J Gen Plant Pathol (2005) 71:319–325
DOI 10.1007/s10327-005-0202-7

VIRAL AND VIROID DISEASES

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Evidence of a new *Tomato yellow leaf curl virus* in Japan and its detection using PCR

Received: November 1, 2004 / Accepted: December 15, 2004

Abstract A new isolate of *Tomato yellow leaf curl virus* (TYLCV) has been identified from tomato plants in Kochi Prefecture in Japan and designated TYLCV-[Tosa]. The complete nucleotide sequence of the isolate was determined and found to consist of 2781 nt. In phylogenetic analyses of entire nucleotide sequences, TYLCV-[Tosa] was delineated as a single branch and was more closely related to TYLCV-[Almeria] than TYLCV isolates Ng, Sz, or Ai reported in Japan, which had spread since 1996. Isolate TYLCV-[Tosa] is suggested to be a newly introduced, novel isolate of TYLCV that dispersed into Kochi Prefecture. In addition, a rapid method using the polymerase chain reaction to separate TYLCV isolates into four genetic groups was established. This method would be useful for reliable diagnosis based on genetic differences among isolates of TYLCV.

Key words Geminivirus · Begomovirus · *Tomato yellow leaf curl virus* · Diagnosis

Introduction

The family Geminiviridae is currently divided into four genera – *Mastrevirus*, *Curtovirus*, *Topocuvirus*, *Begomovirus* – on the basis of their genome organizations and biological properties (Fauquet et al. 2003). *Tomato yellow leaf curl virus* (TYLCV; genus *Begomovirus*) has a monopartite single-stranded DNA (ssDNA) genomic component (DNA-A) encapsidated in geminate particles and transmitted by the whitefly, *Bemisia tabaci* (Genn.). Tomato yellow leaf curl (TYLC) disease caused by TYLCV is one of the most damaging diseases on tomato worldwide (Mansoor et al. 2003; Salati et al. 2002). Infected tomato plants have leaves with chlorotic, curled-up margins; the plants become stunted, and the flowers abscise. Since the first record of TYLCV in Israel in 1939–1940 (Antignus and Cohen 1994), isolates have been reported from various locations in the Mediterranean Basin, Africa, Reunion Islands, Asia, Caribbean islands, and Central America; and they are currently classified into several viral species (Antignus and Cohen 1994; Delatte et al. 2004; Pico et al. 1996; Polston and Anderson 1997). The first complete nucleotide sequences of TYLCV isolates were reported in 1991 for isolates from Israel and Sardinia (*Tomato yellow leaf curl Sardinia virus*, TYLCSV) (Kheyr-Pour et al. 1991; Navot et al. 1991). Many sequences have been obtained for TYLCV isolates from various locations (Moriones and Navas-Castillo 2000).

In Japan, the first evidence of economic damage to tomato caused by TYLCV was recorded in 1996 in Shizuoka and Aichi prefectures in Tokai district in central Japan and in Nagasaki Prefecture in Kyushu located in southwestern Japan (Kato et al. 1998). Since then, TYLCV has been isolated not only from tomato plants but also from *Eustoma russellianum* plants (Haga and Doi 2002; Kato et al. 1998; Onuki et al. 2004; Ueda et al. 2004). The complete nucleotide sequences from various TYLCV isolates in Japan have shown that isolates from Shizuoka (TYLCV-Mld[Shi]) and Aichi (TYLCV-Mld[Aic]) are closely related to TYLCV-Mild isolate (TYLCV-Mld), and those from Nagasaki (TYLCV-[Ng]) are closely related to TYLCV (Kato et al. 1998; Onuki et al. 2004; Ueda et al. 2004).

Another begomovirus, *Tobacco leaf curl Japan virus* (TbLCJV), which is also found on tomato plants in Japan,

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The nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank databases under accession numbers AB192965 and AB192966

induces symptoms similar to those of TYLC disease. TbLCJV rarely affects tomato fruit yield because infection with TbLCJV is restricted to only low percentages of tomato plants in greenhouses in Japan. Until 2002, only TbLCJV had been reported in Shikoku in midwestern Japan. However, in 2003–2004 there was a sudden outbreak of TYLC disease from the early growth stage of seedlings in a tomato greenhouse in Kochi Prefecture in Shikoku. This new TYLCV quickly dispersed into surrounding greenhouses and caused heavy losses to the tomato yield.

In this study, we determined the complete nucleotide sequence of this new TYLCV isolate (TYLCV-[Tosa]) from Shikoku and performed phylogenetic and sequence comparison analyses to understand how this outbreak occurred. The polymerase chain reaction (PCR) followed by agarose gel electrophoresis has been utilized to develop a simple method for detecting and identifying genetic groups of isolates occurring in Japan.

Materials and methods

Viral origin and maintenance of isolates

Naturally infected tomato plants with typical yellow leaf curl disease symptoms were collected from greenhouses in February at Tosa in Kochi Prefecture in Shikoku where the first outbreak of TYLCV took place and at Haruno in Kochi Prefecture in August 2004. The viral isolates were maintained by graft-inoculation onto *Lycopersicon esculentum* cv. Hausumomotaro (tomato). Inoculated plants were grown in an insect-proof greenhouse at a constant temperature of 26°C with 16-h day length.

Sequencing and sequence analysis

Total DNA was extracted from young leaves of plants by an alkaline-lysis preparation followed by precipitation with isopropanol (Palmer et al. 1998). To amplify cDNA of entire viral genome DNA for sequencing, PCR was performed using two sets of primers described by Ueda et al. (2004). Independent fragments amplified by PCR from three distinct infected tomato leaves were used for direct sequence determination. The complete nucleotide sequences were determined using the BigDye terminator cycle sequencing kit version 3.1 (Applied Biosystems, Foster City, CA, USA) and resolving on the ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems). Sequence data were assembled and analyzed with DNASIS software (Hitachi, Tokyo, Japan) and the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). Sequence alignments were generated using programs in Clustal W or X (Jeanmougin et al. 1998; Thompson et al. 1994). Their phylogenetic relations were determined by methods using the neighbor-joining (NJ) and maximum-parsimony (MP) algorithms running of MEGA2.1 software (http://www.megasoftware.net/). The following sequences of TYLCV isolates were used for phylogenetic analyses.

Tomato yellow leaf curl virus (TYLCV, X15656) -Mild (TYLCV-Mld, X76319); -Cuba (TYLCV-[CU], AJ223505); -Dominican Republic (TYLCV-[DO], AF024715); -Egypt (TYLCV-[EG], AY594174); -Mild[Portugal] (TYLCV-Mld[PT], AF105975); -Almeria (TYLCV-[Alm], AJ489258); -Iran (TYLCV-IR, AJ132711); -Puerto Rico (TYLCV-[Puerto Rico], AY134494); -Mild[Spain] (TYLCV-Mld[Spain], AJ519441); -Mild[Spain7297] (TYLCV-Mld[ES72-97], AF071228); -Florida (TYLCV-[Flo], AY530931); -Ng (TYLCV-[Ng], AB110217); -Omura (TYLCV-[Omu], AB116630); -Misumi (TYLCV-[Mis], AB116631); -Miyazaki (TYLCV-[Miy], AB116629); -Mild[Shizuoka] (TYLCV-Mld[Shi], AB014346); -Mild[Sz] (TYLCV-Mld[Sz], AB110218); -Mild[Yaizu] (TYLCV-Mld[SzY], AB116632); -Mild[Aic] (TYLCV-Mld[Aic], AB014347); -Mild[Osuka] (TYLCV-Mld[SzOs], AB116636); -Mild[Daito] (TYLCV-Mld[SzD], AB116635); -Mild[Atumi] (TYLCV-Mld[Atu], AB116633); -Mild[Kisozaki] (TYLCV-Mld[Kis], AB116634)

Tomato yellow leaf curl Sardinia virus (TYLCSV, X61153); -Sicily (TYLCSV-Sic, Z28390).

Tomato yellow leaf curl China virus (TYLCCNV, AF311734)

Tomato yellow leaf curl Thailand virus -[1] (TYLCTHV-[1], X63015)

Tomato yellow leaf curl Gezira virus -[1] (TYLCGV-[1], AY044137)

Tomato yellow leaf curl Malaga virus (TYLCMalV, AF271234)

Primer design and PCR for identification of genetic groups

Based on the complete nucleotide sequence data determined previously (Kato et al. 1998; Onuki et al. 2004; Ueda et al. 2004) and in this study, multiple alignment was performed to design specific primers (Fig. 3). The polymerase chain reaction (PCR) was performed using two sets of virion-sense and complementary-sense primers: 162NgTos(+) (TGTATCGGTGTCTTACTTATAC) and 169SzAi(+) (GATTTTTACACTTATCCCTGG); and 161NgTosSz(−) (AATTTGATTGGTTGACAGTGACG) and 164NgTosSzAi(−) (AGGATGCAATTTGATTGG TT). PCR was carried out using Ex *Taq* polymerase (Takara, Otsu, Japan) according to the manufacturer's instruction. The PCR was done in a reaction volume of 50 µl containing 10 pmol of each set of primers. Viral DNA fragments were amplified for 35 cycles: 96°C for 30 s, 60°C for 30 s, and 72°C for 40 s after an initial denaturation of 96°C for 3 min; the final extension was 72°C for 5 min. Amplified PCR products (5 µl volume) were separated by electrophoresis in 3.0% agarose gel (Agarose X; Wako, Osaka, Japan) in TAE buffer at 100 V for 70 min. The DNA bands on the gel were stained with ethidium bromide and visualized under an ultraviolet transilluminator.

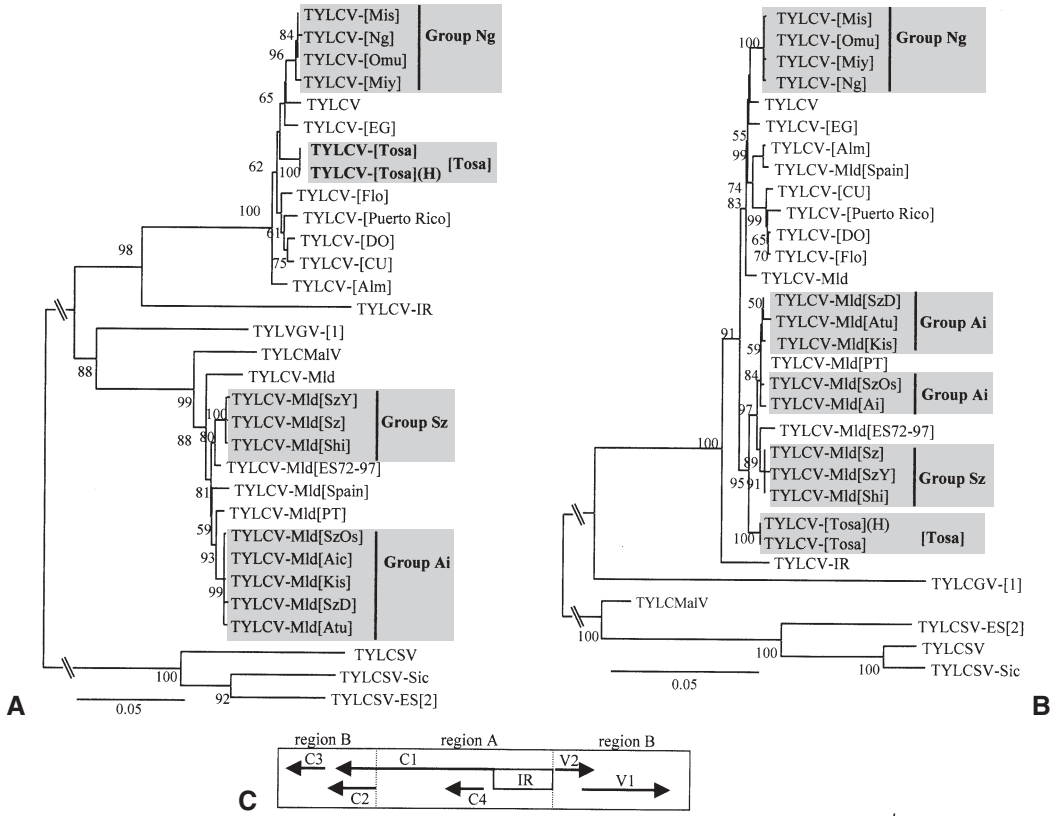

Fig. 1. Neighbor-joining phylogenetic trees of partial nucleotide sequences of 28 isolates of TYLCV, TYLCGV, and TYLCMalV. Two regions of region A (**A**) and region B (**B**) that partitioned in this study are shown at the bottom (C). Numbers at each node indicate the percentage of supporting bootstrap samples (only values > 50 are shown). Horizontal branch length is drawn to scale, bar indicates 0.05 nt replacements per site. Isolates reported from Japan are shaded

Table 1. Percentage nucleotide and amino acid sequence identities of TYLCV-[Tosa] with selected TYLCV isolates

Isolate	Nucleotide sequences			Amino acid sequences					
	Total DNA	IR	V2-C2[a]	V1 (CP)	V2	C1 (Rep)	C2	C3	C4
TYLCV	98	94	98	98	99	97	96	97	96
TYLCV-[Ng]	98	95	98	98	99	97	97	97	97
TYLCV-[Alm]	98	97	98	98	99	98	96	97	95
TYLCV-[DO]	98	96	98	98	99	97	96	97	96
TYLCV-[CU]	98	95	98	98	99	97	96	97	96
TYLCV-[Puerto Rico]	97	94	97	97	99	98	95	97	94
TYLCV-[Flo]	98	95	98	98	99	97	96	97	95
TYLCV-IR	91	86	97	98	98	86	97	94	65
TYLCV-Mld	91	75	98	99	99	87	94	97	48
TYLCV-Mld [Shi]	92	79	99	99	100	88	97	98	47
TYLCV-Mld [Aic]	91	76	99	99	100	88	97	97	47

[a] V2-C2 region (region B) is shown in Fig. 1C

Results and discussion

Sequence analyses of TYLCV-[Tosa]

To determine the complete nucleotide sequences of TYLCV isolates from the site of the first outbreak of TYLCV in Tosa City, we collected infected tomato plants in February 2004. Isolate TYLCV-[Tosa] was determined to be 2,781 nt in length (database accession no. AB192965). The full genome sequence of an isolate collected at another location, Haruno, in August 2004 {TYLCV-[Tosa](H), AB192966} was identical to the sequence of TYLCV-[Tosa] (2,781 nt/2,781 nt). Because the sequences of the isolates from Tosa and Haruno were identical, this isolate would have been dispersed by viruliferous *B. tabaci* in these are as only within a half year. Sequence alignment analyses showed that nucleotide and amino acid sequences of TYLCV-[Tosa] in the intergenic region (IR), C1 (Rep), and C4 were more closely related to isolates of TYLCV strains than those of TYLCV-Mld strains. However, amino acid sequences in coding regions of V1, V2, C2, and C3 and nucleotide sequences between V2 and C2 (region B, Fig. 1C) were similar; thus, it was difficult to distinguish them from the sequence of TYLCV-[Tosa] (Table 1).

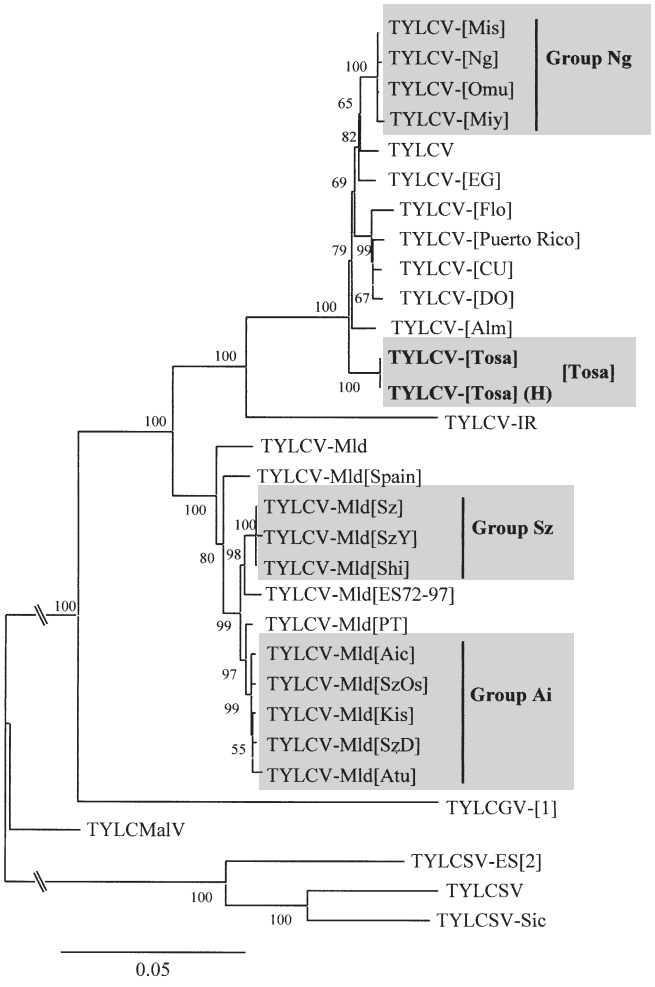

Fig. 2. Neighbor-joining phylogenetic tree calculated from the complete nucleotide sequences of 28 isolates of TYLCV, TYLCGV, and TYLCMalV. *Numbers* at each node indicate the percentage of supporting bootstrap samples (only values > 50 are shown). Horizontal branch length is drawn to scale, *bar* indicates 0.05 nt replacements per site. The homologous sequences of three isolates of TYLCSV were used as the outgroup. Isolates reported from Japan are *shaded*

Phylogenetic analyses using NJ and MP methods were calculated from the entire nucleotide sequences of 28 isolates of TYLCV, TYLCGV, and TYLCMalV, including TYLCV-[Tosa] and -[Tosa](H). Results calculated by NJ and MP were well correlated. Only the phylogenetic tree calculated from the entire nucleotide sequences by NJ is shown in Fig. 2. All isolates from the Tokai district and Kyushu in Japan were separated and classified into three genetic groups: group Sz (type isolate is TYLCV-Mld[Shi]), group Ai (TYLCV-Mld[Aic]), and group Ng (TYLCV-[Ng]), which were 2791, 2787, and 2774 nt in length, respectively (Ueda et al. 2004). Isolates of TYLCV-[Tosa] were in the basal position of the TYLCV group, which consists of: (1) group Ng, previously reported from Kyushu in Japan; (2) TYLCV and TYLCV-[EG], reported from the Near and Middle East; (3) isolates of the Caribbean islands and Florida; and (4) TYLCV-[Alm] from Spain. TYLCV-[Tosa] and TYLCV-[Alm] were placed in two separate branches, apart from the other branches. This grouping was also supported by high bootstrap values (Fig. 2).

In addition, we performed phylogenetic analyses using partial nucleotide sequences. Analysis of region A, which contains sequences of IR, C4, and most parts of C1, showed that isolates of TYLCV-[Tosa] were in a single branch clustered in the TYLCV group; and all TYLCV isolates formed a tight cluster when analyzing with the entire nucleotide sequences (Fig. 1A, 2). However, when analyzing with region B, which included sequences of V2, V1, C3, and C2, all TYLCV and TYLCV-Mld isolates clustered into a large group consisting of two subgroups: (1) most isolates of TYLCV strain and TYLCV-Mld and TYLCV-Mld[Spain]; (2) isolates of group Sz and group Ai from Japan and TYLCV-Mld[ES72-97] and TYLCV-Mld[PT] (Fig. 1B). Only TYLCV-[Tosa] isolates fell into the second subgroup. Navas-Castillo et al. (2000) have already suggested from phylogenetic analyses that TYLCV and TYLCV-IR isolates have chimeric genomes that have arisen by recombination between TYLCV-Is-like and tomato leaf curl virus-like ancestors. More recently, TYLCV-IR has been proposed as a recombinant between TYLCV-Mld and *Tomato leaf curl Iran virus* (Bananej et al. 2004). Although a more detailed investigation for recombination events on TYLCV-[Tosa] was done using the RDP program (Martin and Rybicki 2000), definitive parents of TYLCV-[Tosa] failed to be detected (data not shown).

By graft inoculation onto tomato plants in the greenhouse, TYLCV-[Tosa] induced severe symptoms like those by isolates of group Ng, in contrast to the mild symptoms caused by isolates of group Sz or group Ai (data not shown). TYLCV is known to have been accidentally introduced during the mid-1990s to the Caribbean islands and to the United States (Nakhla et al. 1994; Polston and Anderson 1997). The simplest explanation would be that TYLCV-[Tosa] was also recently introduced to Japan from overseas. Although the actual introductory route of TYLCV-[Tosa] is not known, it probably arrived through international plant trade in certain plant hosts or with viruliferous *B. tabaci*.

PCR-based detection of genetic groups

Knowledge of the complete genome sequences of TYLCV isolates would provide useful information for developing a rapid detection method for efficient disease control and management. Therefore, we established rapid, simple methods to distinguish four genetic groups (Ng, Sz, Ai, Tosa) of TYLCV using their sequences. We first selected the intergenic region (IR) design PCR primers because this region contained variable sequences among the isolates (Fig. 3), but none of those group-specific primer pairs were useful for specific detection (data not shown). Therefore, duplex primers 162NgTos (+) and 169SzAi (+) for the viral sense primer were designed based on the conserved region between Ng and Tos or between Sz and Ai, respectively. For complementary sense primers, we designed triplex primer 161NgTosSz(−), which has perfectly conserved specific sequences complementary to Ng, Tos, and Sz but

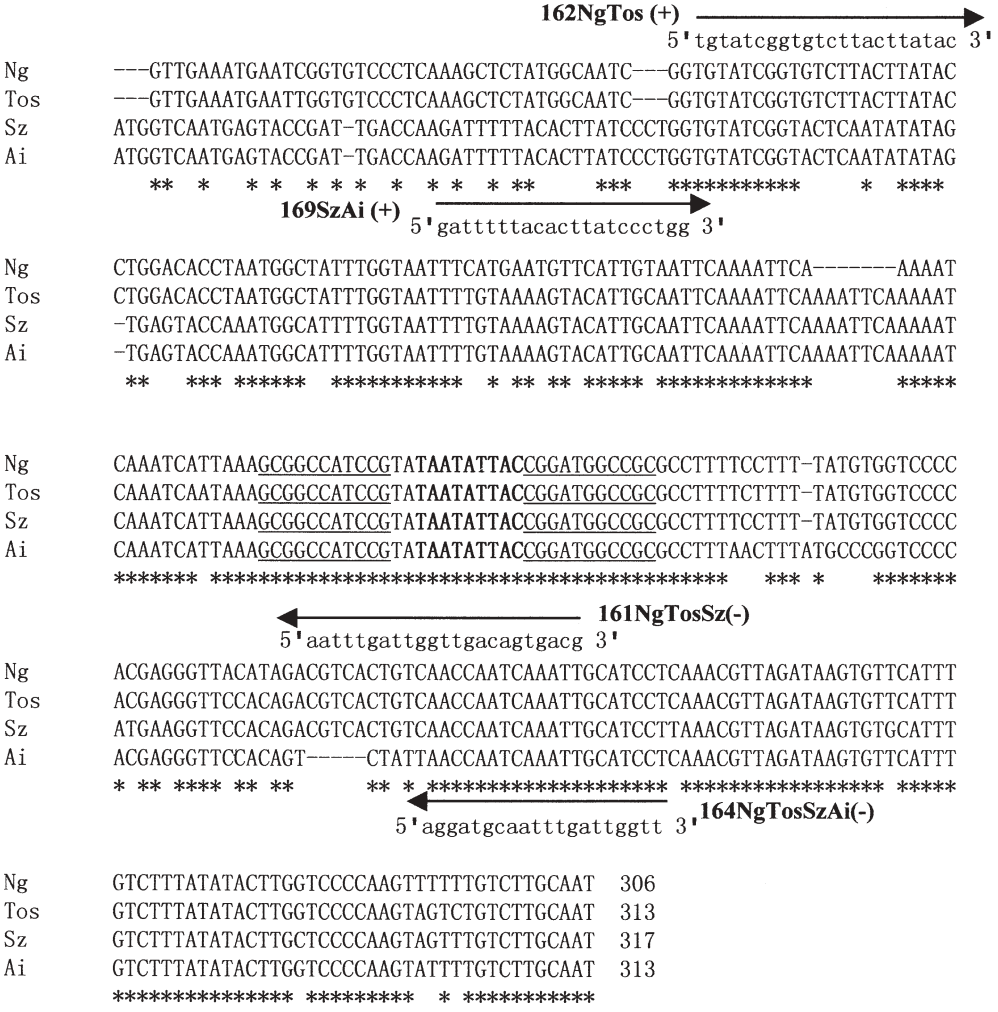

Fig. 3. Nucleotide sequences of the intergenic region of four genetic groups. The positions of designed primers are shown as *arrows*. The conserved stem sequences (*underlined*) and nonanucleotide loop sequences (*bold*) within the stem-loop structures (Robert and Stanley 1994) are indicated. The conserved nucleotides among groups are shown as *asterisks* at the bottom. Sequences are from the following isolates as type sequences of groups: TYLCV-[Ng] (*Ng*), TYLCV-[Tosa] (*Tos*), TYLCV-Mld[Shi] (*Sz*), and TYLCV-Mld[Aic] (*Ai*)

not to Ai. 164NgTosSzAi(–) was also designed from perfectly conserved specific sequences complementary to all four genetic groups. Optimum conditions and specific primer combinations were investigated for discriminating the four genetic groups. Single PCR products were obtained using primer sets 162NgTos(+) and 161NgTosSz(–) or 164NgTosSzAi(–) for TYLCV-[Ng] or TYLCV-[Tosa], which corresponded to the target size of 193 or 200 bp, respectively (Fig. 4A). TYLCV-[Ng] and TYLCV-[Tosa] were also detected using primer sets 162NgTos(+) and 164NgTosSzAi(–), corresponding to the target sizes of 200 or 207 bp, respectively (data not shown). TYLCV-Mld[SzY] in group Sz was detected using primer sets 169SzAi(+) and 161NgTosSz(–) or 164NgTosSzAi(–), corresponding to the target sizes of 220 or 227 bp, respectively (Fig. 4B,C). TYLCV-Mld[Kis] in group Ai was detected using primer set 169SzAi(+) and 164NgTosSzAi(–), corresponding to the target size of 223 bp, although only faint signals were detected using primer sets 169SzAi(+) and 161NgTosSz(–) (Fig. 4B,C). This discrepancy occurred because the sequences of group Ai lack the sequence ACGTCA in the downstream region in IR, and the complementary sense primer 161NgTosSz(–) could not anneal to the target sequences completely during the reaction (Fig. 3). The PCR products visually differed in size with 3.0% agarose gel electrophoresis. Triplex primer sets were also used in combination with 162NgTos(+), 169SzAi(+), and 161NgTosSz(–) or with 162NgTos (+), 169SzAi(+), and 164NgTosSzAi(–) under similar conditions (Fig. 4D,E). Amplified products from these triplex PCRs had the expected target sizes as deduced from the sequence data. Nucleotide sequences of the products from four distinct isolates were confirmed. We tried this method to differentiate TYLCV isolates from various places in Kochi, Wakayama, and Hiroshima prefectures, where TYLC disease had recently been found, to identify genetic groups prior to sequencing analysis. Our PCR results coincided completely with those from subsequent sequencing analyses (data not shown). This PCR-based detection can discriminate closely related isolates of TYLCV and TYLCV-Mld by using primers in approximate combination.

TYLCV has already spread into Kochi Prefecture. TYLC disease was also found in tomato greenhouses in Wakayama Prefecture in Kinki district and Hiroshima Prefecture in Chugoku district in August 2004 for the first time. The sequence analysis of a Hidaka (in Wakayama

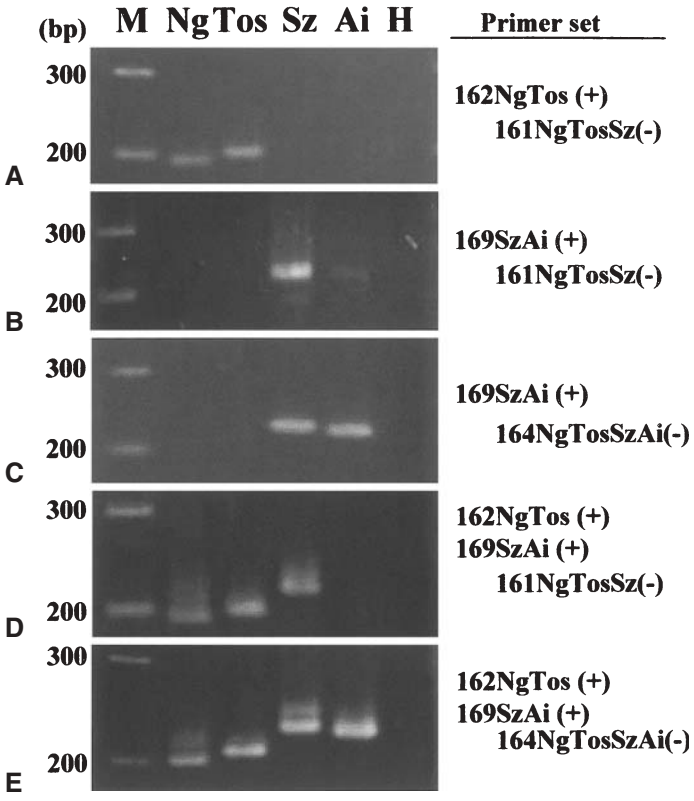

Fig. 4. Polymerase chain reaction (PCR)-based discrimination of genetic groups. Amplified PCR products were separated by agarose gel electorophoresis (3%) and visualized under an ultraviolet transilluminator, after being stained with ethidum bromide. Template DNA extracted from tomato plants infected with TYLCV-[Ng] (*Ng*), TYLCV-[Tosa] (*Tos*), TYLCV-Mld[SzY] (*Sz*), and TYLCV-Mld[Kis](*Ai*), or a healthy tomato plant (*H*). A. 100-bp ladder (Promega) was used as a marker (*M*). PCR primer sets are shown at the right side of each panel

Prefecture) isolate indicated that it had extremely high identity with sequences of isolates in group Ng isolated in Kyushu (unpublished data).

Little analytical work has been done in Japan on the distribution and ecology of the *B. tabaci* biotype. It is important for efficient management of TYLCV to reduce the number of infected plants and viruliferous vector insects. Since the first outbreak of TYLCV in Japan (Kato et al. 1998), control of TYLC disease has been investigated and attempted using screens and ultraviolet-absorbing sheets together with insecticides to limit vector entry (Ogawa and Uchikawa 2004). However, these screens are relatively expensive and cause overheating in the summer. Some growers have also used commercial tomato cultivars tolerant to TYLCV from foreign seed companies.

Conclusions

In 2004 in Kochi Prefecture a population of TYLCV isolates were found to have a unique nucleotide sequence distinct from those of previously reported isolates; they were named TYLCV-[Tosa]. TYLCV-[Tosa] might have been introduced through infected plants or viruliferous vector insects from foreign countries. The PCR-based detection methods that we established here can be useful for rapid, accurate genetic group discrimination of TYLCV. It is important now to control TYLC disease. Combining physical, chemical, and biological control methods, such as TYLCV-resistant cultivars and natural enemies against vector insects, might be the best way to achieve such control.

Acknowledgments The authors thank Mr. Y. Masuda and Mr. S. Matsuura for providing the naturally infected tomato plants and Dr. E. Moriones for advice on Almeria isolate and viral isolates terminology.

References

Antignus Y, Cohen S (1994) Complete nucleotide sequence of an infectious clone of a mild isolate of *Tomato yellow leaf curl virus* (TYLCV). Phytopathology 84:707–712

Bananej K, Kheyr-Pour A, Hosseini Salekdeh G, Ahoonmanesh A (2004) Complete nucleotide sequence of Iranian tomato yellow leaf curl virus isolate: further evidence for natural recombination amongst begomoviruses. Arch Virol 149:1435–1443

Delatte H, Naze F, Granier M, Reynaud B, Peterschmitt M, Lett M (2004) Tomato-infecting begomoviruses in the South Western islands of the Indian Ocean. Presented at the 4th International Geminivirus Symposium, W3-4

Fauquet CM, Bisaro DM, Briddon RW, Brown JK, Harrison BD, Rybicki EP, Stenger DC, Stanley J (2003) Revision of taxonomic criteria for species demarcation in the family Geminiviridae, and an updated list of begomovirus species. Arch Virol 148:405–421

Haga H, Doi M (2002) The frequent occurrences and control of Tomato yellow leaf curl in Shizuoka Prefecture. Shokubutuboueki 56:153–156 (in Japanese)

Jeanmougin F, Thompson JD, Gouy M, Higgins DG, Gibson TJ (1998) Multiple sequence alignment with Clustal X. Trends Biochem Sci 23:403–405

Kato K, Onuki M, Fuji S, Hanada K (1998) The first occurrence of *Tomato yellow leaf curl virus* in tomato (*Lycopersicon esculentum* Mill.) in Japan. Ann Phyopathol Soc Jpn 64:552–559

Kheyr-Pour A, Bendahmane M, Matzeit V, Accotto GP, Crespi S, Gronenborn B (1991) *Tomato yellow leaf curl virus* from Sardinia is a whitefly-transmitted monopartite geminivirus. Nucleic Acids Res 19:6763–6769

Mansoor S, Briddon RW, Zafar Y, Stanley J (2003) Geminivirus disease complexes: an emerging threat. Trends Plant Sci 8:128–134

Martin D, Rybicki E (2000) RDP: detection of recombination amongst aligned sequences. Bioinformatica 16:562–563

Moriones E, Navas-Castillo J (2000) *Tomato yellow leaf curl virus*, an emerging virus complex causing epidemics world wide. Virus Res 71:123–134

Nakhla MK, Maxwell DP, Martinez RT, Carvalho MG, Gilbertson RL (1994) Widespread occurrence of the eastern Mediterranean strain of tomato yellow leaf curl geminivirus in tomatoes in the Dominican Republic. Plant Dis 78:926

Navas-Castillo J, Sanchez-Campos S, Noris E, Louro D, Accotto GP, Moriones E (2000) Natural recombination between *Tomato yellow leaf curl virus-Is* and *Tomato leaf curl virus*. J Gen Virol 81:2797–2801

Navot N, Pichersky E, Zeidan M, Zamir D, Czosnek H (1991) *Tomato yellow leaf curl virus*: a whitefly-transmitted geminivirus with a single genomic component. Virology 185:151–161

Ogawa H, Uchikawa K (2004) Efficacy of physical control of tomato yellow leaf curl disease and silverleaf whitefly. Kyushu Plant Prot Res 50:72–76 (in Japanese)

Onuki M, Ogawa T, Uchikawa K, Kato K, Hanada K (2004) Molecular characterization and strain-specific detection of the *Tomato yellow leaf curl virus* occurring in Kyushu, Japan. Bull Natl Agric Res Cent Kyushu Okinawa Reg 44:55–77 (in Japanese)

Palmer KE, Schnippenkoetter WH, Rybicki EP (1998) Geminivirus isolation and DNA extraction. In: Foster GD, Taylor SC (eds) Plant virology protocols. Humana, Totowa, NJ, pp 41–52

Pico B, Diez MJ, Nuez F (1996) Viral diseases causing the greatest economic losses to the tomato crop. II. The *Tomato yellow leaf curl virus*. Sci Hort 67:151–196

Polston JE, Anderson PK (1997) The emergence of whitefly-transmitted geminiviruses in tomato in the Western Hemisphere. Plant Dis 81:1358–1369

Roberts S, Stanley J (1994) Lethal mutations within the conserved stem-loop of African cassava mosaic virus DNA are rapidly corrected by genomic recombination. J Gen Virol 75:3203–3209

Salati R, Nahkla MK, Rojas MR, Guzman P, Jaquez J, Maxwell DP, Gilbertson RL (2002) *Tomato yellow leaf curl virus* in the Dominican Republic: characterization of an infectious clone, virus monitoring in whiteflies, and identification of reservoir hosts. Phytopathology 92:487–496

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Ueda S, Kimura T, Onuki M, Hanada K, Iwanami T (2004) Three distinct groups of isolates of *Tomato yellow leaf curl virus* in Japan and construction of infectious clone. J Gen Plant Pathol 70:232–238