

Original Article

***In silico* comparative analysis of 'Candidatus Liberibacter asiaticus' isolates based on the Tandem Repeat Variability in a Genomic Locus (CLIBASIA_01645) of Maharashtra and Assam, India**

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Article Info

Article history:

Received: November 1, 2022

Accepted: November 25, 2022

Published: November 26, 2022

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Abstract

The most harmful disease to citrus is huanglongbing (HLB). In numerous citrus growing regions throughout the world, HLB kills millions of citrus plants. Yellow shoots, stunted development, aberrant fruit colouring, bitter-tasting fruit unsuitable for juice production are some of the signs of HLB disease. This HLB disease has potential to destroy trees completely after showing first symptoms of the disease within few years. *Candidatus Liberibacter* spp. is the disease's causative agent, and psyllids are its primary vectors. However, there are differences in the environment and the vector species used to transmit the disease. The examination regarding TRNs in the "*Ca. L. asiaticus*" locus CLIBASIA 01645 connected with the HLB of citrus in the current study reveals that Maharashtra represents a significantly more diverse range of people than the Assam population of "*Ca. L. asiaticus*." The "*Ca. L. asiaticus*" locus, named CLIBASIA 01645, was shown to be a useful tool for identifying and describing the genetic diversity of the bacterial community.

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1. Introduction:

The most devastating of the several diseases that affect citrus trees is known as citrus greening (Huanglongbing, or HLB). Millions of citrus trees are killed by HLB in numerous citrus growing regions across the world. Yellow shoots, stunted development, irregular fruit coloring, bitter-tasting fruit unfit for juice production are some of the symptoms of HLB disease [1]. This HLB disease has potential to destroy trees

completely after showing first symptoms of the disease within few years.

According to estimates, this disease alone has killed more than 60 million citrus trees globally. First off, the disease was identified in China's Guangdong province in the late 1800s. In this region, it was known as yellow shoot disease, and it was confirmed in India in 1967. According to estimates, this disease alone has killed more than 60 million citrus trees globally. First off, the

disease was identified in China's Guangdong Province in the late 1800s. In this region, it was known as yellow shoot disease, and it was confirmed in India in 1967. From then, practically every citrus-growing region in the world has reported disease, including Taiwan, Japan, Florida (United States), Sao Paulo (Brazil), Jamaica, Indonesia, South Africa, Caribbean, North, and Central American countries.[2].

Candidatus Liberibacter spp. is the disease's causative agent, and psyllids are its primary vectors. However, there are differences in the environment and the vector species used to transmit the disease. '*Ca. L. africanus*' is heat-labile as well as is vectored through *Trioza erytreae* Del Guercio, but '*Ca. L. asiaticus*', '*Ca. L. americanus*', and '*Ca. L. asiaticus*' are heat-tolerant as well as are vectored through *Diaphorina citri* Kuwayama. The most common species on the Indian subcontinent is "*Ca. L. asiaticus*." [3]. It's crucial to have methods for differentiating "*Ca. L. asiaticus*" isolates in order to comprehend the genetic makeup of pathogen populations as well as conduct ecological study together with epidemiological research on them. Originally, the populations of "*Ca. L. asiaticus*" were analysed using conserved genomic loci, like 16S rDNA sequences. The locations of the 16S rRNA gene, a gene encoding an outer membrane protein (omp), and a b-operon in ribosomal protein genes (rplAJ) in "*Ca. L. asiaticus*" were then compared. Sequence analysis, however, showed that these genes only had a small number of variants [4]. These loci (16S rDNA, rplAJ gene, and omp gene) generally have modest levels of genetic diversity, making it challenging to identify bacterial populations from various origins with sufficient clarity. Recently, a distinctive genomic area of "*Ca. L. asiaticus*" demonstrating locus mosaicism was identified, allowing for the division of "*Ca. L. asiaticus*" isolates in China into high and low altitude groups [5]. A similar examination of the diversity of the bacterial populations based on the tandem repeat numbers (TRN) of a few genomic regions was made possible by the release of the 1.23 Mb whole genome sequence of the hazardous "*Ca. L. asiaticus*" strain psy62.

Three main genetic groupings of "*Ca. L. asiaticus*" were widely discovered using microsatellite markers

established by Islam et al. in 2012 [6]. The Florida bacterial group was monopolised by a TRN = 5 genotype (84.5%), whereas the Guangdong bacterial community prevalently contained a TRN = 7 genotype, according to Chen et al. 2010's investigation into the variation analysis of TRN at a genomic locus (CLIBASIA 01645) of "*Ca. L. asiaticus*" strains. However, there are only limited amount of biological and genetic data available on the diversity of bacterial communities among India's many geographic origins. The current study was designed to conduct an *in-silico* comparative analysis of "*Candidatus Liberibacter asiaticus*" isolates predicated on Tandem Repeat Variability (TRN) in a Genomic Locus (CLIBASIA 01645) of Maharashtra and Assam state in India. Given the success in separating "*Ca. L. asiaticus*" populations at locus CLIBASIA 016456, this was done.

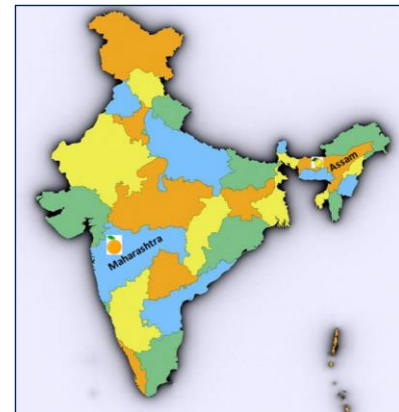


Figure: 1 The Indian map exhibiting the states from where samples taken for the study

2. Materials and Method:

The tandem repeat sequences were retrieved from the NCBI lab (appendix 1; table 1). To ascertain the impact of environment and host on the tandem repeat variation, the sequences were particularly extracted from Maharashtra (Central India) (figure 2; appendix 2) and Assam (North India) (figure 3; appendix 2). All the sequences were extracted from NCBI and the tandem repeat "AGACACA" was calculated using MS word. From each state, fifteen isolates were extracted and screened for tandem repeats.

3. Result and Discussion:

Given the many "environmental circumstances" in its several states, India might potentially sustain a rise in the variety of pathogens there. It is essential for determining the disease risk to understand the genetic

variability regarding "*Ca. L. asiaticus*" through various geographic regions as well as citrus cultivars [7]. Citrus greening has been known to affect all commercialized cultivars in India for many years, but there hasn't been any research on the genetic changes within the "*Ca. L. asiaticus*" bacterial population [8, 9]. *Citrus reticulata*, *Citrus sinensis*, and *Citrus aurantifolia* are the principal crops grown mostly for commercial purposes in Maharashtra.

The productivity of all crops is less as compared to other major citrus cultivating countries including USA, China and Brazil. Citrus greening is an important and most devastating pathogen predominately behind this gloomy scenario [10]. In India, the etiological agent of this disease is "*Candidatus Liberibacter asiaticus*" and which found to be a most devastating [11, 12].

Author contribution: AW: Writing, Concept, Editing, Analysis, VJ,PJ: Verified the content.

Competing interest: Authors declare that no competing interest exists.

Ethical statement: Since the work is in-silico based, no ethical permission required.

Grant Support Details: The authors did not receive funding for this work from any agency.

Acknowledgement: Authors are thankful to Dr. Harishchandra Ramchandra Kulkarni, Principal, Jayawantrao Sawant Commerce and Science College, Hadapsar, Pune, Maharashtra, India to support for publication of this work.

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Cite this article as:

Warghane A, Jaiswal V. In silico comparative analysis of '*Candidatus Liberibacter asiaticus*' isolates based on the Tandem Repeat Variability in a Genomic Locus (CLIBASIA_01645) of Maharashtra and Assam, India. *Int. J. Micro. Sci.* 2022;3(1):54-59.

Appendix 1

Table 1: Tandem repeats of "*Candidatus Liberibacter asiaticus* isolates, location, cultivars, accession numbers and classification

Sr. no	Sample Code	Pathogen	Accession number	Cultivar	State	Country	TRN	Classification
1	'MH-R-CM'	<i>Ca. L. asiaticus</i>	KJ885230	Nagpur mandarin	Maharashtra	India	8	Class II
2	'MH-R-MO'	<i>Ca. L. asiaticus</i>	KJ885231	Mosambi	Maharashtra	India	9	Class II
3	'MH-R-RL'	<i>Ca. L. asiaticus</i>	KJ885232	Rangpur lime	Maharashtra	India	12	Class III
4	'MH-R-AL'	<i>Ca. L. asiaticus</i>	KJ885233	Acid lime	Maharashtra	India	9	Class II
5	'MH-BL3P4'	<i>Ca. L. asiaticus</i>	KJ885234	Mosambi	Maharashtra	India	7	Class II
6	'MH-BL3P7'	<i>Ca. L. asiaticus</i>	KJ885235	Mosambi	Maharashtra	India	13	Class III
7	'MHBL3P21'	<i>Ca. L. asiaticus</i>	KJ885236	Mosambi	Maharashtra	India	17	Class IV
8	'MHBL3P25'	<i>Ca. L. asiaticus</i>	KJ885237	Mosambi	Maharashtra	India	2	Class I
9	'MHBL4P11'	<i>Ca. L. asiaticus</i>	KJ885238	Mosambi	Maharashtra	India	12	Class III
10	'MH-BL5P5'	<i>Ca. L. asiaticus</i>	KJ885239	Mosambi	Maharashtra	India	16	Class IV
11	'IPB-24'	<i>Ca. L. asiaticus</i>	KJ885209	Nagpur Mandarin	Maharashtra	India	9	Class II
12	'IPB-49'	<i>Ca. L. asiaticus</i>	KJ885210	Nagpur Mandarin	Maharashtra	India	9	Class II
13	'IPB-66'	<i>Ca. L. asiaticus</i>	KJ885211	Nagpur Mandarin	Maharashtra	India	9	Class II
14	'IPB-118'	<i>Ca. L. asiaticus</i>	KJ885212	Nagpur Mandarin	Maharashtra	India	5	Class I
15	'IPB-127'	<i>Ca. L. asiaticus</i>	KJ885213	Nagpur Mandarin	Maharashtra	India	8	Class II
16	'KhM-ii'	<i>Ca. L. asiaticus</i>	KJ885191	Khasi mandarin	Assam	India	7	Class II
17	'KhM-iii'	<i>Ca. L. asiaticus</i>	KJ885192	Khasi mandarin	Assam	India	7	Class II
18	'Mo-i'	<i>Ca. L. asiaticus</i>	KJ885193	Mosambi,	Assam	India	8	Class II
19	'AsL-i'	<i>Ca. L. asiaticus</i>	KJ885194	Assam lemon	Assam	India	7	Class II
20	'KhM-i'	<i>Ca. L. asiaticus</i>	KJ885195	Khasi mandarin	Assam	India	9	Class II
21	'KhM-vii'	<i>Ca. L. asiaticus</i>	KJ885196	Khasi mandarin	Assam	India	6	Class II
22	'KhM-vi'	<i>Ca. L. asiaticus</i>	KJ885197	Khasi mandarin	Assam	India	7	Class II
23	'AK2-KhM'	<i>Ca. L. asiaticus</i>	KJ885198	Khasi mandarin	Assam	India	8	Class II
24	'AK3-KhM'	<i>Ca. L. asiaticus</i>	KJ885199	Khasi mandarin	Assam	India	8	Class II
25	'AK4-KhM'	<i>Ca. L. asiaticus</i>	KJ885200	Khasi mandarin	Assam	India	7	Class II
26	'AK5-KhM'	<i>Ca. L. asiaticus</i>	KJ885201	Khasi mandarin	Assam	India	7	Class II
27	'AK7-KhM'	<i>Ca. L. asiaticus</i>	KJ885202	Khasi mandarin	Assam	India	7	Class II
28	'AK8-KhM'	<i>Ca. L. asiaticus</i>	KJ885203	Khasi mandarin	Assam	India	7	Class II
29	'AK9-Mo'	<i>Ca. L. asiaticus</i>	KJ885204	Mosambi	Assam	India	7	Class II
30	'AK10-Mo'	<i>Ca. L. asiaticus</i>	KJ885205	Mosambi	Assam	India	7	Class II

Appendix 2

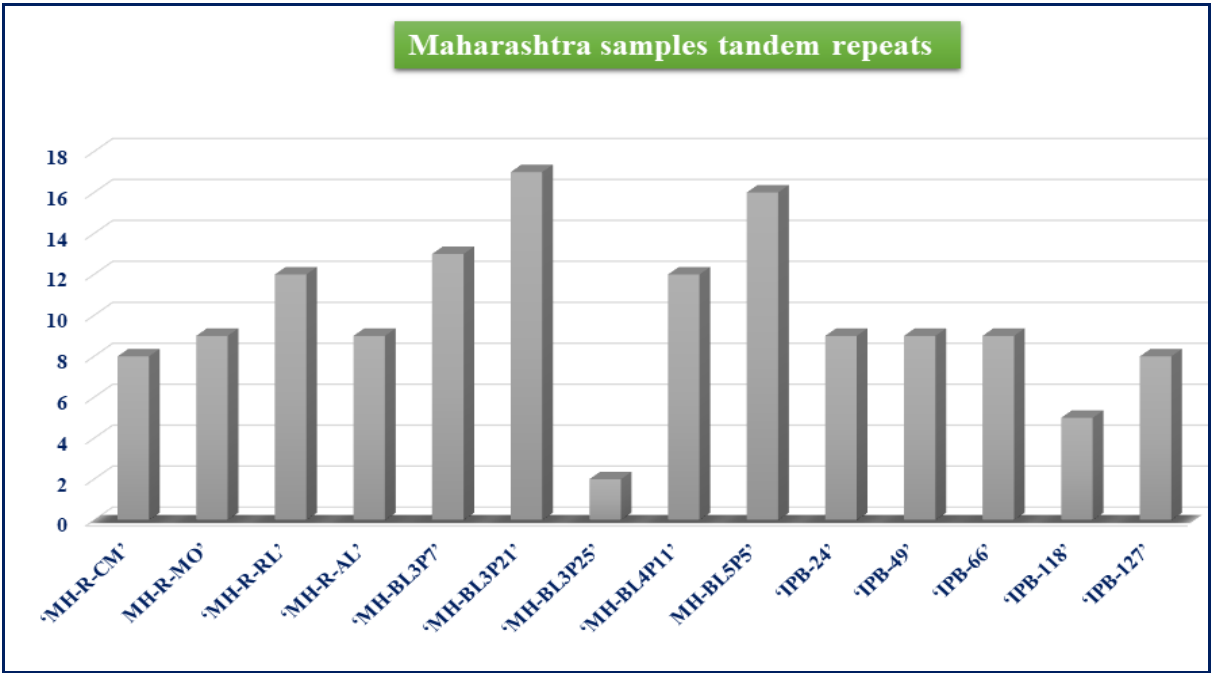


Figure 2: Graphical representation of the tandem repeats observed in Maharashtra state of India.

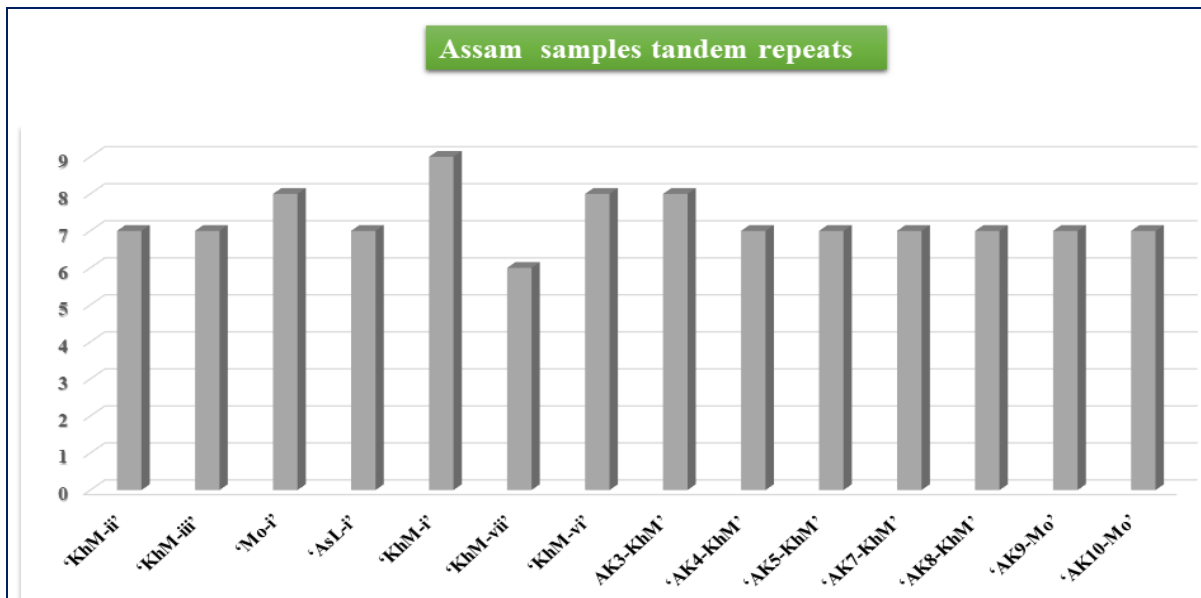


Figure 3: Graphical representation of the tandem repeats observed in the Assam state of India.