Pathological, Bio-chemical and Molecular diversity amongst the isolates of *Xanthomonas axonopodis* pv. c*itri* causing Citrus canker in acid lime from different agro-climatic region of India

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Abstract— *In present investigation isolates of Xanthomonas axonopodis pv. citri (Xac)causing citrus canker were collected from fourteen agro climatic regions of India. The pathogenic variability of Xac was studied on four different varieties of acid lime viz. Sai sarbati, Phule sarbati, Pramalini and PKM-1 by using detached leaf assay. The isolates showed varied reaction in the symptoms development. The isolates viz. Xac- III, Xac- V, Xac- VII, Xac- XI, Xac- XIII and Xac- XIV found highly virulent and showed of typical symptoms at the point of inoculation within 7 to 9 days. The isolates Xac- I, Xac- II and Xac- IV were found less virulent, developed symptoms after 13 to 16 days of inoculation. The isolates Xac- I and Xac- II failed to develop symptom on variety PKM-1. Further all 15 selected isolates were subjected to biochemical characterization; all isolates were found rod shaped, gram –ve, with colony colour ranging from pale yellow to dark yellow. The isolate were positive for Catalase, KOH and H_2S production, hydrolyse starch and gelatin liquefaction. All isolates produce acid from Trahalose. Whereas all isolates fails to produce Indol. The Random Amplified Polymorphic DNA (RAPD) was used to study the variation amongst the 15 isolates of Xac. A total of 27 RAPD primers were screened. Off which 19 primers showed amplification and produced scorable bands with high degree of polymorphism. A total 220 amplicons were obtained of which 218 amplicons were polymorphic with 99.52% level of polymorphism. The banding profile varied from minimum 5 band types (OPB-1) to maximum 21 band type (REP) indicating the high molecular variability amongst all the fifteen isolates of Xac.The similarity coefficient ranged from 0.27 to 0.68. The maximum genetic similarity was found amongst the isolate from Uttar Pradesh (Xac- V) and Shriganganagar (Xac-XIV) i.e. 0.68*

Keywords— Biochemical test , ERIC, Detached leaf assay, REP, Xanthomonas axonopodis pv. Citri.

I. INTRODUCTION

The Acid lime is an important fruit crop and grown in varying tropical or subtropical regions in the world. It has enormous therapeutic values (Chaudhry *et al.*, 1992). It belongs to family Rutaceae. The worldwide production of acid lime is threatened by a number of biotic and abiotic factors. The citrus canker which is one of the major constraints in cultivation was first reported from Punjab (Luthra and Sattar, 1942). Its occurrence was further recorded in Tamil Nadu (Ramakrishnan, 1954), Andhra Pradesh (Rao,G.P.,1954), Karnataka (Venkatakrishnaiah,1957),Rajasthan (Prasad,1959), Madhya Pradesh (Parsai,1959), Assam(Chowdhury,1951) and Uttar Pradesh (Nirvan, 1960).The bacterium, *Xanthomonas* causes different types of the symptoms ranging from pustules to necrotic lesions consisting of erumpent corky tissue surrounded by water soaked tissues and yellow halo on leaves, stems and fruits, results in defoliation, dieback, premature fruit drop and blemished fruit, which consequently decrease fruit production and market value of the fruits both qualitatively and quantitatively (Zekri *et al.*, 2005; Graham *et al.*,2004; Das, 2003). There are many types in citrus canker disease caused by various pathovars and variants of the bacterium *Xanthomonas axonopodis* (Graham *et al.*, 2004). Recently canker has been detected in kinnow mandarin nursery in Punjab state (Anonymous, 2000). In India, occurrence of strains (pathotypes) of the pathogen has been reported by Rangaswami and Soumini (1957) and Hamlin(1967). Khan and Hingorani (1970) grouped 15 isolates of the pathogens into 3 strains by their reaction on *Murraya exotica*. Kishore

and Chand (1972) studied the reaction of eight isolates on *C. aurantifolia, C. sinensis* and *C. jambhiri* and showed the presence of more than one strain of the pathogens in Harayana. Recently Das (2002) reported the existence of pathogenic variability within the 'A' strain of *X. axonopodis pv. citri*. The molecular variability amongst the *X. axonopodis pv. Citri* can be detected by the Serology (Alvarez *et al.*, 1991), plasmid fingerprints(Pruvost *et al.*, 1992), DNA-DNA homology(Egel *et al.*,1991) and by various RFLP (Restriction Fragment Length Polymorphism) and PCR (Polymerase Chain Reaction) analyses (Miyoshi *et al.*, 1998; Cubero and Graham, 2002). When the DNA-based assays were unavailable, strains of *X. axonopodis* pv. *citri* can be distinguished from other pathovars by infecting a panel of susceptible and resistant citrus hosts or as a bioassay on detached-leaves or leaf-disks (Gottwald *et al.*, 1993). Such pathogenecity test is an essential component in diagnostic programmes for regulation of citrus canker diseases (Schubert *et al.*, 2001). Genetic diversity analyses were performed using two marker systems; Repetitive Polymerase Chain Reaction (Rep-PCR) and Random Amplified Polymorphic DNA (RAPD), (Rezaei, *et al.*, 2012). Hence the present study aimed to understand the Pathogenic and molecular variability amongst the *Xanthomonas axonopodis* pv. *citri* in different agro-climatic regions of India

II. MATERIAL AND METHOD

Collection of symptomatic samples and Isolation of causal agent

The symptomatic samples of Citrus canker were collected from the 14 Agro-climatics region of the India (Table-1). The different plant parts like infected leaves, twigs and fruits were used for isolation of pathogen by tissue isolation method. The isolation of *Xanthomonas axonopodis* pv.*citri* was done on Nutrient Agar (NA) medium.The typical bacterial colonies showing characteristics of *Xanthomonas spp.* were maintained on the slant containing Yeast Extract Glucose Chalk Agar (YGCA) medium and subsequently sub cultured at regular intervals. The fourteen pure bacterial isolates of *Xanthomonas axonopodis* pv.*citri* were inoculated on NA medium.The cultures were incubated at 27 ± 2^0C for 48 hrs. after 48 hrs bacterial suspention of 1 x 10^8 cfu/ml was prepared for each isolate.The pathogenic variation amongst all fifteen isolates of *Xanthomonas axonopodis* pv.*citri;* there reaction were tested by detached leaf method on four different cultivers of acid lime *viz. Sai Sarbati, Phule Sarbati,Pramalini* and *PKM-1* and the isolates were catagerized on the basis of days requried for the development of symptoms.The fully expanded leaves of all four cultivers of acid lime were collected separatly and washed under running water for about 10 min to remove the dirt on the leaves then leaves were soaked in 1% sodium hypochloride for 1 min., after that leaves were washed for 3 times with sterilized distilled water to remove the traches of chemical and leaves were kept for air drying. For –ve control 10µl of sterilized distilled water was placed aseptically onto three leaves of each cultivar at six different sites on each leaf with the help of sterilized syring. For +ve control 10µl of each bacterial suspension was placed onto three leaves of each cultivar at six different sites on each leaf with the help of sterilized syring. Separate syring was used for each isolate. plate incling was wrap and plates were placed at 27 ± 2^0C in a growth cabinate equipped with white light for 12 hrs exposure to white light and 12 hrs for dark. Observations were recorded from 4^{th} day of inoculation upto 25^{th} day of inoculation to record development of symptoms.

Biochemical characterization of *Xanthomonas axonopodis* pv. *citri:*

All the isolates of *X. axonopodis* pv. *citri* were characterized on the basis of their biochemical reactions as per by Aneja (2003).The different biochemical tests performed *viz.* Gram staining, KOH test, Starch hydrolysis, Gelatin liquefaction, H_2S Production, Indol Production, Acid and gas production, Catalase test.

Molecular differentiation among the different isolates of *Xanthomonas axonopodis* pv. *citri.*

The Random amplified polymorphic DNA (RAPD) analysis was used to detect the variations among the different isolates of *Xanthomnas axonopodis* pv. *citri.* The DNA obtained after extraction was confirmed by running it on 0.8% agarose gel containing ethidium bromide @ 0.5 mg/ml in a horizontal gel electrophoresis system. Genomic DNA (2 µl) of each isolate + 3µl loading dye + 5µl sterile water loaded in each well. After completion of 5 cm run, the gel was observed under UV light and the DNA yield and quality was confirmed. The master mix for each primer was prepared by dissolving PCR Reaction 10X without $Mgcl_2$ 2.0 µl, $MgCl_2$ 25 Mm 2.0 µl , DNTPs10 mM each 1 µl Taq DNA Polymerase 5 unit μl^{-1} 0.50 µl, Primer 10 uM 1.0 µl, DNA (10ng)10ng/ µl 1.0 µl, Sterile milli Q water 12.50 µl to make the final volum 20 µl.The PCR was performed in Thermo cycler (Applied Biosystem) using a programme for the RAPD primer. The master mix was distributed to PCR tubes and later 10 ng of template DNA of each isolate was added separately to each of the master mix tube. Final volume was made upto 20 µl.The PCR programme consist

of initial denaturation at 94^0C for 5 min for one cycle, fallowed by denaturation at 94^0C for 1 min.Anneling at 37^0C for 1 min and extension at 72^0C for 2 min for a total of 40 cycle,with the final elongation at 75^0C for 5 min and retension of PCR-RAPD product at 4^0C. The PCR-RAPD product analysis was carried out in horizontal gel electrophoresis. The PCR products were separated electrophoretically in 1.5% agarose gel using 1X TBE buffer. The gel was stained with ethidium bromide (Sambrook *et al.*2001). The gel was run for 2 hrs. at 80v. After the run, the gel was removed carefully from the unit and observed under Gel Doc instrument to visualize the amplification.The amplified profiles for all the primers were compared with each other and bands of DNA fragments were scored as '1' for presence and '0' for absence, generating '0' and '1' matrix. Per cent polymorphism was calculated by using the formula.

$$\text{Per cent polymorphism} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

The data was used to generate similarity coefficient using simple matching coefficient based on RAPD bands scoring. The Dice similarity coefficient between each pair of accessions were then used to construct a dendrogram using the Unweighted Pair Group Method with Arithmetic Average (UPGMA).

III. RESULT AND DISCUSSION

Collection of diseased samples

A total of fifteen symptomatic samples of acid lime infected with citrus canker were collected from fourteen agro climatic regions of India as listed in Table -1.The isolation of causal agent was done from various infected plant parts viz. leaf, twig and fruit. The causal agent thus isolated from each location was designated as an 'isolate' *viz.*, *Xac- I, Xac- II, Xac- III, Xac- IV, Xac- V, Xac- VI, Xac- VII, Xac- VIII, Xac- IX, Xac- X, Xac- XI, Xac- XIIA, Xac- XIIB, Xac-XIII* and *Xac- XIV*. Two isolates were taken from 12^{th} agro climatic region of India (West Cost Plane and Ghat Region) so they are designated as *Xac- XIIA* and *Xac- XIIB* (Table 1). Valenchia *et al.* (2004) obtained 123 Isolates of *Xanthomonas axonopodis* pv. *dieffenbachiae* (*Xad*) from Los Banos.Islam *et al.* (2014) who collected 9 disease samples of Citrus canker from different regions of Bangladesh and the isolates were identified based on morphological, cultural and biochemical characteristics. Similarly;

The pathogenic variability among *Xanthomonas axonopodis pv. citri*

The pathogenic variability amongst the fifteen isolates *Xanthomonas axonopodis* pv. *citri*, was studying (Table-2 ,Plate-3)inoculating on four different varieties of acid lime *viz. Sai sharbati, Phule sharbati, Pramalini and PKM-1* by using detached leaf assay technique (Tuite, 1969). All varieties were found susceptible to all the fifteen isolates *Xanthomonas axonopodis* pv. *citri*. The isolates *Xanthomonas axonopodis* pv. *citri* showed varied reaction in the symptoms development. The isolates *viz. Xac- III*, *Xac- V*, *Xac- VII*, *Xac- XI*, *Xac- XIII* and *Xac- XIV* found highly virulent in development of typical symptoms i.e. white crystalline callus formation at the point of inoculation within 7 to 9 days. The isolates *Xac- I, Xac- II* and *Xac- IV* were found less virulent as they developed symptoms after 13 to 16 days of inoculation. The isolates *Xac- I* and *Xac- II* failed to develop symptom on variety *PKM-1*. The categorization of isolates of *Xac* was done on the basis of symptoms development on leaves and days taken for appearance of the symptoms as No canker (-), Weak canker (+), Moderate canker (++) and Strong canker (+++) as presented in Table. 2. Atiq *et al.* (2007) who screened fifteen citrus cultivars for resistance against citrus canker disease incited by *X. axonopodis* pv. *citri* and reported that no immune response was exhibited by any variety in the experiment. Ismail *et al.* (2014) studied the reaction of *Xac* on 5 different host of *Rutaceace* family by detached leaf assay and reported that the pathogen also produced water soaking, followed by necrosis around the wound inoculated surface on grape fruit, Rough lemon followed by Lime.

Biochemical Characterization of *Xanthomonas axonopodis* pv. *citri*

All the fifteen isolates of *Xac* were rod shaped produce typical mucoid colonies with the color variation among all the isolates from pale yellow to dark yellow. They are Gram's positive and showed string formation in KOH test (Table-3). All the fifteen isolates of *Xac* were found positive for hydrolysis of starch in variable degree. The isolates *Xac- 1, Xac- III, Xac- IV, Xac- V, Xac-VII, Xac-IX, Xac-XIV, Xac- XIIB* and *Xac-XIII* showed strong reaction with clear zone around hydrolyzed area when lugol iodine was poured. The isolates *Xac- II, Xac- VI, Xac- VIII, Xac- X, Xac- XI, Xac- XIIA* and *Xac- XIV* showed moderate reaction, showed slow zone formation around the area of hydrolysis when lugol iodine was poured. For the liquefaction of gelatin by fifteen isolates of *Xanthomonas*

axonopodis pv. *citri,* showed variable reaction. The isolates *Xac- V* and *Xac- XIIA* showed strong reaction for gelatin liquefaction as they produced strong proteolytic exo-enzyme due to which gelatin was hydrolyzed. The isolate *Xac- III, Xac- IV ,Xac- VI, Xac- VIII, Xac- IX, Xac- XI, Xac- X, Xac- XIIB* showed moderate reaction for gelatin liquefaction. The isolate *Xac- I, Xac- II, Xac- VII, Xac- XIII* and *Xac- XIV* showed weak reaction for gelatin liquefaction (Table-3). **A**ll the fifteen isolates of *Xac* showed variable reaction for H_2S Production. The isolates *Xac- I*, *Xac-II*, *Xac- III*, *Xac- IV*, *Xac- V*, *Xac- VI*, *Xac- IX*, *Xac- X*, *Xac-XIIA*, *Xac- XIIB*, *Xac- XIII* and *Xac- XIV* were strong H_2S producer, showed black coloration along the line of stab inoculation within 3 to 4 days after inoculation. The isolate *Xac- VII*, *Xac-VIII* and *Xac- XI* were found weak H_2S producer as the black coloration along the line of stab inoculation was formed after 7 days of inoculation. Similarly all isolates produce acid from carbon source Trahalose but they are fails to produce gas from the same carbon source. All isolates were negative for Indol production. All the fifteen isolates of *Xac* were found positive for Catalase test as bubble formation was observed after addition of 3% hydrogen peroxide in the 48 hrs old incubated bacterial culture. All the isolates were rod shaped, Gram negative showed circular pale yellow colonies on nutrient agar as earlier reported by Patel (1950) who observed bacterial colonies of *X .malvacearum* as flat glistering pale yellow on nutrient agar also identified the organism is short rod with rounded ends, Gram negative. The results obtained are in confirmation with those reported by Manjula (2002) who reported that, the bacterium *Xanthomonas axonopodis* pv. *punicae* causing oily spot pomegranate were small rods, appeared singly, Gram negative. Gottwald *et al.* (2002) who reported that *Xanthomonas axonopodis* is a rod shaped Gram negative bacterium.The cultures showed variable reaction among the isolates of *Xanthomonas axonopodis* pv. *citri.* Similar variation among the isolates has been earlier noted by Raut (1990) he studied 15 isolates of *Xanthomonas axonopodis* pv. *mangiferae indicae* for different physiological and biochemical properties viz. H_2S production , action on carbohydrates, gelatin test, KOH test etc. Das (2003) reported that the bacterial cells of *Xanthomonas citri* are positive for hydrolysis of starch, liquefaction of gelatin, catalase. Das (2005) studied different isolates of *Xanthomonas axonopodis* pv. *citri* for different physiological and biochemical properties viz. H2S production, gelatin liquefaction, KOH test, catalase test, acid production from different sugars.Bhardwaj *et al.* (2014) collected 20 isolates of *Xanthomonas axonopodis* pv. *citri* were collected from various regions of Varanasi. Isolates were characterized with the help of morphological, pathogenicity and biochemical analysis. All the isolates showed similar morphological and biochemical characteristic and all were found pathogenic on citrus, thus confirming the identity of isolates as belonging to those of *Xanthomonas axonopodis* pv. *citri.*

Molecular characterization of *Xanthomonas axonopodis* pv. *citri*

Out of 27 primers screened, 19 primers showed amplification and produced scorable bands with high degree of polymorphism. A total 220 amplicons were obtained of which 218 amplicons were polymorphic with 99.52% level of polymorphism was observed (Table-4). The banding profile varied from minimum 5 band types (OPB- 1) to maximum 21 band (REP) indicating the high molecular variability amongst all the fifteen isolates of *Xac*. The amplification profile of selected primer on 1.5% agarose gel were showed in Plate-1and 2. The information on banding pattern of all primers was used to determine genetic distance between the fifteen isolates of *Xac* and the dendrogram was constructed by using Un-weighted Pair Group Arithmetic Mean method (UPGMA). Based on simple matching coefficient a genetic similarity matrix was constructed to access the genetic relatedness amongst the fifteen isolates of *Xac*. The genetic similarity coefficient of fifteen isolates given in Table 5. The similarity coefficient ranged from 0.27 to 0.68 showed high genetic diversity. The maximum genetic similarity was found between the isolate from Uttar Pradesh (*Xac- V*) and Shriganganagar (*Xac- XIV*) *i.e* 0.68 and both isolates in a same cluster B_1. The least similarity was found between the isolates from Uttarakhand (*Xac- I*) and Dhule, Maharashtra (*Xac- IX*) 0.27.The dendrogram (Fig-1) showed that both isolates falls in different cluster *i.e. Xac- I* in cluster A and *Xac- IX* in sub cluster B_2 of cluster B. Further the dendrogram constructed by UPGMA clearly showed the main two clusters *viz.* Cluster A and Cluster B. the Cluster B was again divided into two sub clusters namely Cluster B_1 and Cluster B_2. Two isolates *Xac- I* and *Xac- III* were falls under cluster A as they are more similar to each other and much differs with other isolates of *Xac*. Isolate *Xac- II, Xac- IV, Xac- V, Xac- XIV , Xac- XIII, Xac- XIIA ,Xac- VI, Xac- VII, Xac- XI, Xac- VIII* and *Xac- X* were falls under same cluster in cluster B_1 and remaining two isolates *Xac- IX* and *Xac- XIIB* were falls under same cluster B_2. The dendrogram showed matched results with similarity coefficient values; as the isolates showed high similarity coefficient value falls

under same cluster (*Xac- V* and *Xac- IVX*) and the isolates showed less similarity coefficient values (*Xac- I* and *Xac- IX*) falls in a different cluster (Fig. 1).The size of banding pattern was found variable for each primer shown in Table - 5. The present findings corroborates with the findings of Yenjerappa (2009) who studied molecular variation amongst the 20 isolates of *Xanthomonas axonopodis* pv. *punicae*, by using Random Amplified Polymorphic DNA (RAPD) technique by using primers belonging to OPA, OPB and OPF series. Among the 20 primers used 11 primers exhibited amplification. The primers, OPA- 20, OPB- 03, OPF- 07 and OPF- 10 showed 100 per cent polymorphism that helped to identify the isolates and served as a basis for identification of specific primers. Arshiya *et al.* (2014) studied the rep-PCR fingerprint profiles, obtained with the REP, ERIC and BOX primers confirmed that all 20 isolates of *X. axonopodis* pv *citri.* were amplified with the Rep- PCR by using the universal primers of REP, ERIC and BOX to identify genetic diversity in *Xanthomonas axonopodis* pv *citri*. REP, ERIC and BOX primers sets gave reproducible genomic PCR profiles consisting of approximately 100- 3kb bands. The bands were clearly differentiated by Agarose gel electrophoresis. These profiles were complex and revealed polymorphic bands among *Xanthomonas axonopodis* pv *citri* also the results obtained in the present investigation were in accordance with the results of Rezaei *et al.* (2012) who reported different RAPD marker was to determine the genetic relationship between Iranian strains of *Xcc*. Primers 211,220,230,232 and OPA11 generated different fingerprints among *Xcc* strains. PCR products of these primers ranged from 100 to 7000 bp. Similarly Rouhrazi (2012) reported that four races of *Xcc* were found in northern Iran (1, 4, 5 and 6),to examine the distribution of dispersed repetitive DNA, Enterobacterial Repetitive Intergenic Consensus (ERIC), BOX, Repetitive Extragenic Palindromic (REP) and Random Amplified Polymorphic DNA (RAPD) sequences in the genome of *Xcc* using conserved primers. The different markers produced characteristic banding patterns and the similarity matrices from binary banding data was derived with the similarity for qualitative data program (SIMQUAL).Present findings corroborates with the findings of Lin *et al.* (2008) reported the similarity coefficient of both a typical symptoms-inducing strains XL16 and XL38 was 0.9–1.0 to *Xac*.

The present investigation clearly concludes that there exist the pathological, biochemical and molecular variation amongst the different isolates of *Xanthomonas axonopodis* pv *citri* collected from the different agro-ecological regions of India.

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Table .1:Details of disease samples collected from Agro climatic regions of India

Sr	Agro climatic regions	State	Location	Designation	Plant part used for isolation
1	Western Himalayan Region	Uttarakhand	G.B.Pant University of Agriculture and Technology Pantnager	*Xac- I*	Leaf
2	Eastern Himalayan Region	Meghalaya	Barapani	*Xac- II*	Leaf
3	Lower Gangentic Plane	West Bengal	B.C. K. V. , Nadia	*Xac- III*	Leaf
4	Middle Gangentic Plane	Bihar	Aria	*Xac- IV*	Leaf
5	Upper Gangentic Plane	Uttar Pradesh	BHU, Varanasi	*Xac- V*	Leaf
6	Trans Gangentic Plane	Panjab	Ludhiana	*Xac- VI*	Leaf
7	Eastern Plateau Hill Region	Nagpur	Dahigoan, Parseoni	*Xac- VII*	Fruit
8	Central Plateau Hill Region	Rajasthan	Bikaner	*Xac- VIII*	Leaf
9	Western Plateau Hill Region	Maharashtra	College of Agriculture, Dhule	*Xac- IX*	Fruit
10	Southern Plateau Hill Region	Andhra Pradesh	Anantpur	*Xac- X*	Twig
11	East Cost Plain and Hill Region	Tamil Nadu	Vamban, Pudukkottai	*Xac- XI*	Leaf
12	West Cost Plane and Ghat Region	Maharashtra	Walawa, Sangali	*Xac- XIIA*	Leaf
13	West Cost Plane and Ghat Region	Maharashtra	MPKV, Rahuri	*Xac- XIIB*	Fruit
14	Gujarat Plane and Hill Region	Gujrat	Anand Agriculture University, Anand	*Xac- XIII*	Fruit
15	Western Dry land	Rajasthan	Shriganga nagar	*Xac- XIV*	Leaf

Table.2: Pathogenicity reaction of isolates Xac on different cultivars of acid lime (By Detached Leaf Assay)

Sr.	Isolates	Location	Reaction on leaves of different cultivars after 20 days of inoculation			
			Sai sarbati	***Phule Sharbati***	***Pramalini***	***PKM- 1***

			Days to initiation of symptoms	**Symptoms**	**Days to initiation of symptoms**	**Symptoms**	**Days to initiation of symptoms**	**Symptoms**	**Days to initiation of symptoms**	**Symptoms**
1	*Xac*-I	Pantnagar, Uttarakhand	16	+	16	+	15	+	-	-
2	*Xac* -II	Barapani, Meghalaya	14	++	15	+	15	+	-	-
3	*Xac* -III	West Bengal	7	+++	8	+++	8	+++	7	+++
4	*Xac* -IV	Aria, Bihar	13	++	12	+++	13	++	13	++
5	*Xac* -V	BHU, Varanasi, U.P.	9	++	8	++	9	++	8	++
6	*Xac* -VI	Ludhiyana, Panjab	12	++	11	++	11	+++	13	++
7	*Xac* -VII	Nagpur, Maharashtra	7	+++	7	+++	8	+++	9	+++
8	*Xac* -VIII	Bikaner, Rajasthan	10	++	11	++	9	+++	10	++
9	*Xac* -IX	Dhule, Maharashtra	12	+++	12	++	13	+++	12	++
10	*Xac* -X	Anantpur, A.P.	12	++	13	++	11	++	12	++
11	*Xac* -XI	Vamban, Tamilnadu	7	+++	8	++	7	+++	8	+++
12	*Xac* -XII A	Sangali, Maharashtra	10	++	11	++	13	+	11	++
13	*Xac* -XII B	MPKV, Rahuri, Maharashtra	10	++	12	++	11	++	10	++
14	*Xac*- XIII	AAU, Anand, Gujarat	8	+++	7	+++	7	+++	7	+++
15	*Xac* -XIV	Shriganganagar, Rajasthan	8	++	9	++	7	+++	8	++

No canker (-), Weak canker (+), Moderate canker (++) Strong canker (+++)

Table.3:The biochemical reaction of the fifteen isolates of Xac

Parameters		***Xac- I***	***Xac-II***	***Xac-III***	***Xac-IV***	***Xac- V***	***Xac-VI***	***Xac- VII***	***Xac-VIII***	***Xac-IX***	***Xac- X***	***Xac-XI***	***Xac-XIIA***	***Xac-XIIB***	***Xac- XIII***	***Xac-XIV***
Shape		Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Colony colour		Pale yellow	Pale yellow	yellow	Pale yellow	Dark yellow	yellow	Dark yellow	Dark yellow	Pale yellow	Yellow	Dark Yellow	Yellow	Yellow	Dark Yellow	Dark Yellow
Gram reaction		-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Catalase		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Indole production		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KOH		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
H_2S Production		+++	+++	+++	+++	+++	+++	+	+	+++	+++	+	+++	+++	+++	+++
Acid and gas production	**Trahalose**	+++	+++	+++	++	+	+++	++	++	+++	+++	++	+++	+++	+++	+++
Hydrolysis	**Gelatine**	+	+	++	++	+++	++	+	++	++	++	++	+++	++	+	+
	Starch	+++	++	+++	+++	+++	++	+++	++	+++	++	++	++	+++	+++	++

\- : Negative Reaction
\+ : Weak Reaction
\+\+ : Moderate Reaction
\+\+\+ : Strong Reaction

Table.4 : Per cent polymorphism observed in RAPD, BOX ,ERIC 1 REP primers

Sr. No.	Primer Name	Total Number of Bands	Polymorphic Bands	Monomorphic Bands	Per cent Polymorphism	Maximum band size (bp)	Minimum band size (bp)
1	OPA-1	15	15	0	100%	7227	364
2	OPA- 2	10	10	0	100%	2777	236
3	OPA- 3	10	9	1	90%	3070	337
4	OPA- 4	10	10	0	100%	2278	231
5	OPA- 7	11	11	0	100%	6401	263
6	OPA- 9	12	12	0	100%	5994	444
7	OPA-10	8	8	0	100%	3792	236
8	OPA-11	12	12	0	100%	3361	396
9	OPB- 1	5	5	0	100%	4476	426
10	OPB- 4	7	7	0	100%	3539	390
11	OPB- 5	10	10	0	100%	4367	236
12	OPB- 6	7	7	0	100%	2943	677
13	OPB- 7	11	11	0	100%	5247	436
14	OPF- 2	13	13	0	100%	3060	335
15	OPF- 4	14	14	0	100%	2900	271
16	OPF- 6	11	11	0	100%	3709	113
17	BOX	17	16	1	94.11%	3999	109
18	ERIC 1	18	18	0	100%	2785	375
19	REP	21	21	0	100%	4221	122
Total No. of Bands		**220**	**218**	**2**	**99.52% (Avg.)**	**-**	**-**

Table 5- Similarity coefficient for RAPD analysis

	Xac-I	*Xac-II*	*Xac-III*	*Xac-IV*	*Xac-V*	*Xac-VI*	*Xac-VII*	*Xac-VIII*	*Xac-IX*	*Xac-X*	*Xac-XI*	*Xac-XIIA*	*Xac-XIIB*	*Xac-XIII*	*Xac-XIV*
Xac-I	1														
Xac-II	0.396	1.000													
Xac-III	0.418	0.363	1.000												
Xac-IV	0.455	0.547	0.521	1.000											
Xac-V	0.301	0.598	0.533	0.535	1.000										
Xac-VI	0.478	0.470	0.461	0.532	0.578	1.000									
Xac-VII	0.514	0.545	0.369	0.365	0.482	0.531	1.000								
Xac-VIII	0.318	0.513	0.252	0.389	0.500	0.500	0.442	1.000							
Xac-IX	0.271	0.464	0.329	0.403	0.361	0.339	0.370	0.300	1.000						
Xac-X	0.325	0.454	0.314	0.347	0.400	0.341	0.487	0.522	0.474	1.000					
Xac-XI	0.375	0.403	0.410	0.386	0.440	0.446	0.585	0.417	0.37	0.464	1.000				
Xac-XIIA	0.447	0.516	0.360	0.480	0.588	0.488	0.436	0.496	0.378	0.360	0.365	1.000			
Xac-XIIB	0.387	0.380	0.368	0.436	0.440	0.392	0.421	0.320	0.434	0.485	0.429	0.376	1.000		
Xac-XIII	0.22	0.46	0.311	0.431	0.641	0.491	0.450	0.464	0.387	0.422	0.460	0.536	0.500	1.00	
Xac-XIV	0.333	0.592	0.406	0.446	0.683	0.590	0.567	0.619	0.392	0.536	0.519	0.540	0.423	0.596	1

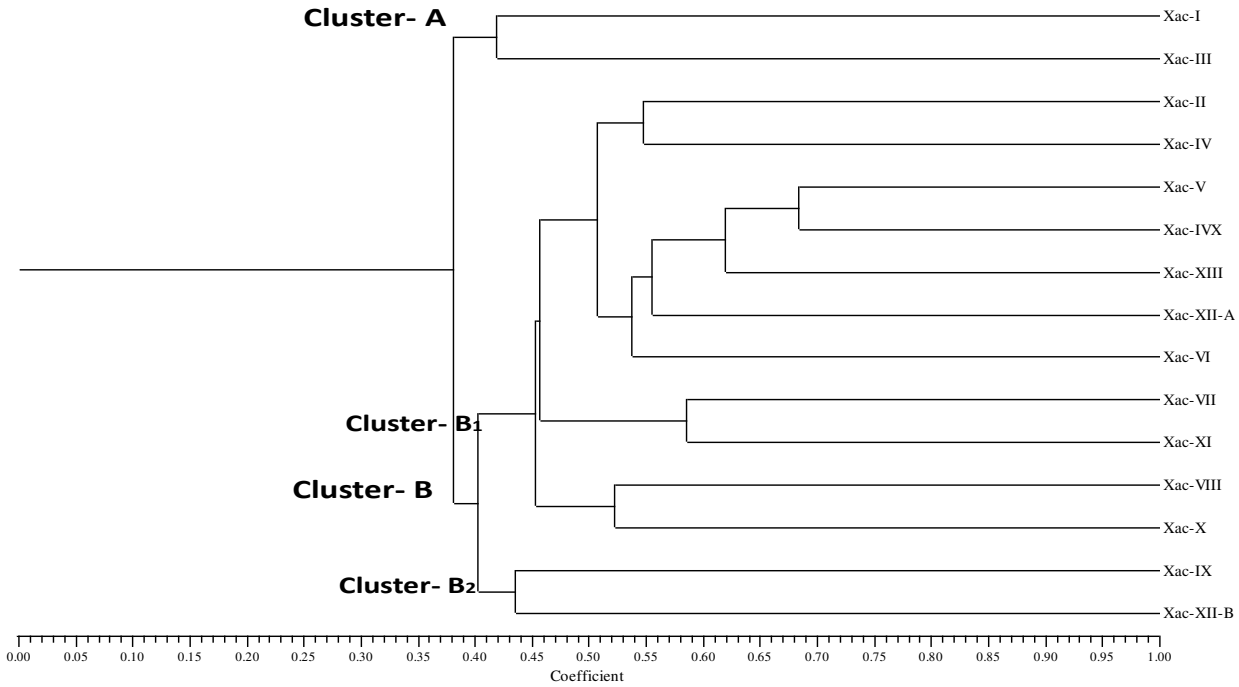


Fig.1:The RAPD UPGMA dendrogram of fifteen isolates of Xanthomonas axonopodis pv. citri based on Jaccards similarity coeffient

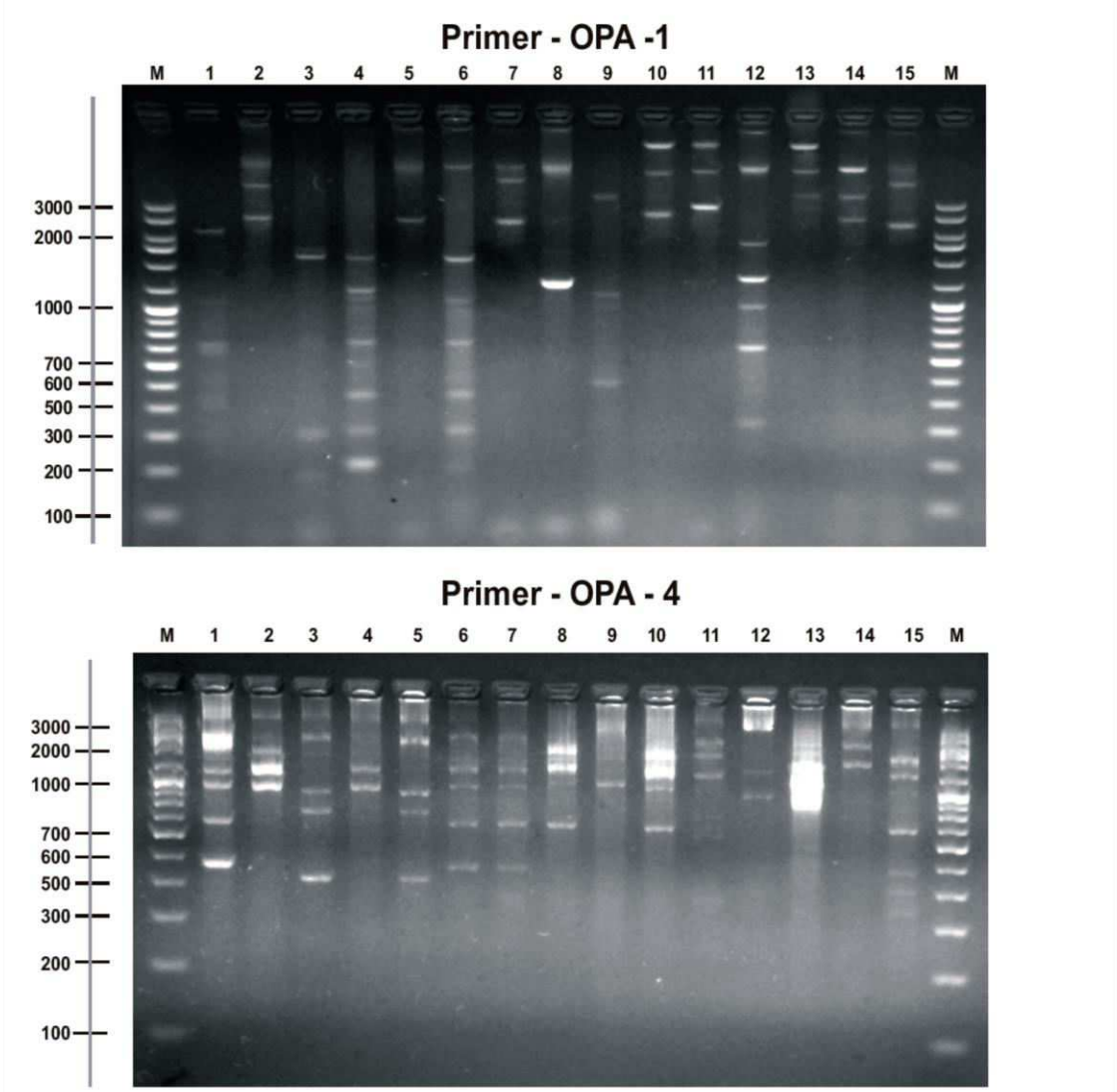
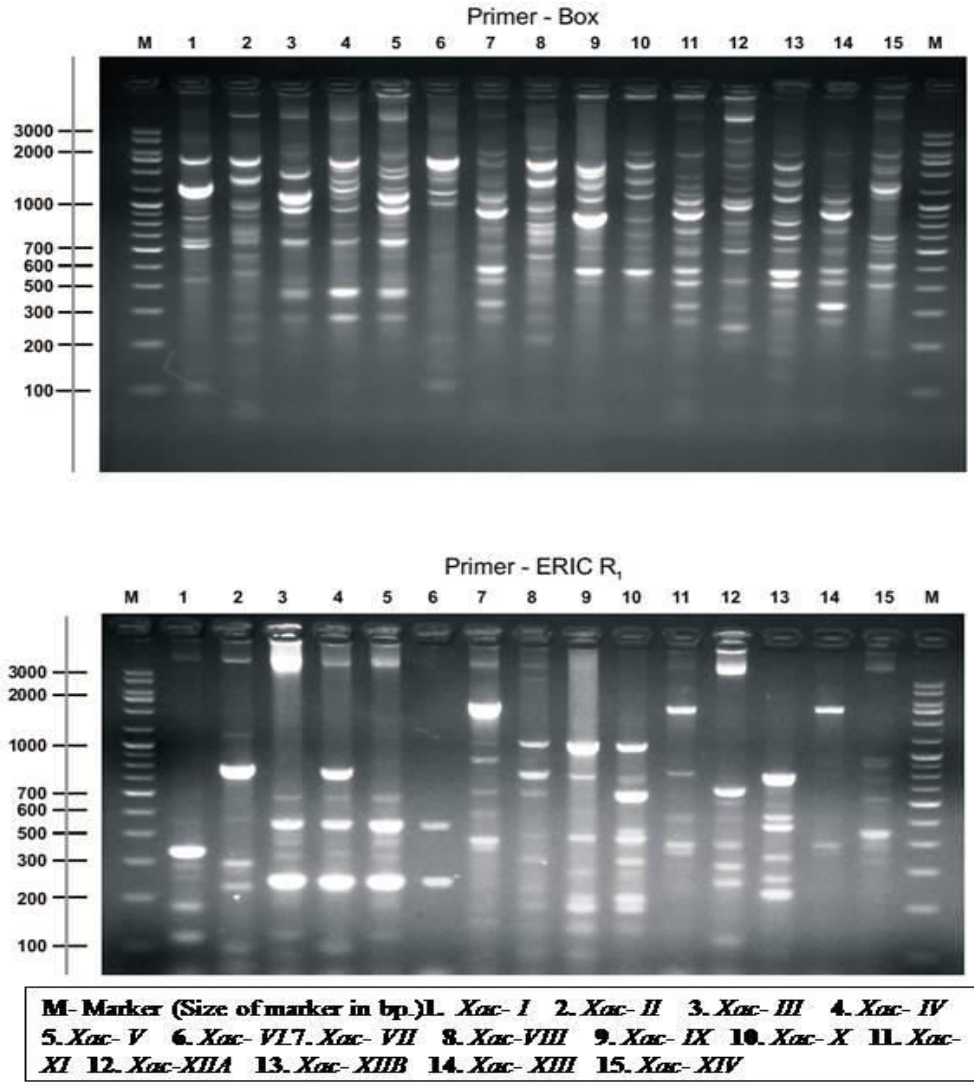


Plate 11 – RAPD banding pattern of primer OPA-1 and OPA-4.

M- Marker (Size of marker in bp.)**1.** *Xac- I* **2.** *Xac- II* **3.** *Xac- III* **4.** *Xac- IV* **5.** *Xac- V* **6.** *Xac- VI* **7.** *Xac- VII* **8.** *Xac- VIII* **9.** *Xac- IX* **10.** *Xac- X* **11.** *Xac- XI* **12.** *Xac- XIIA* **13.** *Xac- XIIB* **14.** *Xac- XIII* **15.** *Xac- XIV*

Plate-1 : The RAPD banding pattern of primer OPA-1 and OPA-4 against 15 isolates of *Xanthomonas axonopodis* pv. *citri*



M- Marker (Size of marker in bp.)1. *Xac- I* **2.** *Xac- II* **3.** *Xac- III* **4.** *Xac- IV* **5.** *Xac- V* **6.** *Xac- VI* **7.** *Xac- VII* **8.** *Xac-VIII* **9.** *Xac- IX* **10.** *Xac- X* **11.** *Xac- XI* **12.** *Xac-XIIA* **13.** *Xac- XIIB* **14.** *Xac- XIII* **15.** *Xac- XIV*

Plate-2: The banding pattern of BOX and ERIC R_1 primer against 15 isolates of *Xanthomonas axonopodis* pv. *citri*

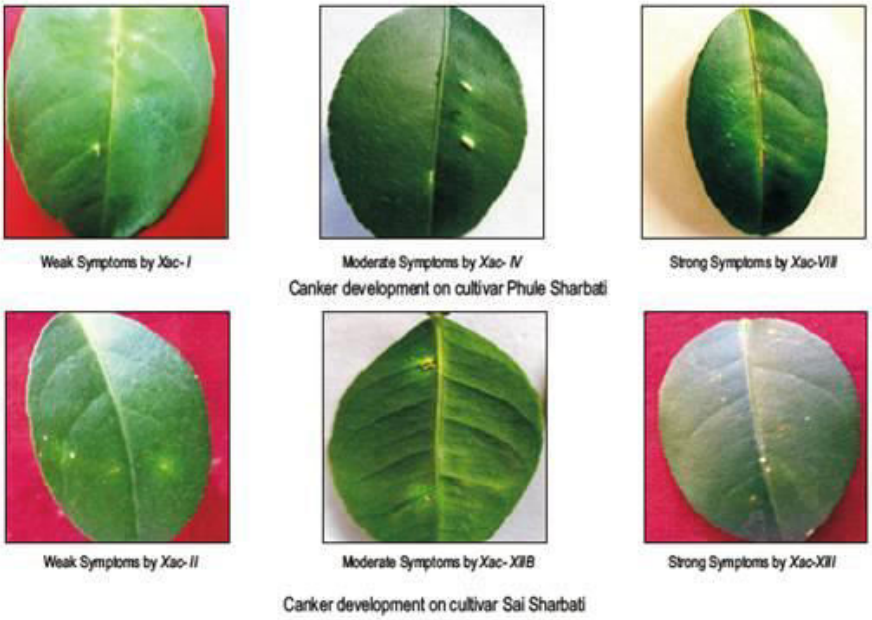


Plate-3: Variation in symptoms development on Phule and Sai Sharbati by Detached leaf Assay

Plate –4 : Pathogenicity on the cultivar Sai Sarbati by Attached Leaf Assay