International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(2): 62-70

Received: 21-01-2020
Accepted: 25-02-2020

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Morphological, biochemical and molecular characterization of *Xanthomonas citri* subsp. *citri* isolates from different agroclimatic zones of Maharashtra

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DOI: https://doi.org/10.22271/chemi.2020.v8.i2a.8857

Abstract
Citrus is one of the important tropical and subtropical fruits in the world as well as in India which belongs to the family *Rutaceae* and sub-family *Aurantioedae*. Among all factors responsible for decline in citrus production, citrus canker is one of the most serious problem which is caused by *Xanthomonas citri* subsp. *citri*. *Xanthomonas citri* subsp. *citri* was successfully isolated on the nutrient agar medium, from the fresh lesions on the leaves of naturally canker infected acid lime leaves collected from different agroclimatic zones of Maharashtra. The fourteen isolates were designated from *Xcc*-1 to *Xcc*-14 representing the agroclimatic zone. The studies on physiological characteristics of *Xanthomonas citri* subsp. *citri*. Showed the maximum growth of pathogen observe at temperature 30^0C and at pH level 7. All isolates of bacteria were found negative test for gram's staining while positive for starch hydrolysis, potassium hydroxide test, catalase test, hydrogen sulphide production, gelatin liquefaction, acid and gas, indole production and nitrate reduction. The genetic variability was studied by using five ISSR and RAPD primers. Among ISSR primers Isolate *Xcc*-11 (Nagpur) had higher value of similarity coefficient (0.85) with *Xcc*-10 (Wardha), whereas *Xcc*-12 (Gadchiroli), had lower value of similarity coefficient (0.42) with *Xcc*-1 (Sindhudurg). The result of RAPD primers reveal that Isolate *Xcc*-11 (Nagpur) had higher value of similarity coefficient (0.81) with *Xcc*-10 (Wardha), whereas *Xcc*-2 (Dapoli), had lower value of similarity coefficient (0.41) with *Xcc*-13 (Gondia).

Keywords: Morphological, biochemical, molecular characterization, *Xanthomonas citri*

1. Introduction
Citrus fruit has been cultivated all over world since ancient time. It grown in the areas with tropical or subtropical climates. It is used as best source of vitamin C, sugars, amino acids and other nutrients. It is one of the important fruit crops of the world. It occupies an important place in the wealth and economy of India as third largest fruit industry after mango and banana. India ranks 6th position in the production of citrus fruit cultivation in the world (Aslin, 2014) [7]. In India, 10.6 mha area is under citrus cultivation with production of 125.10 lakh Metric Ton (Anonymous 2018). It occupies 14.9 per cent area of the total fruit area and 12.5 per cent of the production with productivity of 10.3 mt/hectare. In Maharashtra, area under acid lime is 2.42 Mha with productivity of 218.40 thousand mt (Anonymous, 2015) [5]. Among the different factors responsible for decline in citrus production, citrus canker is one of the most serious problem which is caused by *Xanthomonas citri* subsp. *citri* (Xac). The disease caused raised necrotic lesions on leaves, twigs and fruits hampering the quality.
The presence of different forms of the bacterium creates many problems in proper detection and management techniques. There are many approaches which allow discrimination of the different forms of CBC causal agent such as physiological, biochemical and serological tests, phage typing, restriction enzyme analysis, total soluble protein profile etc. Also, the genome diversity of bacterial plant pathogens including *Xanthomonas* is widely studied with PCR-based methods to point detection, genotypic characterization, ecological distribution and evolutionary process. The variability present can be detected by various techniques like random amplified polymorphic DNA, inter-simple sequence repeat,

amplified fragment length polymorphism, sequence related amplified polymorphism and simple sequence repeat etc. The RAPD and ISSR primers are more frequently used for detecting the genetic variability present among the bacteria.

Knowledge of pathogen diversity can also assist in developing effective disease management strategies, including host resistance (Adhikari, *et al*., 1999) [2]. Therefore, for management of disease accurate detection and study of variability becomes very important. Keeping this in view, the present investigation was planned for biochemical characterization and study of genetic diversity using RAPD and ISSR primers between isolates of *Xanthomonas citri* subsp. *citri*. Collected from different agroclimatic zones of Maharashtra.

2. Material and Methods

A) Isolation

Fourteen samples of acid lime leaves infected with citrus canker were collected from the nine agroclimatic zones of Maharashtra. Infected samples were surfaced sterilized by dipping in 1% sodium hypochloride solution for 30 sec. and rinsed in sterile distilled water three times. The leaves were cut into small pieces with sterilized scalpel and crushed into drop of sterilized water. A loopful of sap was streak on NA plate with zigzag fashion with same loop (without recharging with sap again and again). The streaked plates were kept in BOD at 28 ^{0}C for 72-96 hours. After incubation, pinhead sized single, light yellow colony from the plate was pick up and streak on NA plate and slant.

Table 1: The isolates of *Xanthomonas citri* subsp. citri representing different agroclimatic zones of Maharashtra.

S. No.	Agroclimatic zone	Location	Code no.
1.	South konkan	Sindhudurg	*Xcc*-1
2.	North konkan	Dapoli	*Xcc*-2
3.	Sub mountain Zone	Kolhapur	*Xcc*-3
4.	Western Maharashtra Plateau	Ahmadnagar	*Xcc*-4
5.	West Maharashtra scarcity zone	Solapur	*Xcc*-5
6.	Central Maharashtra plateau	Akola	*Xcc*-6
		Amaravati	*Xcc*-7
7.	Western ghat zone	Nashik	*Xcc*-8
		Nandurbar	*Xcc*-9
8.	Central Vidarbha	Wardha	*Xcc*-10
		Nagpur	*Xcc-11*
9.	Eastern Vidarbha	Gadchiroli	*Xcc-12*
		Gondia	*Xcc-13*
		Yavatmal	*Xcc-14*

B) Pathogenicity test

Preparation of bacterial culture

The isolates were tested for the pathogenicity reaction on acid lime. The isolates were inoculated on NA medium. The cultures were incubated at 27±2 ^{0}C for 3 to 5 days prior to inoculation. The 48 hrs old culture was used for the inoculation.

Inoculation of bacterial culture

The seedlings of acid lime were used for inoculation of isolate. Inoculation was done by syringe inoculation method. The plants were maintained under humid condition. The observations were recorded on the basis of number of inoculations made and number of spots exhibited diseased symptoms. Uninoculated injured plants treated with sterilized water served as control.

Reisolation

The pathogen was reisolated from the artificially inoculated plant under the aseptic condition. The isolation yielded a yellow color of *Xanthomonas citri* subsp. *citri* on NA medium.

C) Physiological characterization

1. Temperature requirement

The study was conducted to know the optimum temperature requirement for the growth of *Xanthomonas citri* subsp. *citri* using nutrient agar broth as a basal medium. A loop full of 48 hours old bacterial culture was mixed in 50 ml of broth containing in 100 ml flasks. Then inoculated flasks were incubated at different temperature level viz., 5, 10, 15, 20, 25, 30, 35 and 40^{0}c respectively for 72 hours. Observations were recorded for the growth of bacterial colonies in the inoculated flasks kept at specific temperature levels. The growth of isolates were studied turbidometrically after 72 hours using spectrophotometer at 600 nm.

2. pH requirement

Effect of hydrogen ion concentrations of the growth of *Xanthomonas citri* subsp. *citri* was studied by adjusting the pH of the medium (NA broth) to various levels viz., 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 using appropriate phosphate buffer. A loop full of 72 hours old bacterial culture was mixed in 50 ml broth containing in 100 ml flask. Inoculated flasks were incubated at 28±2^{0}c temperature for 72 hours. After the incubation period observations were recorded for the growth of the bacterium in the media having different pH level. The growths of isolates were studied turbidometrically after 72 hours using spectrophotometer at 600 nm.

D) Biochemical characterization

All the isolates of *X. citri* subsp. *citri* will be characterized on the basis of their biochemical reactions as per by Aneja (2003). The different biochemical tests will be performed viz. Gram staining, KOH, acid and gas production, catalase test etc.

E) Molecular characterization

1. Bacterial DNA extraction

The bacterial DNA extraction was done by using the thermo scientific genomic DNA purification kit with slight modification in standard protocol. The DNA was suspended in TE buffer and quality is determined by 1% agarose gel electrophoresis.

3. Molecular variability

A) RAPD primers

The total five RAPD primers synthesized by IDT Technologies, New Delhi, were used for detecting the genetic variability among the fourteen isolates of *Xanthomonas citri* subsp. *citri*. The PCR reaction was carried out with thermal cycler (Bio-Rad T-100). The PCR mixture was consisting of 10x PCR Buffer (with Mgcl2) 2.5 µl. dNTP's 0.5 µl, Primer 2 µl, BSA 0.2 µl, Taq polymerase 0.2 µl, 17.6 µl nuclease free water and 2 µl bacterial genomic DNA making final volume of 25 µl. The PCR program were set for initial denaturation at 94^0C for 5 min for one cycle, followed by denaturation at 94^0C for 1 min. Annealing 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.

B) ISSR primers

The DNA isolated from fourteen bacterial samples was subjected to polymerase chain reaction amplification with 5 random 10-mer RAPD primers (synthesized by IDT technologies, New Delhi, India). Amplification of genomic DNA was carried out in 25 µl reaction mixture containing 2µl genomic DNA as template, 10x PCR buffer (with $Mgcl_2$) 2.5 µl. dNTP,s 0.5 µl, Primer 2 µl, BSA 0.2 µl, Taq polymerase 0.2 µl and 17.6 µl nuclease free water. DNA amplification was performed in a DNA thermal cycler. (T100 Thermal cycler). PCR conditions were set as first cycle of 5 min at 93^0C for template denaturation, followed by 40 cycles of 45 sec at 93^0C, annealing for 45 sec at respective temperature,1 min at 72^0C. An additional cycle of 5 min at 72^0C was used for final primer extension. Amplified products were analyzed by electrophoresis on 1.5 per cent agarose gel.

4. Analysis of the profile of the amplified fragment

Pair-wise genetic similarity coefficient between the fourteen test isolates was estimated by Jaccards similarity coefficient. Clustering was done using the symmetric matrix of similarity coefficient and cluster obtained based on unweighted pair group arithmetic mean (UPGMA) using sequential agglomerative hierarical nested (SAHN) cluster analysis of NTSYS-PC version 2.0 (Rohlf, 1998) [24].

Table 2: List of ISSR primers with their sequence used for molecular variability.

Primer pair	Sequence (5' – 3')	Annealing (^{0}c)
UBC-840	GAG AGA GAG AGAGAG A(CT)T	54.40
UBC-841	GAG AGA GAG AGA GAG A(CT)C	56.60
UBC-846	CÁC ACA CÁCACACÁCA(AG)T	54.40
UBC-888	(GCT) (AGT) (GCT)CÁCACACACACA CA	44.60
UBC-890	(ACT)(AGC) (ACT) GTG TGTGTGTGTGT	61.10

Table 3: List of RAPD primers with their sequence used for molecular variability.

Primer	Sequence (5' – 3')	Annealing (^{0}C)
OPA-1	CAGGCCCTTC	37
OPA-9	GGGTAACGCC	37
OPF-4	GGTGATCAGG	37
OPF-2	GAGGATCCCT	37
OPB-7	GGTGACGCAG	37

5. Results and Discussions

A) Disease samples collected

Xanthomonas citri subsp. *citri* was successfully isolated on the nutrient agar medium, from the fresh lesions on the leaves of naturally canker infected acid lime leaves, These isolates were maintained on NA slants and catalogued as under table 1. The similar tissue isolation method was adopted for the isolation of *Xanthomonas citri* subsp. *citri* by using nutrient aggar medium by Abhang *et al.* (2015) [1] and Katkar *et al.* (2016) [16]. While Jabeen *et al.* (2012) [15] isolated the *Xanthomonas* by using yeast dextrose calcium carbonate agar medium (YDCA).

B) Pathogenicity test

The characteristic symptoms of canker appeared after 15-22 days on acid lime leaves depending upon the isolate (Table 4) (Plate1). The symptoms started with the slightly raised small blister- like lesions within 17 days of inoculation and started turning tan to brown and a water-soaked margin appeared around the leaves surrounded by a yellow halo forming the visible lesions resembling canker symptoms. Pathogenic ability of all different isolates of *Xcc* were confirmed and found that isolate *Xcc*-6, *Xcc*-7 *Xcc*-10, *Xcc*11 and *Xcc*-12 showed highly pathogenic to initiate minute canker lesion and fully developed symptoms after 17- 22 days. While *Xcc*-1, *Xcc*-2, *Xcc*-3, *Xcc*-4, *Xcc*-5, *Xcc*-8, *Xcc*-9 and *Xcc*-13 were found to produce very poor in virulent symptoms. The Katkar *et al.* (2016) [16] and Jabeen *et al.* (2011) also confirmed the bacterium in similar manner as performed in this study.

Table 4: Pathogenic ability of *Xanthomonas citri* subsp. *citri* on acid lime leaves.

S. No.	Isolates	No. of days required for development of symptoms	Reaction
1	*Xcc*-1	17	Weak
2	*Xcc*-2	17	Weak
3	*Xcc*-3	15	Weak
4	*Xcc*-4	18	Weak
5	*Xcc*-5	20	Weak
6	*Xcc*-6	19	Strong
7	*Xcc*-7	17	Strong
8	*Xcc*-8	21	Weak
9	*Xcc*-9	17	Weak
10	*Xcc*-10	19	Strong
11	*Xcc*-11	21	Strong
12	*Xcc*-12	22	Strong
13	*Xcc*-13	18	Weak
14	*Xcc*-14	19	Weak

C) Physiological characterization

1. Effect of temperature regimes on growth of *Xanthomonas citri* subsp. *citri*

The data in Table 5 indicates the effect of various temperature level on the growth of *Xanthomonas citri* subsp. *citri* was recorded by measuring their optical density value. The maximum optical density value of bacterial growth was at 30^0C with an optical density value ranging from 0.79 to 0.93 The next favorable temperature for the growth of bacterium was 25^0C with an OD value range 0.60 to 0.70 followed by 35^0C with an OD value range 0.41 to 0.54. The moderate growth of bacteria was observed at 20^0C, 15^0C, 10^0C. There was the least growth of the bacterium at 5^0C and 40^0C.

Table 5: Effect of temperature regimes on growth of *Xanthomonas citri subsp. Citri*

Isolates	Optical density (72 h) at 600nm							
	Temperature							
	5	10	15	20	25	30	35	40
Xcc-1	0.08	0.16	0.30	0.33	0.60	0.80	0.50	0.18
Xcc-2	0.07	0.18	0.33	0.35	0.64	0.90	0.48	0.17
Xcc-3	0.06	0.19	0.27	0.36	0.62	0.91	0.41	0.13
Xcc-4	0.08	0.20	0.31	0.38	0.63	0.85	0.61	0.12
Xcc-5	0.05	0.24	0.29	0.34	0.60	0.84	0.47	0.17
Xcc-6	0.09	0.21	0.27	0.37	0.64	0.92	0.41	0.17
Xcc-7	0.07	0.18	0.31	0.34	0.62	0.79	0.49	0.19
Xcc-8	0.06	0.20	0.25	0.38	0.65	0.84	0.54	0.20
Xcc-9	0.09	0.15	0.24	0.40	0.69	0.83	0.41	0.14
Xcc-10	0.07	0.14	0.21	0.37	0.70	0.93	0.43	0.18
Xcc-11	0.06	0.12	0.26	0.29	0.62	0.87	0.48	0.19
Xcc-12	0.05	0.18	0.27	0.34	0.64	0.86	0.52	0.12
Xcc-13	0.03	0.19	0.29	0.41	0.67	0.84	0.46	0.13
Xcc-14	0.04	0.15	0.24	0.34	0.68	0.84	0.47	0.14

2. Effect of pH levels on growth of *Xanthomonas citri subsp. citri*

The data presented in Table 6 revealed that maximum growth of the bacterium was recorded at pH level 7.0 with OD value ranging from 0.76 to 0.89 followed by pH 6 with an OD value range between 0.58 to 0.68. The growth of bacteria were moderate at pH 5 and pH 8. The least growth of pathogen was recorded at pH level of 3.0, 4.0 and 9.0. The present results are confined with the findings of Eugenia *et al.* (1995) that the temperature between 25-27^{0}C and pH 6-7.5 were optimum for the growth. Kiran Kumar (2007) also find that, the optimum temperature for the growth of bacterium was found to be 28-32^{0}C and minimum and maximum temperature were 10 and 40^{0}C respectively. The optimum pH required for the growth was 7.0-7.2, while, minimum and maximum pH was 5 and 9 respectively. Similar result were also reported earlier by Hingorani and Mehata (1952) [13], Manjula (2002) [21], Giri (2009) [12] and Yenjerappa (2009) [25].

Table 6: Effect of pH levels on growth of *Xanthomonas citri subsp. citri*

Isolates	Optical density (72 h) at 600nm						
	pH levels						
	3	4	5	6	7	8	9
Xcc-1	0.12	0.25	0.42	0.60	0.82	0.54	0.22
Xcc-2	0.07	0.23	0.41	0.66	0.84	0.56	0.19
Xcc-3	0.14	0.24	0.38	0.66	0.87	0.55	0.19
Xcc-4	0.17	0.22	0.39	0.64	0.85	0.51	0.27
Xcc-5	0.18	0.19	0.37	0.68	0.86	0.59	0.18
Xcc-6	0.14	0.28	0.41	0.61	0.88	0.62	0.21
Xcc-7	0.16	0.27	0.43	0.62	0.78	0.54	0.18
Xcc-8	0.20	0.31	0.46	0.68	0.89	0.64	0.22
Xcc-9	0.13	0.24	0.42	0.63	0.76	0.51	0.19
Xcc-10	0.12	0.26	0.39	0.59	0.79	0.59	0.16
Xcc-11	0.15	0.29	0.37	0.67	0.84	0.63	0.17
Xcc-12	0.17	0.30	0.39	0.61	0.88	0.52	0.20
Xcc-13	0.19	0.28	0.34	0.58	0.81	0.58	0.18
Xcc-14	0.18	0.24	0.32	0.61	0.79	0.52	0.21

D) Biochemical test

The studies on biochemical characteristics of *Xanthomonas citri* subsp. *citri.* Showed their positive reactions for potassium hydroxide solubility test, catalase test, starch hydrolysis, gelatin liquefaction, acid and gas production, H_2S test, nitrate reduction and gives negative response for gram's staining. The results of the biochemical characterization from this study are in agreement with the findings of Bhardwaj *et al.* (2001), Islam *et al.* (2014) [14], Abhang *et al.* (2015) [1], Mubeen *et al.* (2015), Labhasetwar *et al.* (2018) [18], Bhure *et al.* a (2018) [9] and Ali *et al.* (2017) [3] who reported the bacterium *Xanthomonas citri* were positive for starch hydrolysis, KOH test, catalase test, H_2S production, gelatin liquefaction, indole production, acid and gas production and negative for the gram reaction confirming the bacterium as gram negative.

Table 7: Morphological and biochemical characteristics of *Xanthomonas citri* subsp. *citri* isolates

Isolate	S	CC	GR	C	KOH	SH	IN	GL	AG	H2S	NR
Xcc-1	Rod	Yellow	-	+	+	+	+	+	+	+	+
Xcc-2	Rod	Yellow	-	+	+	+	+	+	+	+	+
Xcc-3	Rod	Yellow	-	+	+	+	+	+	+	+	+
Xcc-4	Rod	Pale yellow	-	+	+	+	+	+	+	+	+
Xcc-5	Rod	Pale yellow	-	+	+	+	+	+	+	+	+
Xcc-6	Rod	Yellow	-	+	+	+	+	+	+	+	+
Xcc-7	Rod	Pale yellow	-	+	+	+	+	+	+	+	+
Xcc-8	Rod	Pale yellow	-	+	+	+	+	+	+	+	+
Xcc-9	Rod	Pale yellow	-	+	+	+	+	+	+	+	+
Xcc-10	Rod	Yellow	-	+	+	+	+	+	+	+	+
Xcc-11	Rod	Pale yellow	-	+	+	+	+	+	+	+	+
Xcc-12	Rod	Pale yellow	-	+	+	+	+	+	+	+	+
Xcc-13	Rod	Pale yellow	-	+	+	+	+	+	+	+	+
Xcc-14	Rod	Pale yellow	-	+	+	+	+	+	+	+	+

(S: Shape, CC: Colony color, G: Grams reaction, C: Catalase test, KOH: Koh test, SH: Starch hydrolysis, IN: Indole test, GL: Gelatin hydrolysis, AG: Acid and gas production, H2S: H_2S test, NR: Nitrate reduction test)

E) Molecular variability

ISSR primers

5 selected of ISSR primers screened for amplification of DNA of fourteen isolates of *Xanthomonas citri* subsp *citri*. Three ISSR primers of produced scorable bands with high degree of polymorphism. The Jaccard's similarity coefficient ranged from 0.42 to 0.85. Maximum similarity value of 0.85 was noticed in between Nagpur is (*Xcc*-11) and Wardha isolate (*Xcc*-10). The low similarity coefficient 0.42 noticed in between Gondia *(Xcc*-1) and Sindhudurg *(Xcc*-13) isolates.

On UPGMA based clustering analysis of 14 *Xcc* isolates generated with NTSYSpc 2.0i program. There were 2 major clusters observed in dendrogram, one of the cluster comprising only 2 isolates from Dapoli and Sindhudurg indicating that they were distinct from all other isolates. All other isolates showed minimum genetic variation and found to be present in same cluster.

The similar study was carried out by Raghuvanshi *et al.* (2013) [23] used the ISSR primers for detection of variability and conclude that ISSR based-tree and 2D PCO scatter plot the isolates Deola-Nashik and Sangamner Ahmednagar were closely placed with each other as compared to Pandharpur-Solapur isolate While isolate from Akkalkot-Solapur region was distinct from remaining three isolates.

Madavi *et al.* (2016) asses the genetic diversity among eight *Xanthomonas* isolates using inter simple sequence repeat (ISSR) PCR based techniques. ISSR techniques revealed high degrees of polymorphisms among the studied isolates. In dendrogram the ISSR analysis revealed four major clusters viz. I, II, III, IV of the test isolates. In terms of percentage similarity values, the genomic variation was found to be in the range of from 0.37 to 0.93 across eight isolates indicating high degree of genetic variation.

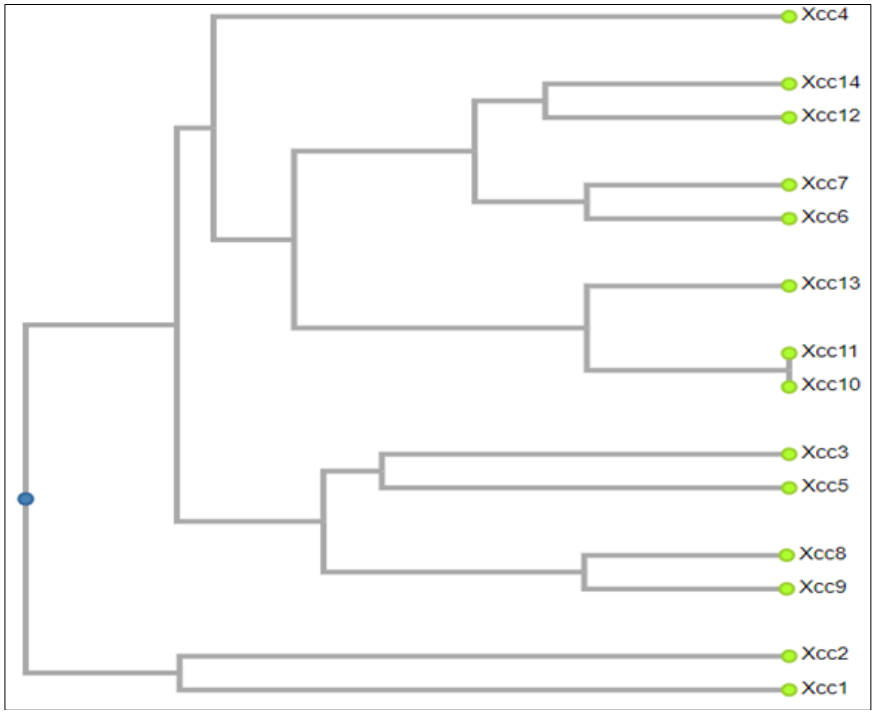


Fig 1: ISSR UPGMA dendrogram of fourteen isolates *Xanthomonas citri* subsp. *citri* based on Jaccard's similarity coefficient.

Table 8: Binary similarity matrix of ISSR analysis.

	***Xcc*1**	***Xcc*2**	***Xcc*3**	***Xcc*4**	***Xcc*5**	**Xa6**	***Xcc*7**	***Xcc*8**	***Xcc*9**	***Xcc*10**	***Xcc*11**	***Xcc*12**	***Xcc*13**	***Xcc*14**
*Xcc*1	1.00	0.80	0.50	0.61	0.61	0.49	0.50	0.60	0.63	0.64	0.51	0.48	0.42	0.52
*Xcc*2		1.00	0.67	0.51	0.58	0.43	0.43	0.56	0.46	0.57	0.49	0.47	0.51	0.59
*Xcc*3			1.00	0.59	0.62	0.59	0.51	0.51	0.54	0.48	0.45	0.49	0.51	0.58
*Xcc*4				1.00	0.64	0.44	0.49	0.50	0.56	0.43	0.51	0.46	0.54	0.57
*Xcc*5					1.00	0.43	0.55	0.61	0.60	0.47	0.49	0.51	0.48	0.61
*Xcc*6						1.00	0.82	0.54	0.57	0.63	0.64	0.56	0.57	0.71
*Xcc*7							1.00	0.57	0.67	0.74	0.81	0.57	0.64	0.69
*Xcc*8								1.00	0.71	0.59	0.51	0.58	0.61	0.51
*Xcc*9									1.00	0.51	0.57	0.49	0.59	0.49
*Xcc*10										1.00	0.85	0.74	0.71	0.74
*Xcc*11											1.00	0.75	0.78	0.79
*Xcc*12												1.00	0.79	0.68
*Xcc*13													1.00	0.67
*Xcc*14														1.00

RAPD primers

Among the five RAPD primers used, three primers OPA-1, OPA-2 and OPB-7 produced scorable bands with high degree of polymorphism while two primers OPF-4 and OPF-2 failed to produce scorable bands. A total of 28 amplicons were obtained with the three primers. All the bands produced were polymorphic with 100 per cent polymorphism. Isolate *Xcc*-11 (Nagpur) had higher value of similarity coefficient (0.81) with *Xcc*-10 (Wardha), whereas there was low similarity coefficient of 0.41 noticed in between Dapoli (*Xcc*-2) and

Gadchiroli (*Xcc*-12) isolate.

On UPGMA based clustering analysis of 14 *Xcc* generated with NTSYSpc 2.0i program. It was observed that three major clusters (A, B and C) were produced. Among these, cluster (A) is divided into two subcluster (A1 and A2), cluster (B) is divided in two subcluster (B1 and B2) while cluster C consist of only two isolates. Cluster A1 comprised of 3 isolates viz. *Xcc*-4, *Xcc*-8 and *Xcc*-9 while another subcluster (A2) contained two isolates *Xcc*-3 and *Xcc*-5. Cluster B was again divided into two sub-clusters representing isolates *Xcc*-6, *Xcc*-7, *Xcc*-10, *Xcc*-11, *Xcc*-12, *Xcc*-13 and *Xcc*-14.The cluster C comprises only two isolates *Xcc*-1 and *Xcc*-2.

The results were consistent with those previously described by Lin *et al.* (2005) [19] which studied 46 isolates of *Xanthomonas citri* from Taiwan. The various primers were used to confirm the identity of organism. The ERIC primers were evaluated for genetic variability analysis which showed the high degree of variability among the isolates. Arshiya *et al.* (2014) [6] which includes the utilization of BOX and ERIC primers for determining the genetic diversity from different regions of Marathwada of Maharashtra. The clusters analysis showed the formation of two major clusters and respective sub-clusters. The minimum genetic similarity coefficient were 0.674 observed between *Xac*-19 and *Xac*-20 whereas the maximum genetic similarity coefficient were 0.902 observed between *Xac*-18 and *Xac*-4. Giri *et al.* (2011) [12] analysed the RAPD profiles which showed a high level of genetic variability among the strains of *X. axonopodis* pv. *punicae*. However, result reflected that the variation exhibited by the strains of *X. axonopodis* pv. *punicae* were independent of geographical location. The results also showed similarity with findings of Gadhe *et al.* (2016) [11] Peerjade *et al.* (2017) [22] and Kharde *et al.* (2018) [17].

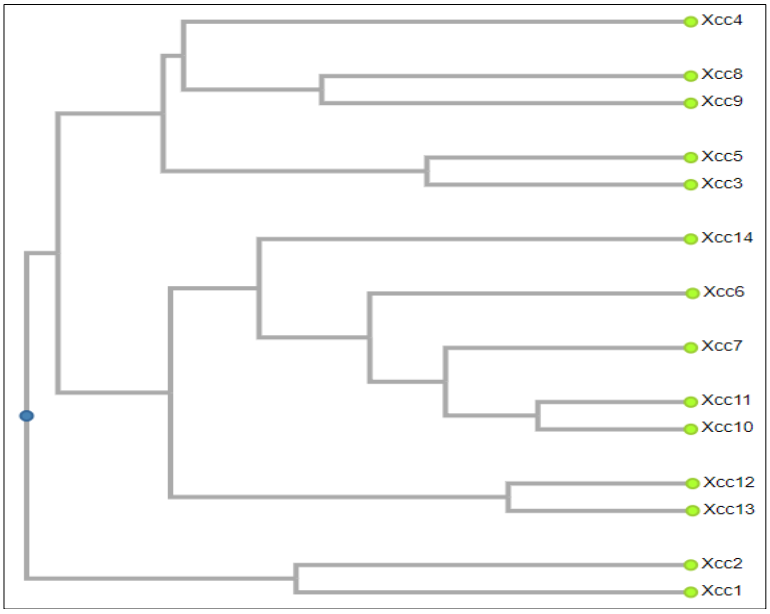


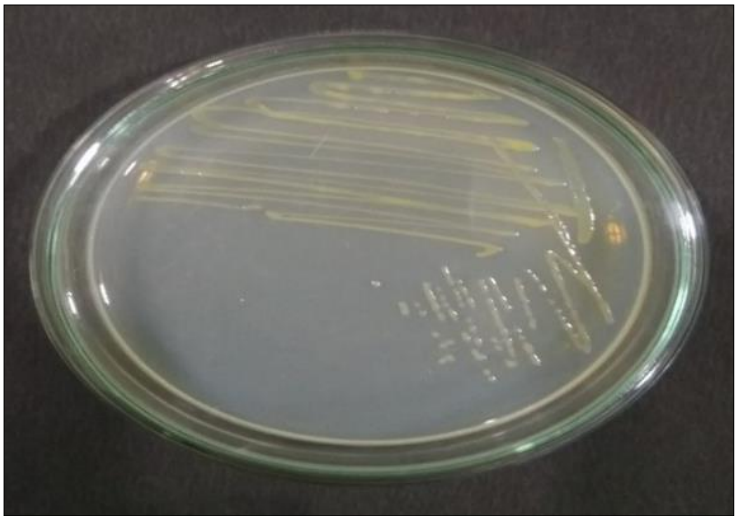
Fig 2: RAPD UPGMA dendrogram of fourteen isolates *Xanthomonas citri* subsp. *citri* based on Jaccard's similarity coefficient

Table 9: Binary similarity matrix of RAPD analysis

	***Xcc*1**	***Xcc*2**	***Xcc*3**	***Xcc*4**	***Xcc*5**	**Xa6**	***Xcc*7**	***Xcc*8**	***Xcc*9**	***Xcc*10**	***Xcc*11**	***Xcc*12**	***Xcc*13**	***Xcc*14**
*Xcc*1	1.00	0.62	0.64	0.49	0.58	0.45	0.49	0.58	0.52	0.49	0.47	0.48	0.48	0.52
*Xcc*2		1.00	0.52	0.48	0.54	0.44	0.51	0.49	0.51	0.51	0.52	0.41	0.43	0.57
*Xcc*3			1.00	0.59	0.71	0.49	0.49	0.59	0.61	0.56	0.49	0.51	0.44	0.51
*Xcc*4				1.00	0.51	0.52	0.47	0.63	0.64	0.61	0.69	0.47	0.51	0.53
*Xcc*5					1.00	0.57	0.62	0.50	0.51	0.48	0.46	0.51	0.51	0.58
*Xcc*6						1.00	0.62	0.51	0.50	0.69	0.71	0.74	0.69	0.62
*Xcc*7							1.00	0.49	0.49	0.74	0.76	0.62	0.67	0.68
*Xcc*8								1.00	0.62	0.49	0.51	0.51	0.49	0.56
*Xcc*9									1.00	0.51	0.56	0.49	0.51	0.43
*Xcc*10										1.00	0.81	0.67	0.68	0.64
*Xcc*11											1.00	0.74	0.71	0.71
*Xcc*12												1.00	0.79	0.61
*Xcc*13													1.00	0.50
*Xcc*14														1.00

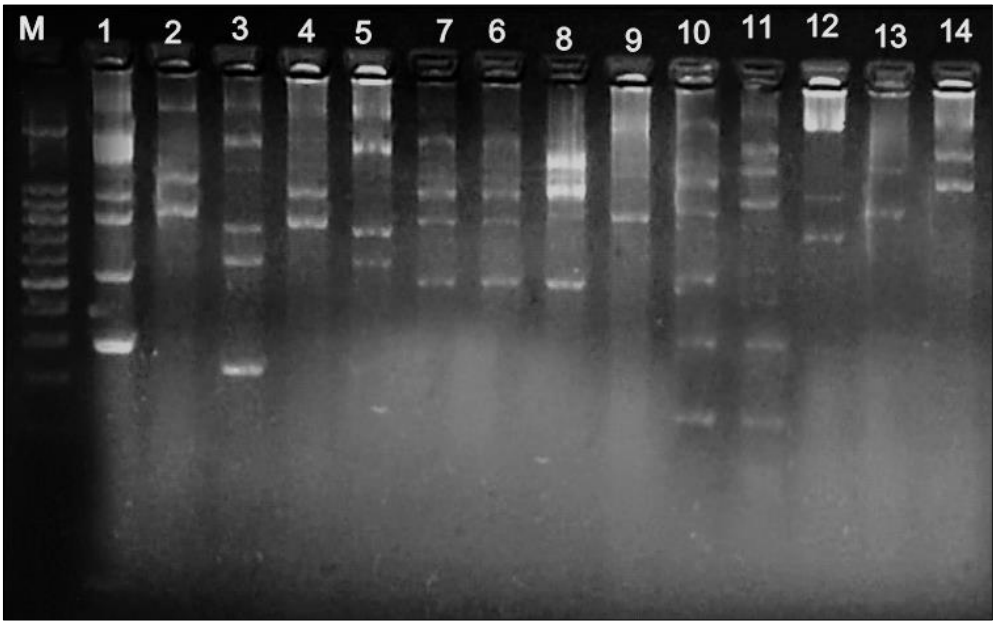
6. Conclusion

The result of the present study concludes that there is variability present among the different isolates of *Xanthomonas citri* subsp. *citri*. The knowledge of physiological, biochemical and molecular variability present among the isolates of different locations and their correlation with virulence of bacterium can be used as tool or basis for designing the suitable management strategies.

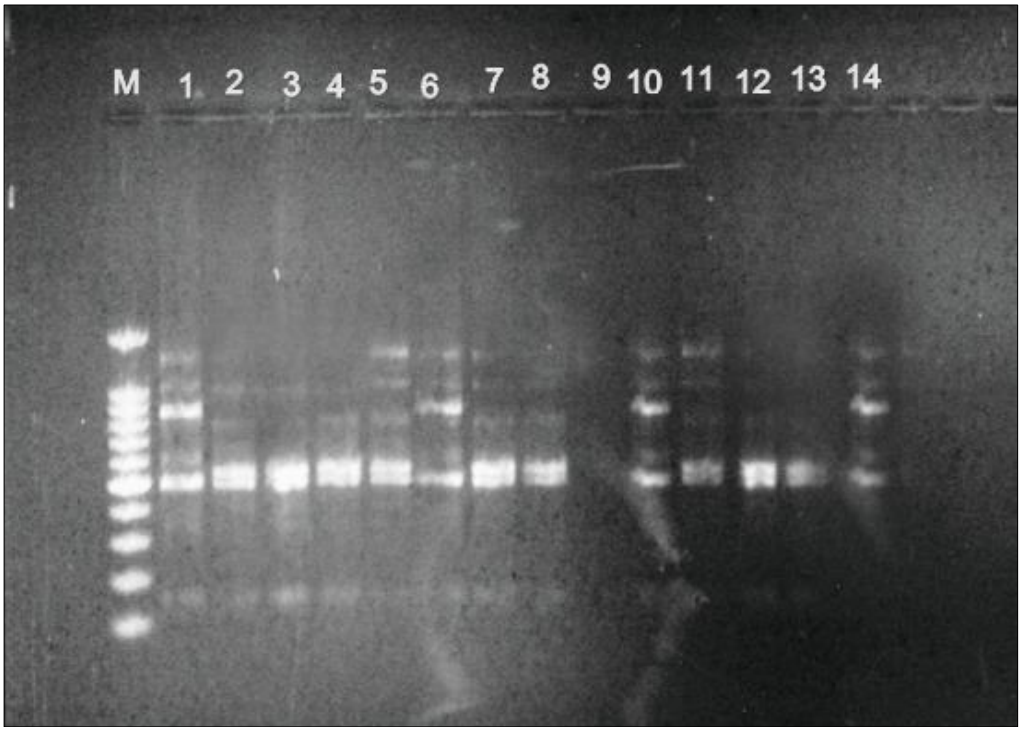


A) Pure culture of *Xanthomonas citri* subsp. *Citri*

Plate 1: Pathogenicity test on acid lime.



A) PCR fingerprinting pattern of genomic DNA of *X. citri* subsp. *citri* primer OPA-1. Lane M, molecular marker Gene Ruler TM 100 bp DNA ladder (Fermentas). Lane 1-14, *Xanthomonas citri* subsp. *citri*.



B) PCR fingerprinting pattern of genomic DNA of of *X. citri* subsp. *citri* primer UBC-846. Lane M, molecular marker GeneRulerTM 100 bp DNA ladder (Fermentas). Lane 1-14, *Xanthomonas citri* subsp. *citri*.

Plate 2: PCR fingerprinting pattern of genomic amplified DNA of *Xanthomonas citri* subsp. *citri* for molecular varibility

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