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Molecular variability among the isolates of *Xanthomonas axonopodis* pv. *citri*. By using ISSR marker

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Abstract
The present investigation was carried for molecular variability of *Xanthomonas axonopodis*pv. *citri*causing citrus canker. Eight isolates of *Xanthomonas* were obtained on NA medium from the canker infected leaves collected from different places.The variability was studied among the eight isolates of *Xanthomonas axonopodis* pv. *citri* by using 7 ISSRprimers *i.e.*ISSR8, ISSR12, ISSR817, ISSR820, ISSR 827, ISSR841, ISSR57101. 7 primers produced 22 scorable bands with an average of 3 bands per primer. Out of 22 bands, 19 bands were polymorphic and level of polymorphism was 68%.The UPGMA analysis showed that Isolate Xac6 (Pune) had higher value of similarity coefficient (0.91) with Xac7(Rahuri), whereas Xac1(Akola),had lower value of similarity coefficient (0.50) with Xac8(Dapoli).

Keywords: primers, *Xanthomonas*, variability

Introduction
Citrus is a member of Rutaceae family and grown in varying areas in countries with tropical or subtropical climates.The most important commercial citrus cultivars in India are the mandarin followed by sweet orange and acid lime.
In India, citrus cultivated commercially acid lime is grown in Khara district of Gujarat, Akola and Amravati districts of Maharashtra and Periyakulam in Tamil Nadu.
Citrus canker is caused by *Xanthomonas axonopodis* pv. *Citri* that is probably the worst enemy to citrus plants (Sahi *et al*., 2007) [5].
The present investigation was undertaken to existence of morphological and molecular variations among *Xanthomonas axonopodis* pv. *Citri.* The ISSR markers are DNA sequences delimited by two inverted SSR composed of the same units which are amplified by a single PCR primer. Inter simple sequence repeats (ISSR) markers involve amplification of the DNA segment between two identical microsatellite repeat regions
In the present investigation, the genetic diversity of the genus *Xanthomonas axonopodis* pv. *citri* was assessed by neutral ISSR markers, using universal primers based on microsatellite motifs.

Materials and Methods
Molecular variability among the isolates of *Xanthomonas axonopodi s*pv. *citri*
The Inter Simple Sequence Repeat (ISSR) analysis was used to detect the variations among the different isolates of Xanthomnas*axonopodis*pv. *citri.*

Results and Discussion
Molecular Variability
ISSR marker selected for molecular study
Seven ISSR primer of ISSR series were selected to evaluate the molecular variability in eight isolates of *Xanthomonas axonopodis*pv.*citri.* The PCR (Polymerases Chain Reaction) amplified product of each primer was resolved on 1.5% agarose gel electrophoresis and the amplified product was compared with DNA molecular weight marker 1 Kb of Biotechnology Grade from Fermentas.
Variation was detected among eight isolates of *Xanthomonas axonopodis*pv.*citri* using ISSR marker.Seven selected primers screened for amplification of DNA of eight isolates of *Xanthomonas axonopodis*pv.*citri.* Seven primers produced scorable bands with high degree of polymorphism.

A total of 22 amplicons were obtained with the 7 primers. Out of 22 bands 19 were found to be polymorphic and 3 were monomorphic and the level ofaverage polymorphism was 68 percent.

Table 1: Percent polymorphism observed in ISSR primers

Sr. no.	Primer	Total Bands	Monomorphic Bands	Polymorphic Bands	% polymorphism
1	ISSR8	1	1	0	00
2	ISSR12	5	1	4	80
3	ISSR817	2	0	2	100
4	ISSR820	1	1	0	00
5	ISSR827	5	0	5	100
6	ISSR841	3	0	3	100
7	ISSR857 101	5	0	5	100
Total		22	03	19	68

ISSR banding pattern

The banding pattern observed in primer ISSR8 is presented in table no. 9 and Plate no. 6.-.The primer amplified 1 amplicons among 8 isolates of *Xanthomonas axonopodis*pv.*citri*. The size of amplicons amplified with primer ISSR8on 985 bp. The details of the 1 bands types of ISSR bands were:

Band type 1: 985 bp Theband observed in Xac1, Xac2, Xac3, Xac4, Xac5, Xa6, Xac7, Xac8

The banding pattern observed in primer ISSR12 arePresented in table no. 9 and Plate 6-. The primer amplified 5 amplicons among 8 isolates of *Xanthomonas axonopodis*pv.*citri* and their size amplified with primer ISSR12 ranged from 277bp to 1995 bp. The details of the 5 bands types of ISSR bands were:

Band type 1: 1995bp The band observed in Xac2, Xac6 and Xac7.
Band type2: 983bpThe band observed in Xac1, Xac3 Xac4, Xac5,Xac6, Xac7 and Xac8.
Band type 3: 896bpTheband observed in Xac1, Xac3, Xac4,Xac5, Xac6, Xac8
Band type 4: 389bpTheband observed in Xac2, Xac3, and Xac7
Band type 5: 277bpTheband observed in Xac1, Xac4, Xac5, Xac7, Xac8

The banding pattern observed in primer ISSR817 is Presented in table no. 9 andPlate 7-. The primer amplified 2 amplicons among 8 isolates of *Xanthomonas axonopodis*pv.*citri* and their sizeamplified with primer ISSR817 ranged from 164bp to 246 bp. The details of the 2 bands types of ISSR bands were:

Band type 1: 246bp The band observed inXac1, Xac6,Xac8.
Band type 2: 164bpThe band observed in Xac1, Xac2,Xac3, Xac4, Xac5, Xac6 and Xac7

The banding pattern observed in primer ISSR820 is Presented in table no. 9 and Plate 8-. The primer amplified 1 amplicons among 8 isolates of *Xanthomonas axonopodis*pv.*citri* and their size of amplified with primer ISSR820 ranged on 657bp. The details of the 1 bands types of ISSR bands were:

Band type:657 The band observed in Xac1, Xac2, Xac3, Xac4, Xac5, Xac6, Xac7, Xac8

The banding pattern observed in primer ISSR827 is Presented in table no. 9 and Plate 7-. The primer amplified 5 amplicons among 8 isolates of *Xanthomonas axonopodis*pv.*citri* and their size of amplified with primer ISSR827 ranged from 157 bp to 970bp.The banding pattern are not observed in isolate Xac4. The details of the 5 bands types of ISSR bands were:

Band type 1: 970b The band observed in Xac8
Band type 2: 615bp The band observed in Xac1, Xac2, Xac3, Xac5, Xac6 and Xac7. Xac8
Band type3: 381bp The band observed in Xac8
Band type 4: 243bpThe band observed in Xac2, Xac3, Xac5
Band type 5:157bp The band observed in Xac5, Xac6

The banding pattern observed in primer ISSR841 is Presented in table no. 9 and Plate 9-. The primer amplified 3 amplicons among 8 isolates of *Xanthomonas axonopodis* pv. *citri* and their size of amplified with primer ISSR841 ranged from 193 bp to 780 bp. However banding pattern not observed in isolate Xac4 and Xac5 The details of the 3 bands types of ISSR bands were:

Band type 1: 780bp The band observed in Xac6, Xac7
Band type 2: 307bp The band observed in Xac1, Xac6 andXac7
Band type3: 193bp The band observed in Xac2, Xac3, Xac6, Xac7, Xac8

The banding pattern observed in primer ISSR857101 is Presented in table no. 9 and Plate 8-. The primer amplified 5 amplicons among 8 isolates of *Xanthomonas axonopodis* pv. *citri* and their size of amplified with primer ISSR857101 ranged from 103 bp to 410 bp. The details of the 5 bands types of ISSR bands were:

Band type 1: 410bpThe band observed in Xac2
Band type 2: 307bpThe band observed in Xac1, Xac2,Xac3, Xac4 and Xac6.
Band type3: 202bpThe band observed in Xac5
Band type4: 161bp, The band observed in Xac7, Xac8
Band type5: 103bpThe band observed in Xac1

Binary similarity matrix of ISSR analysis

A binary similarity matrix of combined data from 7 primers for the eight isolates of *Xanthomonas axonopodis*pv.*citri* were prepared by scoring bands for presence or absence and the DNA bands of same mobility (molecular weight) were assumed to be identical. Genetic similarity estimate (jaccard's coefficient) based on ISSR banding pattern used for cluster analysis to present genetic relationship in the form of dendrogramJaccard's coefficient value for eight isolates of *Xanthomonasaxonoodis* pv. *citri* are presented in Table 2

Table 2: Binary similarity matrix for ISSR analysis

Isolates	Xac1	Xac2	Xac3	Xac4	Xac5	Xac6	Xac7	Xac8
Xac1	1.00							
Xac2	0.64	1.00						
Xac3	0.77	0.86	1.00					
Xac4	0.86	0.68	0.73	1.00				
Xac5	0.77	0.68	0.73	0.82	1.00			
Xac6	0.77	0.77	0.82	0.64	0.64	1.00		
Xac7	0.68	0.77	0.82	0.64	0.64	0.91	1.00	

Xac8	0.59	0.50	0.55	0.55	0.64	0.55	0.55	1.00

In present study, the similarity coefficient value ranged from 0.50 to 0.91 among eight isolates which indicated a high range of genetic diversity. Results on differentiation of isolates revealed that, 7 primers showed the amplification. The highest genetic similarity to an extent of 91% was recorded in Xac6 and Xac7 isolates. Least genetic similarity 50% was observed in Xac2 and Xac8.

The dendrogram of ISSR analysis revealed four major clusters *viz*. I, II, III, IV of the test isolates. The cluster I comprised the isolates Nagpur (Xac1), Amravati (Xac3) Bhandara (Xac5) with about 0.67 similarity coefficient. Whereas The cluster II comprised of Akola (Xac2), Washim (Xac4),cluster III comprised of Pune(Xac6), Rahuri(Xac7) and cluster IV include Dapoli (Xac8) indicating their distinctness from all other isolates under study.

There are numerous DNA based molecular marker systems suitable for genetic diversity assessment. RAPD-, ISSR- and repPCR makers are most popular as prior knowledge about the sequences of genomes is not required, comparatively easy to use and inexpensive. However, RAPD is often criticised for lack of reproducibility. Hence, in the present study ISSR and rep-markers were used.The ISSR technique has widespread acceptance because it is relatively simple, well suitable assay when the nucleotide sequence is unknown.

Nagaoka and Ogihara (1997) [3] reported the ISSR primers produced several times more information than RAPD markers. PCR was carried out with primers that annealed to simple sequence repeats. The resultant products were subjected to agarose-gel electrophoresis, and the banding patterns were compared among six wheat accessions containing diploid,ntetraploid, and hexaploid members. Out of 100 examined, 33 primers produced distinguishable as well as polymorphic bands in each of the six accessions. Although most of the primers that gave distinct bands (30 primers out of 33) contained dinucleotide repeats, each of the primers with tri-, tetra-, and penta-nucleotide motifs also yielded discrete bands. Primers based on (AC)/ repeats gave the most polymorphic bands.

Fatima *et al.* (2012) [1] measured genetic diversity with inter-intra species variation of some indigenous *Xanthomonas*sp. using RAPD and ISSR markers and revealed that seven isolates showed 29 to 100% genetic variation among them.

Madavi *et al* (2017) [2] *studied* the genetic diversity among eight of Xanthomonas isolates using inter simple sequence repeat (ISSR) PCR based techniques and revealed high degrees of polymorphisms among the studied isolates. The genomic variation was found to be in the range from 0.37 to 0.93 across eight isolates indicating high degree of genetic variation.

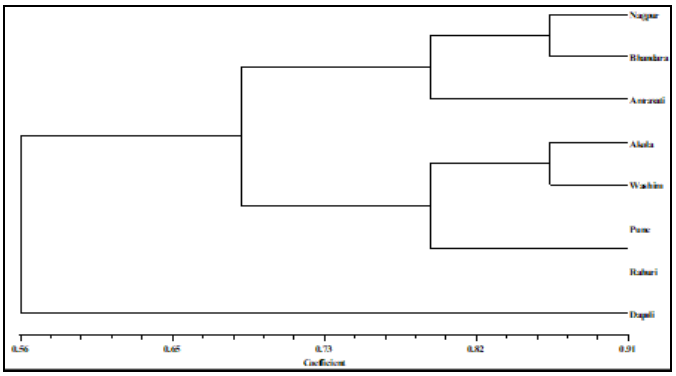

Fig 1: ISSR UPGMA dendrogram of Eight **Isolates of *Xanthomonas axonopodis pv. citri* based on Jaccard's similarity coefficient.**

Present findings are in consequences of Sabin *et al*. (2012) who studied the genetic diversity among seven *Xanthomonas* isolates representing four species using RAPD and ISSR PCR-based techniques. Both techniques revealed high degrees of polymorphisms among the test isolates. A cluster dendrogram based on the combined data of RAPD and ISSR exhibited existence of genetic diversity amonglocal isolates of *Xanthomonas*. The percent similarity values in respect of, the genomic variations were ranged from 29% to 100 percent among the isolates. *X*. *campestris*pv. *Mangiferaeindicae* remained unclustered in cluster dendrogram and revealed a unique genomic profile compared to other isolates tested.

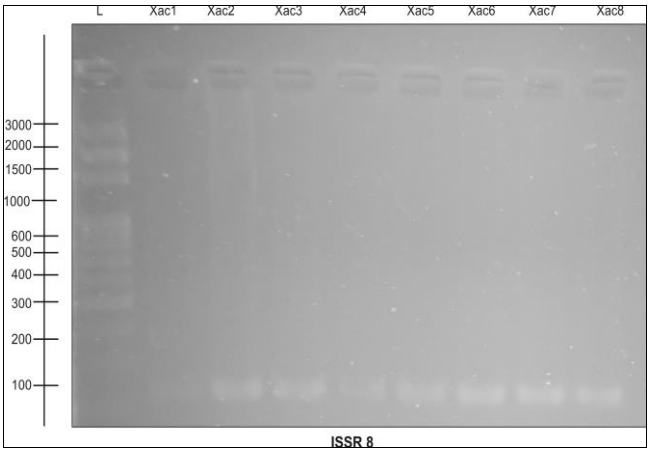

Fig 2: Banding pattern of primer ISSR 8

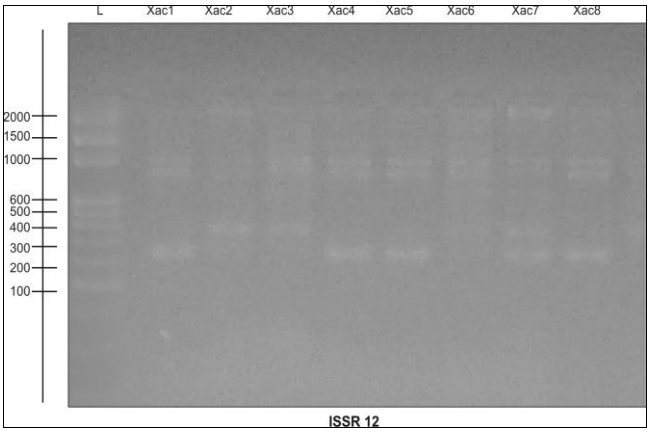

Fig 3: Banding pattern of primer ISSR 12

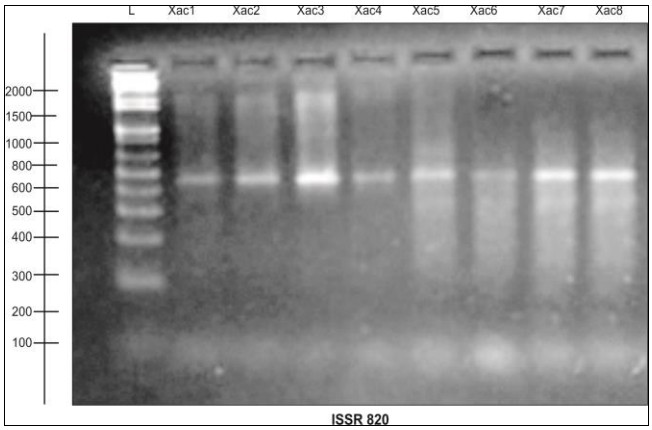

Fig 4: Banding pattern of primer ISSR 820

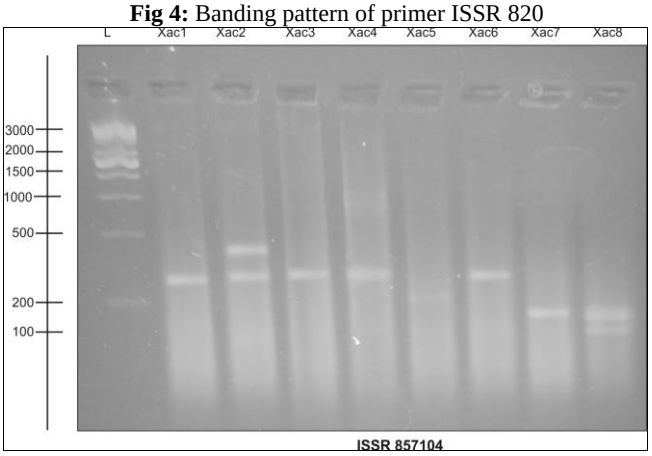

Fig 5: Banding patternof primer ISSR 857104

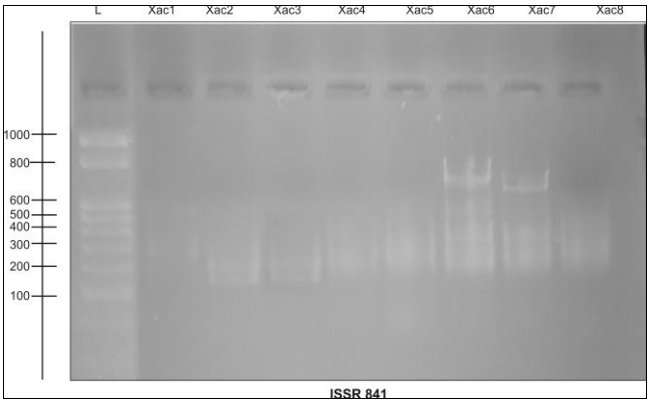

Fig 6: Banding pattern of primers ISSR 841

Conclusions

The variability was studied among the eight isolates of*Xanthomonas axonopodis*pv.*citri* by using 7 ISSR primers *viz* ISSR8, ISSR12, ISSR817, ISSR820, ISSR827, ISSR841, ISSSR857101 which produced 22 scorable bands with an average of 3 bands per primer. Out of 22 bands, 3 bands were monomorphic and 19 bands were polymorphic and level of polymorphism was 68%.Isolate Xac6 had higher value of similarity coefficient (0.91) with Xac7, whereas Xac2,had lower value of similarity coefficient (0.50) with Xac8.

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