Identification of Tobacco Leaf Curl Virus Infecting *Lonicera japonica,* an Ornamental Plant Common in Japan

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ABSTRACT

A begomovirus, tentatively named *Tobacco leaf curl Japan virus*-JpU (TbLCJV-JpU), was isolated from *Lonicera japonica* (Honeysuckle) Plants Grown in Utsunomiya showing veinal chlorosis symptoms. The TbLCJV-JpU genome with 2,761 nt showed a highest identity with TbLCJV-Jp3 and was also close to TbLCJV and as well to TbLCJV-Jp2. The overall nt identity with TbLCJV-Jp3 amounted to 92.94%, while the identities in encoded amino acid (aa) sequence of Coat Protein (CP) and putative products of AC1 and AV2 ORFs were as high as 98.05, 92.54 and 93.96%, respectively. Low sequence identities were observed in the Intergenic Region (IR) of TbLCJV-JpU as compared with TbLCJV, *Ageratum yellow vein Taiwan virus*-Kochi isolate and *Honeysuckle yellow vein virus*-Kagoshima isolates. Recombinations were detected in the 5′end (2650 to 2761) and extreme 3′ portion of the genome (220 to 350). Both regions demonstrated high identities with AYVTV-Kochi and HSYVV-Kagoshima. To the best of our knowledge this is the first report of isolation of TbLCJV from *L. japonica*.

INTRODUCTION

The family *Geminiviridae* is a group of plant viruses with circular single–stranded DNA genomes, encapsidated in twinned particles. It is currently divided into four genera: *Mastrevirus, Curtovirus, Topocovirus* and *Begomovirus*, on the basis of their genome organizations and biological properties (Fauquet *et al.*, 2003). Geminiviruses are not a major problem in Japan, although their existence has been known for a long time as one poem, attributed to the Empress Koken and written in the summer of 752 AD, describes the autumnal appearance of eupatorium plants in summer and is reputedly the earliest written record of the symptoms of a plant virus disease (Saunders *et al.*, 2003). In Japan, one monocot-infecting virus, *Miscanthus streak virus* (MSV) (Yamashita *et al.*, 1985), and five dicot-infecting geminiviruses; *Abutilon mosaic virus* (AbMV) (Osaki and Inouye, 1981), *Sweet potato leaf curl virus* (SPLCV) (Shinkai, 1983), *Tomato yellow leaf curl virus* (TYLCV) (Kato *et al.*, 1998), *Honeysuckle yellow vein mosaic virus* (HY12, SP1 and KG5Tob) and *Tobacco leaf curl Japan virus* (TbLCJV) (Kitamura *et al.*, 2004) have been reported so far. A high rate of recombination among geminiviruses is being resulted in the recent emergence of new geminivirus disease outbreaks (Padidam *et al.*, 1999; Zhou *et al.*, 1998) in new crops in diverse agricultural regions. An increase in the insect vector population in Japan during the recent past (Matsui, 1993) may have resulted in the dissemination of begomoviruses to new, previously

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unaffected areas. *Bemisia tabaci* is a polyphagus insect vector for which at least 506 plant species in 74 families (dicotyledonous and monocototyledonous) have been reported as hosts, including 96 host species in *Fabaceae*, 56 in *Compositae*, 35 in *Malvaceae*, 33 in *Solanaceae*, 32 in *Euphorbiaceae*, 20 in *Convolvulaceae* and 17 in *Cucurbitaceae* (Cock, 1986). Moreover, movement of plants by humans is also partially responsible for the changes in population dynamics of *B. tabaci.* Introduction of an insect vector in an area, coupled with the presence or introduction of any virus species, may cause unpredicted outbreaks in a short time. In addition, acquisition of new nucleic acid molecules may change the virus pathogenicity and the infection of a new host may be initiated very quickly. Therefore, possible effects of such recombinations as alteration in host range due to some selective advantage should be a concern (Garcia-Arenal *et al.*, 2001). TbLCV is a serious pathogen causing substantial yield losses in tobacco and tomato crops in tropical, subtropical and temperate regions of the Old World (Paximadis *et al.,* 1997; Paximadis *et al.,* 1999). TbLCJV from Japan (TbLCJV-Jp) is a distinct geminivirus classified in the genus *Begomovirus*. Substantial losses are reported to have been caused by begomoviruses (Briddon and Markham, 2000; Morales and Anderson, 2001). In some cases economic losses of up to 100% have been reported with rapid spread of the diseases in new areas (Pico *et al.*, 1996). No strategy for control of whitefly-transmitted geminiviruses has proved effective in practice. Whiteflies are difficult to control through insecticides and are often resistant to pesticides (Gerling, 1990). Transgenic resistance gives a ray of hope, but the harvest of genetically engineered plants is not acceptable to consumers. Therefore, under the current situation "prevention is better than control" sounds as the best option. It is very important to know the viral species prevalent in the area and to study the distribution pattern of these viruses, making

every possible effort to avoid mixing and movement of viruses and of their vectors in different growing regions. Recently, monopartite begomoviruses have been reported to require a satellite-like molecule termed DNA 1 (Mansoor *et al.*, 1999, 2001; Saunders and Stanley, 1999). These satellite-like molecules encode a single product with similarity to the replication-associated protein (Rep; a rolling-circle replication initiator protein) of nanoviruses, another family of plant infecting single-stranded DNA viruses. Consequently, DNA 1 molecules are of the potential of autonomous replication in the cells of the host plants, but require the helper begomovirus for spread in plants and insect transmission. DNA 1 appears to have no role in the disease process, being dispensable both for infectivity and symptom inductions in host plants.

Complete characterization of viral species present in a locality is important to forecast the spread and as well to develop effective control measures. Of the reported geminiviruses in Japan, the complete nucleotide sequence has been determined for MSV, TbLCJV, and TYLCV (Onuki and Hanada, 2000; Kitamura *et al.*, 2004).

Lonicera japonica Thunb., popularly known as honeysuckle, is a common perennial indigenous to Japan and widely grown as an ornamental plant. Veinal chlorosis in honeysuckle has been known for a long time (Osaki *et al.*, 1979), but the infecting virus has not been characterized. In this study, the complete nucleotide (nt) sequence of the virus infecting *L. japonica* was determined. Sequencing and a BLAST search identified the virus as TbLCJV, which will be named hereafter TbLCJV-JpU. This virus (TbLCJV-JpU isolate) is a monopartite geminivirus and has high nucleotide sequence identity homology with the TBLCJV-Jp3 isolate, reported from the Nara region, which was found in infected tomatoes. Simplot analysis showed that the Intergenic Region (IR) of the virus exhibits some degree of recombination. Using the universal primers (Bull *et al.*, 2003) for

DNA 1, the DNA product of expected size (1.3 kb) was also amplified. To our knowledge this is the first report of TbLCJV infecting *Lonicera japonica.*

MATERIALS AND METHODS

Sample Collection

Samples of *L. japonica* were collected from the vineyard at Utsunomiya University, Japan. Leaves were collected from the plants exhibiting symptoms (thought to be caused by a geminivirus) such as veinal chlorosis, vein banding and dark green enation on the abaxial leaf surface. They were transferred to the Plant Virology Laboratory, Utsunomiya University. The samples were stored at -80°C until being further processed.

Polymerase Chain Reaction (PCR)

Total DNA was extracted from the selected samples using the Nucleon PHYTO-pure plant DNA extraction kit (Amersham Pharmacia Biotech, UK) as instructed by the manufacturer's instructions. The Coat Protein (CP) region of the virus was amplified using degenerate begomovirus universal primers (Roja's *et al.*, 1993). Each 50µl PCR reaction mixture contained 1 µl total-DNA, PCR buffer for *Taq* polymerase, 0.2 mM of each dNTP, 1 unit *Taq* DNA polymerase and 25 pmol of

each set of primers (Table 1). Following the initial denaturation step at 94°C for 2 minutes, PCR was performed for 35 cycles, each at 94°C for 1 minute, 58°C for 1 minute and 72°C for 2 minutes, followed by a final extension step at 72°C for 3 minutes. The amplified product was cloned in the pUC19 vector and sequenced in both directions. A BLAST search revealed the identity of TbLCJV. Extending primers (Table 1) were designed from the sequence of TbLCJV in GenBank, in order to amplify and sequence the full length DNA-A.

Efforts were made to amplify a DNA-B component homologue using the degenerate universal primers PBL1v 2,040 and PCRc1 (Roja's *et al.*, 1993) known to amplify the DNA-B fragment from a range of bipartite viruses, but no amplification products were obtained. Universal primers UN 101 and UN 102 (Table 1) were used to amplify the full-length DNA 1 molecules using PCR amplification for 35 cycles each with a melting temperature of 94°C for 1 minute, 50°C for 1 minute, and 1.5 minutes at 72°C after an initial melting temperature of 94°C for 2 minutes and final extension of 72°C for 3 minutes, as reported previously (Bull *et al.*, 2003).

Cloning and Sequencing

Following PCR amplification, the products were electro-eluted using standard procedures (Sambrook and Russell, 2001)

Table 1. Set of primers employed to detect and amplify the genome.

Name	Sequence of Primer	Amplified segment (bp)
PAL1v1978[a]	*GCATCTGCAGGCCCACATYGTCTTYCCNGT*	1.6 kb (CP, AV1
PAR1c496	*AATACTGCAGGGCTTYCTRTACATRGG*	and AC1)
F-312	*AAGAACTATGTCGAAGCGTC*	1.6 kb (CP, AC1,
R-1992	*CCATGAGTATTGTGATTGAG*	AC2 and AC3)
PBL1v2040[a]	*GCCTCTGCAGCARTGRTCKATCTTCATACA*	DNA-B
PCR1c154	*GGTAATATTATAHCGGATGG*	
UN 101[b]	*AAGCTTGCGACTATTGTATGAAAGAGG*	1.3 kb DNA 1
UN 102	*AAGCTTCGTCTGTCTTACGAGCTCGCTG*	

[a] Set of degenerate universal primers reported to amplify the DNA A and DNA B of a diverse group of geminiviruses by Rojas *et al.,* 1993, [b] Set of degenerate universal primers reported to amplify the DNA 1 of a diverse group of geminiviruses by Bull *et al.,* 2003.

and ligated into *Sma* I-cut dephosphorylated pUC19 vector, as reported previously (Ali *et al.*, 2004). Plasmids with the desired DNA insert were selected and sequenced. Sequencing was done using dideoxy sequence analysis (Sambrook and Russell, 2001). At least three clones were sequenced in both directions using a DSQ 1,000 L (Shimadzu, Kyoto, Japan) sequencer.

Sequence Analysis

Amino acid (aa) and nt sequences, representing the CP, AC1 and AV2 ORFs and full length DNA-A, were compared with the sequences reported in the DNA database. Multiple sequence alignments and an investigation of phylogenetic relationships were achieved with CLUSTAL W (Thompson *et al.*, 1994), the Mac Vector package (Oxford Molecular Ltd, Oxford, UK) and using the neighbor-joining method. An unrooted tree was constructed using TREEVIEW and a bootstrap analysis with 1,000 replicates was performed on the data. Recombination analysis used SimPlot (Ray, 2003) to create a plot of similarity versus position. SimPlot calculates and plots the percentage identity of the query sequence to a panel of reference sequences in a sliding window, which is moved across the alignment in steps. Different window and step sizes were tested and regions of more than 50% gaps removed. Several windows and step sizes were tested. Sequence comparisons were performed without any correction for multiple substitutions.

RESULTS

Lonicera japonica develops yellow vein mosaic symptoms (Figure 1-a), often accompanied by enations along the veins, on the abaxial surface of the leaves (Figures 1-b and -c). These symptoms are typical of geminivirus infection hence universal primers (Rojas *et al.*, 1993) for geminiviruses were used to amplify the CP
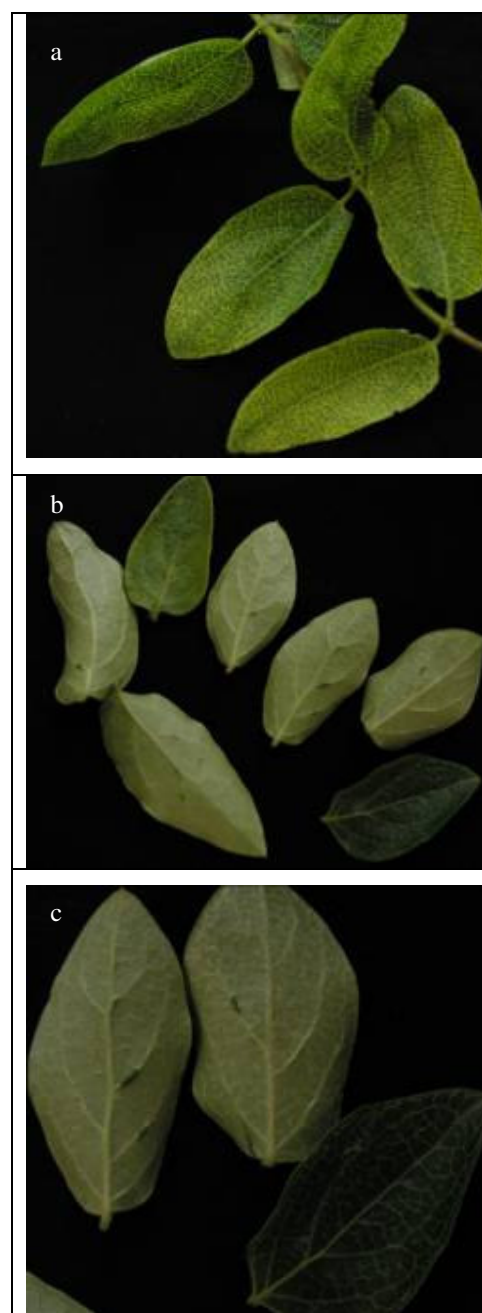

Figure 1. Leaves of *Lonicera japonica* showing the typical symptoms of begomovirus infection. (a) Veinal chlorosis caused by TbLCJV-JpU, (b and c) Dark green enations and vein thickening, a characteristic symptom caused by begomovirus, on abaxial surface of the leaf infected with TbLCJV-JpU.

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and replication-associated protein (Rep) region. The primer pair PAL1v1978 and PAR1c 496 amplified a region of approximately 1.6 kb. Ligation, sequencing and a BLAST search revealed the highest sequence homology with TbLCJV-Jp3 isolated from infected tomatoes from Nara, Japan. Extending primers (Table 1) were designed to amplify the complete genome. The total size of the TbLCJV-JpU DNA is 2761 nt (Accession number EF 620536). No DNA-B segment was detected in infected *L. japonica* samples. Computer translation showed the coding capacity of six ORFs from the genome of TbLCJV-JpU. Two potential proteins were encoded in the virion sense orientation and four ORFs found on the complementary sense. A 31 nucleotide potential stem-loop forming region was found in the Intergenic Region (IR). It included the conserved nonanucleotide sequence TAATATTAC, characteristic of all geminiviruses sequenced so far (Argüello-Astorga *et al.*, 1994; Lazarowitz, 1992). Most of the conserved sequence

motifs known for such begomoviruses as the TATA box, rep binding motif, AG motif, C box and nonanucleotide sequence, were found conserved in the genome. The iteron sequence GGTGTXXXGGTGT was the same as that observed in most begomovirus sequences. In the TbLCJV-JpU Rep protein sequence, the putative DNA-nicking motifs reported by Koonin and Ilyina (1992) were also recognized. Furthermore, a putative NTP-binding motif (EGXXXXGKTX33DD) was located in the Rep encoding region, as found in the helicases of several viruses.

In nt and putative protein sequence comparisons, the highest homology was observed with *Tobacco leaf curl Japan virus*-Jp3 (Kitamura *et al.*, 2004). The overall nt identity was 92.94%, while the identities in encoded amino acid (aa) sequence of the CP region and putative product of AC1 and AV2 ORFs were as high as 98.05, 92.54 and 93.96%, respectively (Table 2). TbLCJV-JpU was also very close to TbLCJV-Jp, TbLCJV-Jp3,

Table 2. The percentage identity in sequence of TbLCJV-JpU with other TbLCJV, HSYVV and AYVV isolates reported in the GenBank.

Virus	Nuc.	CP(AV1)	AC1	AV2	IR-1[a]	IR-2[b]
TbLCJV-Jp3*	**92.94**	**98.05**	**92.54**	93.96	**92.14**	88.19
TbLCJV-Jp2	92.51	96.86	92.01	**94.82**	92.14	86.95
TbLCJV-Jp	89.77	96.86	92.28	90.51	85.00	59.14
AYVV-Kochi	89.72	96.88	91.16	92.03	84.28	**91.76**
HSYVV-Kagoshima	87.94	84.82	90.63	85.96	87.85	86.33
AYVCV-G68	74.10	74.70	80.66	72.41	52.17	66.20
AYVTV	72.50	75.48	72.57	75.86	52.50	57.76
AYVSLV-Srilanka	72.57	70.03	84.27	74.13	61.53	61.37
AYVTV-Taiwan	72.50	75.48	72.57	75.86	52.50	57.76
AYVV-China	73.80	74.70	83.97	61.78	50.70	56.14
EpYVV	83.01	87.54	85.91	85.34	67.36	54.38
EpYVV-MNS2	80.51	87.93	86.77	84.48	65.73	51.41
EpYVV-SOJ3	79.43	87.54	80.16	86.20	61.97	72.87
EpYVV-Yamaguchi	81.22	88.71	86.77	86.20	62.23	50.56
TYLCCNV	73.74	74.40	82.82	69.82	56.00	59.72
TYLCTHV-A	75.51	73.15	82.30	76.10	51.22	63.33
TYLCV-Aichi	71.22	70.54	77.31	67.24	56.84	63.46
TYLCV-Shizuoka	71.08	70.54	77.03	67.24	60.00	63.46

[a] Intergenic region from nucleotide 1 to 150 of the genome, [b] Intergenic region from nucleotide 2,600 to 2,761 of the genome.
*Bold letters indicate the highest identities in the respective genes. Amino acid sequence was employed for calculating the percent identities in different genes.

Ageratum yellow vein Taiwan virus-Kochi isolate (AYVTV-Kochi) and *Honeysuckle yellow vein virus-Kagoshima isolate* (HSYVV-Kagoshima) reported from Nara, Kochi and Yamaguchi Prefectures, Japan. The percentage identities of TbLCJV-JpU with TbLCJV-Jp2 in total nt, CP, AC1 and AV2 coding region were 92.51, 96.86%, 92 and 94.82%, respectively, while 89.77, 96.86, 92.28 and 90.51% identities observed with the TbLCJV-Jp in the same regions. TbLCJV-JpU was more closely similar to AYVTV-Kochi (96.88%) than HSYVV-Kagoshima (84.82%) as based on comparison of the CP region.

In the AC1 and AV2 encoding regions TbLCJV-JpU shares 91.16 and 92.03% identities with AYVTV-Kochi (Table 2). TbLCJV- JpU was also close to the *Eupatorium yellow vein virus* (EpYVV) reported from Fukuoka and Yamaguchi Prefectures, Japan, having identities well above 80% in most parts of the genome (Table 2). The identities with AYVV reported from Sri Lanka and Taiwan and with TLCV reported from China, Thailand and Japan were comparatively low (Table 2). Using universal primers for the amplification of DNA 1, a product of around 1.3 kb was amplified as reported previously (Bull *et al.,* 2003), but needs confirmation through sequencing.

Low sequence identities were observed in the IR region of TbLCJV-JpU compared to TbLCJV-Jp, AYVTV-Kochi and HSYVV-Kagoshima isolates. At the 5´ end of the characteristic nonanucleotide sequence (TAATATTAC) in the IR region, the identity of TbLCJV-JpU was highest (91.76%) with AYVTV-Kochi, while it was only 59.14% with TbLCJV-Jp. In most parts of the genome, identities of TbLCJV-JpU were above 90% with TbLCJV-Jp, but were drastically reduced in the N-terminal IR region. The sequence identities in the C-terminal 150 nt of IR were 84.28% with AYVTV-Kochi and 85% with TbLCJV-Jp. These data strongly indicate that TbLCJV-JpU, TbLCJV-Jp-2 and TbLCJVJp3 acquired this region, which is very important

in replication, from AYVTV-Kochi. The recombination analysis done by Simplot also supports this hypothesis (data not shown). Recombination was detected in the IR region from nucleotide position 2,650 to 2,761 and 220 to 350. Both these regions showed high homologies with AYVTV-Kochi and HSYVV-Kagoshima.

Another region of recombination was detected at positions 2450–2650 (data not shown). In the phylogenetic analysis based upon the CP sequence, TbLCJV-JpU converged with EpYVV and TbLCJV isolates from Japan (Figure 2). Within this cluster, TbLCJV and EpYVV isolates were grouped separately. Although AYVV isolates produced a well defined cluster, AYCSLV diverged clearly from this grouping. TYLCCNV and TYLCTHC-A, which were close in the phylogenetic tree based on the complete nt sequence, were both separated in the phylogeny based on CP aa sequences.

The same topology was obtained from a phylogenetic analysis of complete nucleotide sequences. TbLCJV and EpYVV isolates clustered together, but within this cluster each represented a separate lineage. AYVV produced a separate cluster in this analysis (Figure 3). It was interesting to note that AYVTV from Kochi, Japan, clustered with TbLCJV and not with AYVV. It is speculated that AYVTV is a variant strain of TbLCJV and not of AYVV. High variabilities were observed among isolates of TLCV from different regions. AYVSLV from Sri Lanka clustered with TLCV isolates from India. Two TYLCV isolates, from Aichi and Shizuoka, Japan, clustered with TLCV from Puerto Rico, Portugal and Spain. All Japanese isolates of TbLCJV were closely related.

Consistency was observed in the phylogenetic relationships of the AV2 and AC1 putative product sequences (data not shown). In the analysis of the nucleotide sequence of the AV2 ORF, EpYVV-SOJ3 was markedly divergent from the main cluster, due to a probable recombination event in this part of the genome. AYVV
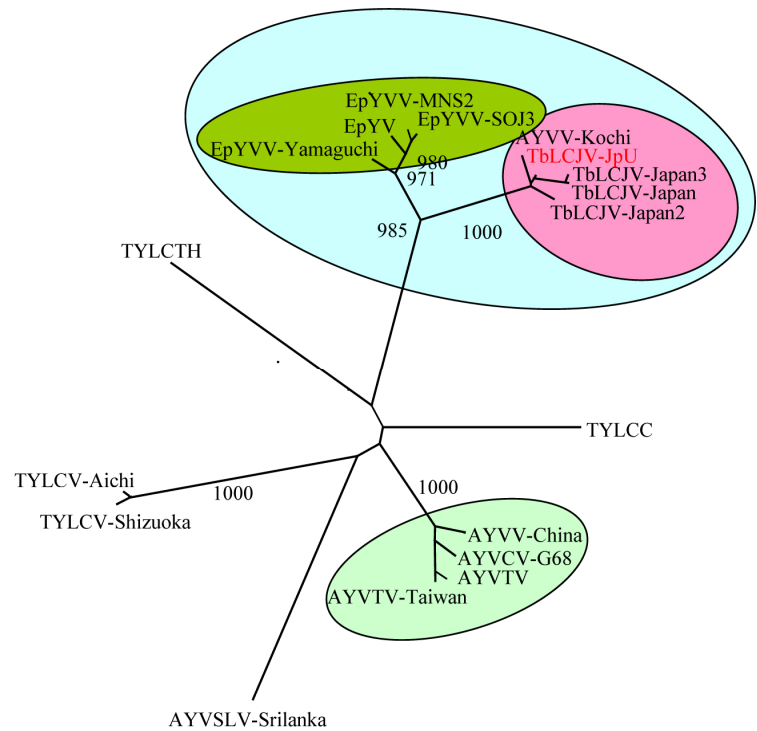
Figure 2. Phylogenetic relationship in aa sequence of CP of TbLCJV-JpU isolate, with TbLCJV, TYLCV, EpYVV and AYVV isolates reported in the GenBank, using neighbour joining method with a bootstrap value of 1,000.
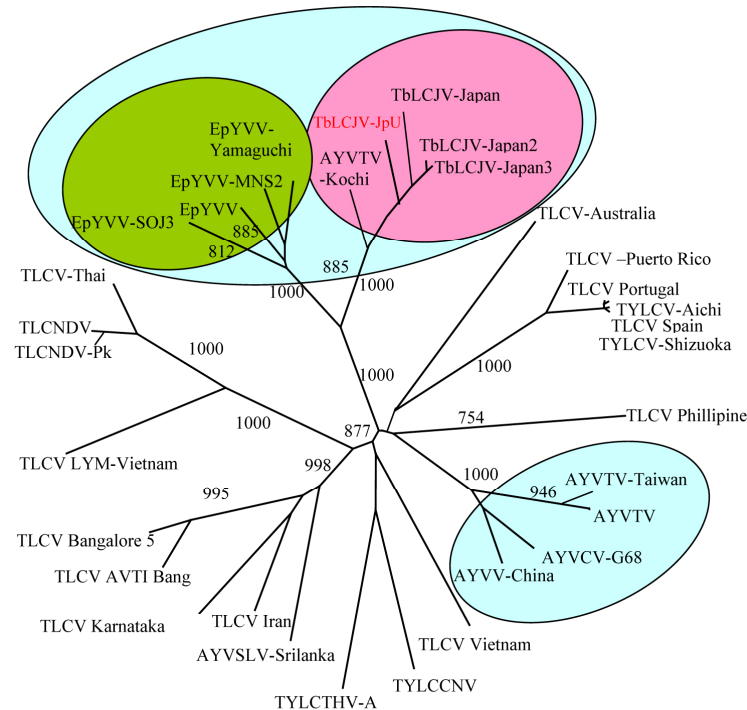

Figure 3. Phylogenetic relationship constructed upon total nt sequences of TbLCJV-JpU isolate, with TbLCJV, TLCV, TYLCV, EYVV and AYVV isolates reported in the GenBank, using neighbour joining method with a bootstrap value of 1,000.

isolates did not produce a well-defined cluster as was observed in other parts of the genome. In the phylogenetic tree for the AC1 encoding region the AYVV-China isolate was somewhat divergent within the AYVV-isolates cluster, showing some variability in its genome.

DISCUSSION

TbLCV is a serious pathogen that has been reported from tropical, subtropical and temperate climatic regions of the Old World (Paximadis *et al.*, 1997; Paximadis *et al.*, 1999), causing substantial yield losses in tobacco and tomato. TbLCJV from Japan is a distinct geminivirus (Sharma *et al.*, 1998) with strains infecting a number of plants.

TbLCJV-JpU, isolated here from honeysuckle, was genetically very close to isolates of TbLCJV, TbLCJV-Jp2 and TbLCJV-Jp3 previously reported. Most begomoviruses reported from Japan, including EpYVV and TbLCJV, showed high level (percentage) identities among themselves and hence have are assumed to very close evolutionary relationships. TbLCJV-JpU was almost identical to TbLCJV-Jp2 and TbLCJV-Jp3 across the entire genome. All the three isolates exhibited high identities with TbLCJV, except in the IR region, where homology was very low near the end of the genome. In the IR region TbLCJV-JpU showed high sequence identity with AYVTV-Kochi, suggesting that

TbLCJV-JpU, TbLCJV-Jp2 and TbLCJV-Jp3 might have originated as a result of recombination in the IR region of TbLCJV. There is considerable evidence that many geminiviruses have arisen *via* recombination (Padidam *et al.*, 1999). Recombinant geminivirus species have been identified in cotton (Zhou *et al.*, 1998), tomato (Kirthi *et al.*, 2002) and cassava (Pita *et al.*, 2001). It has been documented that recombination events have played a role in the emergence of new geminiviral diseases. The severe outbreak of cotton leaf curl disease in

Pakistan is thought to be the result of recombination between *Cotton leaf curl virus* (CLCuV) and *Okra yellow vein mosaic virus* (OYVMV) (Zhou *et al.*, 1998). The high level of identities among the isolates of TbLCJV-JpU, TbLCJVJp2 and TbLCJV-Jp3 suggests that either this recombinational event occurred at an early stage after the introduction of geminiviruses to Japan or all these isolates might have been the result of a very recent introduction of a single mutated isolate.

The tight clustering of geminivirus isolates from Japan indicates the relationship to be of a geographical basis. Some inconsistency in phylogenetic trees based on different parts of the genome also strongly indicates a frequent occurrence of recombinations in the genome, consistent with the previous suggestions that the location and length of recombinant sequences occur throughout the genome (Padidam *et al.*, 1999). These results also strongly support the idea of using the entire genome, rather than only a portion of it, for taxonomic demarcation of virus species. AYVTV-Kochi clustered with the TbLCJV isolates reported from Japan, and it seems to be a strain of TbLCJV, rather than AYVV. TbLCJV-JpU not only clustered with TbLCJV isolates reported from Japan, but also with EpYVV isolates in all the regions of the genome, including the complete nt sequence, indicating that these groups might have a common ancestor.

Efforts were made to detect a DNA-B component for TbLCJV-JpU, but no amplification products were obtained using the degenerate universal primers (Rojas *et al.*, 1993) reported to amplify the DNA-B segment from a diverse group of begomoviruses. Hence, it is speculated that TbLCJV-JpU is a monopartite begomovirus, in complete agreement with the other reported TbLCJV isolates from Japan (Kitamura *et al.*, 2004).The authors were able, throughout the ongoing study, to amplify putative DNA 1, making use of the universal set of primers.

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شناسائی ویروس پیچیدگی برگ تنباکو (Tobacco leaf curl virus) بیمارگر پیچ امین‌الدوله (Lonicera Japonica)، گیاه زینتی متداول در ژاپن

ا. علی، م. احمد، ه. نیشیگاوا، و ت نات سواکی

چکیده

ویروسی از بگومووویروسی (begomoviruses) که فعلاً (بطور آزمایشی) ویروس ژاپنی پیچیدگی برگ تنباکوی اوتسونومیای ژاپن (Tobacoo leaf curl Japan virus- Jup (TbLCJV-JpU نامگذاری می‌شود از گیاه پیچ امین‌الدوله (Honeysuckle) در اوتسونومیا (Utsumomia)، ژاپن که دارای علائم مرض کلرز رگبرگی (Veinal chlorosis) بودند جدا گردید. ژنوم TbLCJV-JₚU با تعداد ۲۷۶۱

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نوکلئوتید بیشترین شباهت را به TbLCJV-J$_P$3 داشت و با TbLCJV و همچنین TbLCJV-J نیز دارای شباهت نزدیک بود. شباهت روی‌هم رفته نوکلئوتیدی با TbLCJV3 به میزان ٪۹۲/۹۴ بود، در حالیکه شباهت‌های توالی اسید آمینه‌ای (aa) کددار (coded) مربوط به پروتئین پوششی (Coat protein) و نیز تولیدات فرضی ا آر اف‌های (ORFs) مربوط به AC$_1$ و AV2 به ترتیب با مقادیر ٪۹۸/۰۵ و ٪ ۹۲/۵۴ و ٪ ۹۳/۹۶ برآورد شدند. شباهت‌های کمی در توالی ناحیه بین ژنی (Intergenic Region) مربوط به TbLCJV-JpU در مقایسه با جدایه‌های TbLCJV، ویروس تایوانی زردی رگبرگ گل ابری کوچی (Ageratum yellow vein Taiwan virus Kochi) و ویروس زردی رگبرگ در پیچ امین‌الدوله – کاگوشیما (Honeysuckle yellow vein virus- Kagoshima) مشاهده شد. نوترکیبی‌هائی (Recombinations) در انتهای ′۵ و بخش انتهائی ′۳ (۳۵۰- ۲۲۰) ژنوم رویایی گردید. هر دو ناحیه شباهت‌های زیادی با AYVTV-Kochi و HSYVV-Kagoshima نشان دادند بطور کلی و تا جائیکه اطلاع حاصل است این اولین گزارش در رابطه با جداسازی TbLCJV از پیچ امین‌الدوله می‌باشد.