+	-	+	-	+	+
Fructose Utilization	+	-	-	-	-	+	-	+	-	+	+	-	-	+	-	+	-	+	-	+	-	+	-	+

+ = Positive test, - = Negative test

*Xac*19, *Xac*20, *Xac*21, *Xac*22, *Xac*23, *Xac*24 were positive for starch hydrolysis test while strain *Xac*3 and *Xac*6 are negative, all strains were negative for Tween 80 lipolysis , all strains were positive for gelatin liquefaction and urease production , strain number *Xac*1, *Xac*6, *Xac*8, *Xac*14, *Xac*16, *Xac*20, *Xac*22 were positive for milk proteolysis while *Xac*2, *Xac*3, *Xac*4, *Xac*5, *Xac*7, *Xac*9, *Xac*10, *Xac*11, *Xac*12, *Xac*13, *Xac*15, *Xac*17, *Xac*18, *Xac*19, *Xac*21, *Xac*23, *Xac*24 were negative. All strains shows positive test for H_2S production, for Arabinose utilization *Xac*1, *Xac*6, *Xac*8, *Xac*10, *Xac*11, *Xac*14, *Xac*16, *Xac*18, *Xac*20, *Xac*22, *Xac*24 were positive, while *Xac*2, *Xac*3, *Xac*4, *Xac*5, *Xac*7, *Xac*9, *Xac*12, *Xac*13, *Xac*15, *Xac*17, *Xac*19, *Xac*21, *Xac*23 were negative. All strains shows mannose utilization positive except *Xac*6. Strain number *Xac*2, *Xac*3, *Xac*4, *Xac*5, *Xac*6, *Xac*7, *Xac*9, *Xac*10, *Xac*11, *Xac*12, *Xac*13, *Xac*15, *Xac*17, *Xac*18, *Xac*19, *Xac*21, *Xac*23, *Xac*24 were positive for galactose utilization while *Xac*1, *Xac*8, *Xac*14, *Xac*16, *Xac*20, *Xac*22 were negative. all strains were positive for Trehalose utilization, strain number *Xac*2, *Xac*3, *Xac*4, *Xac*5, *Xac*7, *Xac*9, *Xac*10, *Xac*11, *Xac*12, *Xac*13, *Xac*15, *Xac*17, *Xac*18, *Xac*19, *Xac*21, *Xac*23, *Xac*24 were Positive for cellobiose utilization, while *Xac*1, *Xac*6, *Xac*8, Xcac14, *Xac* 16, *Xac*20, *Xac*22 were negative & Strain number *Xac*1, *Xac*6, *Xac*8, *Xac*10, *Xac*11, *Xac*14, *Xac*16, *Xac*18, *Xac*20, *Xac*22, *Xac*24 were positive for fructose utilization, while *Xac*2, *Xac*3, *Xac*4, *Xac*5, *Xac*7, *Xac*9, *Xac*12, *Xac*13, *Xac*15, *Xac*17, *Xac*19, *Xac*21, *Xac*23 were negative.

On fruit e.g. healthy citrus, necrosis developed around (i.e.1-2mm) the inoculation wound after 10-15 days. Sometimes the necrotic spots tended to enlarge and some fruit fell prematurely. The inoculated leaf lamina and veins started to develop necrotic areas 7-11 days after inoculation. Some strains are more aggressive in the leaf lamina veins, where large necrotic spots were developed. In control no symptoms were developed.

Two primers were selected to assess the diversity among the 24 *X. axonopodis* pv *citri*. The primer OPA-2 yielded fragments ranging from 200bp to approximately 2kb. In RAPD analysis total seven bands were observed. Band first having molecular wt 1000kb were observed in most of the of *Xanthomonas* strain like *Xac*1, *Xac*2, *Xac*3, *Xac*4, *Xac*5, *Xac*6, *Xac*7, *Xac*10, *Xac*12, *Xac*13, *Xac*16, *Xac*17, *Xac*18, *Xac*22, *Xac*23 while in other it was absent. Band II of molecular wt 900bp observed in *Xac*9, *Xac*12, *Xac*14, *Xac*15, *Xac*20, *Xac*21 while absent in others. Band III of molecular wt 800bp was observed in *Xac*3, *Xac*6, *Xac*7, *Xac*8, *Xac*10, *Xac*13, *Xac*16, *Xac*22 only. Band IV of molecular wt 500bp was observed in *Xac*1, *Xac*7 *Xac*11, *Xac*12, *Xac*14, *Xac*17, *Xac*21, *Xac*22, *Xac*23 while absent in others. Band V of molecular wt 400bp was observed in *Xac*3, *Xac*5, *Xac*9, *Xac*14, *Xac*17, *Xac*22. Band VI of molecular wt 300bp were observed in *Xac*3, *Xac*5, *Xac*8, *Xac*9, *Xac*10, *Xac*12, *Xac*13, *Xac*14, *Xac*21, *Xac*22 while absent in others. Band VII of molecular wt 200kb were observed in *Xac*2, *Xac*3, *Xac*4, *Xac*11, *Xac*13, *Xac*15, *Xac*17, *Xac*18, *Xac*20, *Xac*22, *Xac*23.

The primer OPA-20 yielding fragments again ranging from 200bp-2kb. In RAPD analysis seven bands were observed. Band I of molecular wt 1000kb observed in *Xac*18 and *Xac*22. Band II

Table 2. Strains of *Xanthomonas axonopodis* pv *citri* used in this study

Strains	Location	Virulence	HR	Year
*Xac*1	Latur –I	+	+	2010
*Xac*2	Hingoli –II	++	+	2010
*Xac*3	Osmanabad –I	+	+	2011
*Xac*4	Nanded –I	+++	+	2011
*Xac*5	Latur-III	++	+	2011
*Xac*6	Parbhani –II	+	+	2010
*Xac*7	Jalna –II	+	+	2010
*Xac*8	Beed –II	++	+	2010
*Xac*9	Beed –III	++	+	2010
*Xac*10	Jalna-III	+++	+	2011
*Xac*11	Beed-I	++	+	2010
*Xac*12	Aurangabad-I	+++	+	2010
*Xac*13	Parbhani-III	+	+	2011
*Xac*14	Hingoli –I	++	+	2011
*Xac*15	Nanded-III	+++	+	2010
*Xac*16	Jalna-I	++	+	2011
*Xac*17	Osmanabad–III	+	+	2011
*Xac*18	Nanded-II	++	+	2011
*Xac*19	Aurangabad-II	+++	+	2010
*Xac*20	Parbhani-I	++	+	2011
*Xac*21	Hingoli-III	+	+	2010
*Xac*22	Osmanabad –II	++	+	2011
*Xac*23	Latur –III	+++	+	2011
*Xac*24	Aurangabad-III	++	+	2010

(+ = Mild virulent, ++ = moderate Virulent, +++ = Strong virulent) (HR + = positive)

Table 3. Similary matrix RAPD banding pattern 24 strains of *Xanthomonas axonopodis* pv *citri* species used for differentiating various strains :

	*Xac*1	*Xac2*	*Xac3*	*Xac4*	*Xac5*	*Xac6*	*Xac7*	*Xac8*	*Xac9*	*Xac10*	*Xac11*	*Xac12*	*Xac13*	*Xac14*	*Xac15*	*Xac16*	*Xac17*	*Xac18*	*Xac19*	*Xac20*	*Xac21*	*Xac22*	*Xac23*	*Xac24*
*Xac*1	0	0.833	0.750	0.800	0.800	0.857	0.667	0.857	0.833	0.857	0.600	0.667	0.875	0.889	1.000	0.833	0.714	0.875	1.000	1.000	0.857	0.625	0.500	1.000
Xac2		0	0.778	0.250	0.600	0.714	0.500	1.000	1.000	0.500	0.857	0.714	0.571	0.900	0.600	0.667	0.571	0.333	0.750	0.500	1.000	0.800	0.833	0.800
*Xac*3			0	0.750	0.750	0.500	0.800	0.500	0.625	0.667	0.625	0.800	0.375	0.727	0.889	0.625	0.556	0.700	1.000	0.800	0.800	0.125	0.889	1.000
*Xac*4				0	0.800	0.667	0.667	1.000	1.000	0.667	0.833	0.667	0.500	1.000	0.500	0.600	0.500	0.500	0.667	0.667	1.000	0.778	0.800	0.750
Xac5					0	0.857	0.667	1.000	0.833	0.667	1.000	0.857	0.875	0.750	1.000	0.833	0.714	0.714	1.000	0.857	1.000	0.778	0.800	1.000
Xac6						0	0.571	0.571	0.875	0.333	0.875	0.571	0.167	0.800	0.857	0.200	0.625	0.625	0.800	0.750	0.750	0.556	0.857	0.833
Xac7							0	0.889	1.000	0.333	0.875	0.571	0.625	0.800	0.857	0.500	0.625	0.625	0.800	0.750	0.889	0.700	0.667	0.833
Xac8								0	0.714	0.750	0.714	0.889	0.625	0.800	1.000	0.714	0.900	0.900	1.000	0.889	0.750	0.556	1.000	1.000
Xac9									0	0.875	0.857	0.714	0.889	0.625	0.833	1.000	0.889	1.000	1.000	0.875	0.714	0.667	1.000	1.000
*Xac*10										0	1.000	0.571	0.429	0.800	0.857	0.500	0.778	0.625	0.800	0.750	0.889	0.700	0.857	0.833
Xac11											0	0.875	0.750	0.778	0.833	0.857	0.571	0.750	1.000	0.714	0.714	0.500	0.833	1.000
Xac12												0	0.625	0.667	0.667	0.714	0.625	0.778	0.800	0.750	0.571	0.700	0.667	0.833
Xac13													0	0.818	0.714	0.333	0.500	0.500	0.833	0.625	0.778	0.444	0.875	0.857
Xac14														0	0.889	0.900	0.700	0.818	1.000	0.667	0.500	0.636	0.750	0.875
Xac15															0	0.833	0.714	0.714	0.667	0.400	0.857	0.900	1.000	0.750
Xac16																0	0.571	0.571	0.750	0.714	0.875	0.667	0.833	0.800
Xac17																	0	0.500	0.833	0.625	0.778	0.444	0.714	0.857
Xac18																		0	0.833	0.429	0.778	0.727	0.875	0.857
Xac19																			0	0.800	1.000	1.000	1.000	0.500
Xac20																				0	0.750	0.818	1.000	0.833
Xac21																					0	0.700	0.857	1.000
Xac22																						0	0.778	1.000
Xac23																							0	0.750
Xac24																								0

of molecular wt 900 observed in *Xac*2, *Xac*5, *Xac*7, *Xac*10, *Xac*14, *Xac*18, *Xac*20. Band III of molecular wt 800 observed only in *Xac*8 while in other bands was absent. Band IV of molecular wt 500bp were observed in *Xac*14, *Xac*23, *Xac*24 only and absent in others. Band V of molecular wt 400kb observed in *Xac*3, *Xac*6, *Xac*8, *Xac*11, *Xac*13, *Xac*14,*Xac*16, *Xac*17, *Xac*18, *Xac*20, *Xac*21, *Xac*22 were observed. Band VI of molecular wt 300kb observed in *Xac*1, *Xac*3, *Xac*8, *Xac*9, *Xac*11, *Xac*22, *Xac*23. Band VII of molecular w 200kb were observed in *Xac*2, *Xac*4,*Xac*6,*Xac*7,*Xac*10,*Xac*12,*Xac*13, *Xac*15, *Xac*16, *Xac*17,*Xac*18, *Xac*19, *Xac*20, *Xac*24.

All the test strains were identified as *Xanthomonas* by UPGMA analysis these strains divided into main two cluster, cluster A and cluster B. In cluster A isolates *Xac*1, *Xac*23, *Xac*5, *Xac*3, *Xac*22, *Xac*17, *Xac*11, *Xac*8, *Xac*9, *Xac*14 and *Xac*21 are included. This cluster A again divided into two subgroups, subgroup A_1 and subgroup A_2. Subgroup A_1 contains strain *Xac*1, *Xac*23, *Xac*5 and subgroup A_2 contains strain *Xac*3, *Xac*22, *Xac*17, *Xac*11, *Xac*8, *Xac*9, *Xac*14, *Xac*21. From the dendrogram it is clear that the cluster A is divided into two subgroups namely A_1 and A_2, A_1 isolates belongs to Nanded district, A_2 isolates belongs to Parbhani, Hingoli and Latur district . This indicate that in cluster A those isolates are present they are very close with each other because of the district wise distribution. Cluster B also divided into subgroup B_1, B_2, B_3. Subgroup B_1 contain the isolates *Xac*2, *Xac*4, *Xac*18, *Xac*15, *Xac*20, subgroup B_2 contains the isolates *Xac*6, *Xac*13, *Xac*16, *Xac*7, *Xac*10, *Xac*12 and in subgroup B3 two isolates are coming *Xac*19 and *Xac*24. Cluster B which divided into three subgroups, subgroup B_1 contains those isolates present that belongs to Osmanabad and Beed districts, those present in subgroup B_2 belongs to Jalna and Aurangabad districts and subgroup B_3 containing those isolates belongs to the Beed and Hingoli districts.

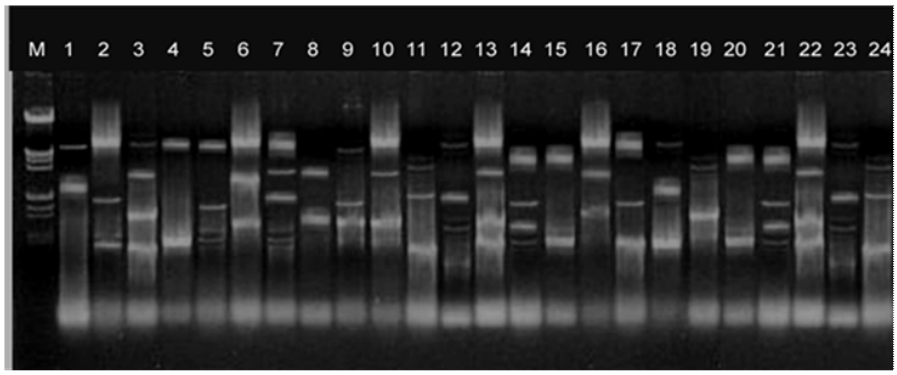

Fig. 1. RAPD banding of *Xanthomonas axonopodis* pv *citri* for differentiating various strains using OPA02

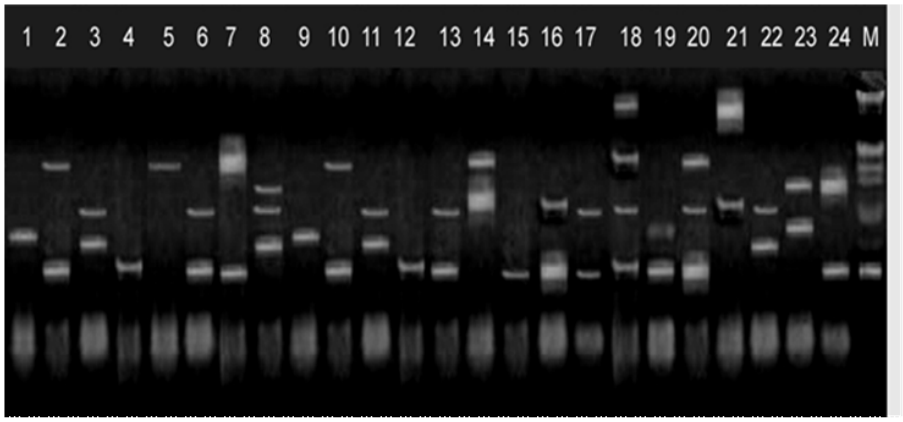

Fig. 2. RAPD banding of *Xanthomonas axonopodis* pv *citri* for differentiating various strains using OPA 20

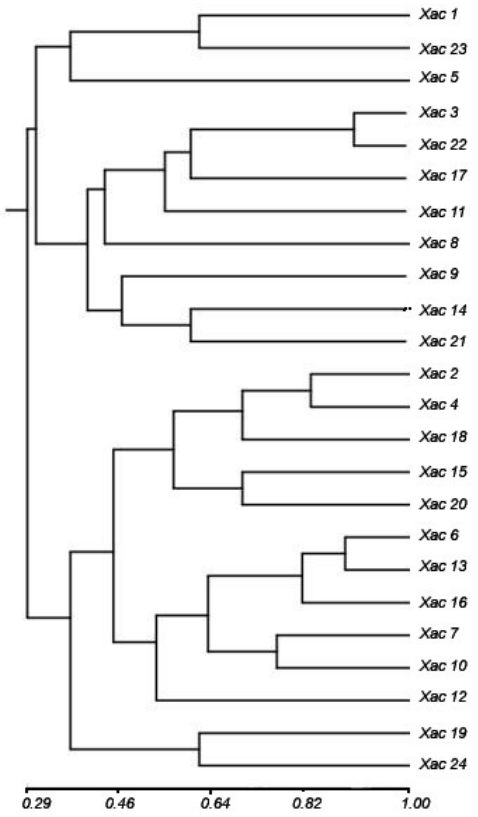

Fig. 3. Dendrogram of *Xanthomonas axonopodis* pv *citri* species generated on RAPD banding by UPGMA

DISCUSSION

A number of morphological and molecular methods have been used for characterization of species of *Xanthomonas.* Genetic studies based on DNA technique have played important role in deriving diversity, relationship, variability within the genus and species of *Xanthomonas*. *Xanthomonas campestris* pv *citri* has been changed to *Xanthomonas axonopodis* pv *citri* owing to insight due to molecular studies[17]. The RAPD results show *Xanthomonas axonopodis* pv *citri* has very small amount of variability within this pathovars. The polymorphism emerged is being results of modification, insertion and deletion of nucleotides at initiation sites. Low genetic diversity of *Xanthomonas axonopodis* pv *citri* from Syria[10] was observed, which is similar to results found in the present study.

The determination of phenotypic characteristics led to the conclusion that all strain identified as *Xanthomonas axonopodis* pv *citri*. The biochemical characterization confirmed the identification consistently. All the strains had similar biochemical properties and varied within these strains. However, these test were not enough capable to demonstrate differences in *Xanthomonas axonopodis* pv *citri* strain from Marathwada[3]. The results of such studies were not similar and the biochemical test based differentiation was limited to three to four test[9].

Disease severity and virulence of these strains could not differentiate within the strains. This may be due to the host range and host reaction to the pathogen[18,19]. In earlier study whole cell protein profiling was used to characterize *Xanthomonas campestris* pv *citri* pathovar from Marathawada region and could substantially differentiate among the isolated speices[3]. The genetic diversity analysis of *Xanthomonas axonopodis* pv *citri* strains using RAPD markers have not been yet studied. The screening of primers is an essential step for reproducible and consistent fingerprints[20]. The genetic similarity among the strains was estimated from the data, from the bands obtained from combination of two RAPD primers. The pairwise matrix (Table-3) of genetic distance was calculated from 24 strains with distinct banding pattern. However, the estimate of substitution of nucleotide per site among the strains are smaller than 0.167 which is considered to be limit to express genetic distance based on RAPD data[21].

In the present study RAPD primer were tested and used which gave consistent and reproducible results. The discriminative power of RAPD was considerably higher than that of biochemical test, virulence and disease severity of whole cell protein profiling[9]. The presently used primer could resolve genetic diversity and geographical distribution in the study of *Xanthomonas axonopodis* pv *citri* and RAPD marker gave reproducible and reliable results.

REFERENCES

1. Anonymous. The Agriculture Diary., Maratwada Agriculture University Parbhani 2011.
2. Agrios G. N.. Plant Pathology, Publication VI, Elsevier Academic, Press. New York 2009.
3. Suryawanshi A. R., More S.M. and. Baig M.M.V. Molecular characterization of *Xanthomonas citrii* pathovars from Marathwada

region. *J Pure Appl Microbio*. 2011; **5**(2): 807-812.

4. Pooler MR, Ritchie DF, Hartung JS.Genetic relationships among strains of *Xanthomonas fragariae* based on random amplified polymorphic DNA PCR, repetitive extragenic palindromic PCR, and enterobacterial repetitive intergenic consensus PCR data and generation multiplexed PCR primers useful for the identification of these phyopathogen. *Appl Environ. Microbial*. 1996; **62**: 3121-3127.
5. Roberts, P.D., N.C. Hodge, H. Bouzar, J.B. Jones, R.E. Stall, R.D. Berger and A.R. Chase.. Relatedness of strains of *xanthomonas fragariae* by restriction fragment length polymorphism, DNA–DNA reassociation, and fatty acid analyses. *Appl. Environ. Microbiol.* 1998; **64**: 3961–3965.
6. Gilling MR, Fahy PC, Bradley J. Identification of *X. fragariae* the cause of an outbreak of angular leaf spot on strawberry in south Australia, and comparison with the cause of previous outbreaks in new south wales and New Zealand. *Australas.Plant Path*., 1998; **27**: 97-103.
7. Lauri M., Wladimir P. S. , Andrea B. M., Claire T.V., AFLP analysis of *X. axonopodis* and *x. arboricola* strains used in xanthan production studies reveal high levels of polymorphism. *Brazilian Journal of Microbiology.* 2010; **41**: 741-748
8. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphism amplified by arbitary primers are useful as genetic markers. *Nucleic Acid Res*., 1990; **8**: 6531-6535.
9. Razaei M.K., Shams-Bakesh M.,Alizadeh A.,.Genetic diversity among *Xanthomonas citri* subsp.*citri* strains in IRAN. *Journal of plant protection research.* 2012; 52(1):
10. Abdo-Hasan, M.,H. Khalil, B. Debis and N. Mir Ali.. Molecular Characterization of Syrian races of *xanthomonas axonopodis* pv. *malvacearum. J. Plant Pathol*, 2008; **90**(3): 431-439.
11. Bradbury J.F.. Genus I. *Xanthomonas* Dowson.In Bergey's Manual of Systematic Bacteriology, 1984; **1**: 63-90. Edited by N. R. Krieg & J. G. Holt.Baltimore: Williams & Wilkins.
12. Goszczynska T., Serfontein J.J. & Serfontein S**.**.Introduction to practical phytobacteriology. 2000; SAFRINET SDC Switzerland.
13. Louw F.J., Bell J., Medinamora C.M., Smart C.D., Opgenorth D., Ishimara C.A. , Hausbeck F. J. de Bruijn and Fulbright C.A**.**. Rep-PCR mediated Genomic fingerprinting; A rapid and effective method to identify *Clavibacter michiganesis*, *Phytopathology* 1998; **88**: 862-868.
14. Scortichini, Marchesi M.U. and Prospero P.D.I. Genetic diversity of *Xanthomonas arboricola pv.juglandis* (synonyms ;*X.campestris* pv.*Juglandis;X. Juglandis* pv *Juglandis*) strain from different geographical areas shown by repetitive polymerase chain reaction genomic fingerprinting. *J.Phytopathology*. 2001; **149**: 325-332.
15. Ausubel, F.M.; Brent, R.; Kingston, R.E.; Moore, D.D.; Seidman, J.G.; Smith, J.A.; Struhl, K. Current protocols in molecular biology. New York: Greene Publishing Association; Wiley-Interscience, 1992 ; v.1.
16**.** Tanveer K., Apastambh A.R., More D.R. and Baig M.M.V. Diversity of plant growth promoting *Pseudomonads* from Banana rhizosphere. *J Pure Appl Microbio*. 2010; **4**(2): 551-556.
17. Vauterin L., Hoste B.,Kersters K., Swings J.,. Reclassification of *Xanthomonas*. *International J Syst Bacter.* 1995; **45**: 472-489.
18. Vernier C., Hartung J.S., Pruvost O.P., Civerolo E.L.,Alvarez A.M.,Maestri P., Luisetti J.,. Characterization of phenotypically distinct strains of *Xanthomonas axonopodis* pv *citri* from Southwest Asia. *Eur.J.Plant Pathol*. 1998; **104**(5): 477-487.
19. Al-Saadi A., Reddy J.D. , Duan Y.P, Bruning A.M., Yuan Q., Gabrial D. W.,. All five host range variants of *Xanthomonas citri* carry one *pth*A homolog with 17.5 repeats that determines pathogenicity on citrus, but not host range variation. *Mol. Plant Microbe Interaction* 2007; **20**(8):934-943.
20. Trebol G., Manceau C., Tirilly Y., Boury S.Assesement of the genetic diversity among strains of *X. cynarae* by randomly amplified polymorphic DNA analysis and development of specific characterized amplified regions for the rapid identification of *X. cynarae. Appl. Environ. Microbiol*. 2001; **67**(8): 3379-3384.
21. Leite, R. P.,. Egel, JR., D. S and. Stall, R. E. Genetic Analysis of hrp-Related DNA Sequences of *Xanthomonas campestris* strains Causing Diseases of Citrus. *Appl and Enviro Microbio*, 1994, **60**(4): 1078-1086.