

Complete Nucleotide Sequence and Genome Analysis of Bipartite Tomato Yellow Leaf Curl Virus in Thailand

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ABSTRACT

The genome of Thai-isolate of tomato yellow leaf curl virus (TYLCV-THL), a bipartite geminivirus, was cloned and the nucleotide sequence was analysed. The genome designated component A and B are 2751 and 2737 nucleotides long, respectively. The component A contains seven open reading frames (ORFs) with coding potential for proteins of molecular weight more than 10,000 Daltons. The component B has totally three ORFs on both strands. Computer-based analysis to compare nucleotide sequence of TYLCV-THL DNAs and the predicted amino acids with other selected geminiviruses indicated high degree of similarity with geminivirus which infected tomato in Australia and Israel for the component A. The component B showed greater homology of amino acid sequence with those of cassava latent and squash leaf curl geminiviruses.

Key words : tomato yellow leaf curl virus, geminiviruses, nucleic acid sequence

INTRODUCTION

Tomato yellow leaf curl virus (TYLCV) in Thailand is classified into a member of geminivirus group, possessing two components of about 2.7 kb genomes (Attathom *et al.*, 1990, Rochester *et al.*, 1990). The virus is transmitted by whitefly and caused yellow leaf curl disease of tomato, *Lycopersicon esculentum* Mill. (Thongrit *et al.*, 1986). The disease was also recognized in the Mediterranean basin (Navot *et al.*, 1991), Sardinia and Italy (Kheyr-Pour *et al.*, 1991) but the causal virus had been characterized as monopartite geminivirus. Agroinoculation with the component A of TYLCV-Thailand isolate can cause systemic infection in plants, in the absence of the component B (Rochester *et al.*, 1990).

Here we report the DNA sequence and genome organization of TYLCV-Thailand isolate (TYLCV-THL). For sequencing the dideoxy chain termination method and an automated DNA sequencer were employed. The nucleotide sequence and amino acid sequence were compared with other published geminivirus genomes.

MATERIALS AND METHODS

Virus and DNA clones

TYLCV was purified from infected tomato tissues and viral single stranded DNA was extracted from virus particles. Circular double stranded DNA was synthesized and cloned into Bluescript plasmid at EcoRI site. Full length cloned DNAs of TYLCV components A and B were generated and designed as pTYLCA and pTYLCB (Attathom *et al.*, 1990; Chiemsombat *et al.*, 1990).

Nucleotide Sequencing

Double stranded DNA templates for nucleotide sequencing were prepared by CsCl purification. Fragments of DNAs for sequencing were obtained by using exonuclease deletion method and nucleotide sequences were determined by the dideoxy chain termination method (Sanger *et al.*, 1977) by Taq dye-primer sequencing kits (Perkin Elmer Cetus). The fluorescent-dye labeled DNA fragments were analysed by using an automated DNA sequencer, ABI model 373A. Sequences were further analysed and connected

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TYLCV-A SEQ.

5' 10 20 30 40 50 60
 GGTCAATCGG GTCCTCTCAA ACTTGGCTAT GCAATCGGGG AATGGGTCCT TATTATAGTT
 70 80 90 100 110 120
 GTGGACCTAA ATGGCATAAT TGTAAATAAG TGTATGAAAT TCAAAATTTA AAATTCATC
 130 140 150 160 170 180
 GTGGCCATCC GTATAATATT ACCGGATGGC CGCGATTITT TTAAGTGGT CCCCTTGATG
 190 200 210 220 230 240
 TGATATGTCA TCCAATCAGA ACACCTCTTG AAAGCCTAAT TATTTATGGT CCCCTATTTA
 250 260 270 280 290 300
 AGACTTAGTC CCCAAGTTTC GCGGAAATTC AAAATGTGGA TCCACTACTA AACGAATTTTC
 310 320 330 340 350 360
 CAGAAAACGT CCACGGTTTC CGTTGTATGT TAGCGGTGAA GTATCTGCAA GCGGTGAGAA
 370 380 390 400 410 420
 AGACTTATTC CCCTGATACT CTAGGGTTTG ATCTCATCCG TGATCTCATC GGTGTAATTC
 430 440 450 460 470 480
 GTGCGAAGAA CTATGTCGAA GCGTCCAGCA GATATTCTCA TTTCCACTCC CGTCTCGAAA
 490 500 510 520 530 540
 GTACGTGGCC GTCTGAACTT CGACAGCCCA TACAACAGCC GTGTGCTGT CCCCACTGTC
 550 560 570 580 590 600
 CGCGTCACAA AAGGGCAGGT ATGGAAGAAC CGACCTGCAT ACAGAAAGCC CAGGATCTAC
 610 620 630 640 650 660
 AGAATGTATA GAAGCCCTGA TGTCCCTAAG GGATGTGAGG GCCCATGTAA GGTCCAATCT
 670 680 690 700 710 720
 TTCGATGCGA AGAAGCAGAT TGGACATATG GGCAAGGTAA TATGTCTGTA TGACGTTACC
 730 740 750 760 770 780
 CGTGGTATTG GGCTTACTCA TCGAGTTGGC AAGCGTTTCT GTGTGAAGTC ACTTTATTTT
 790 800 810 820 830 840
 GTCGGGAAGA TCTGGATGGA TGAAAATATT AAGGTTAAGA ATCATACTAA CACCGTTTAA
 850 860 870 880 890 900
 TTCTGGATAG TTAGGGATCG CAGTCCTACT GGAACGCCCT ATGATTTTCA GCAGGTCTTT
 910 920 930 940 950 960
 AATGTATATG ATAATGAACC CAGCACTGCT ACTGTGAAGA ACACACGCGG TGATCGTTTC
 970 980 990 1000 1010 1020
 CAGGTTATAA GGAGGTTCCA GGCAACAGTT ACTGGTGGAC AATATGCAGC TAAGGAGCAG
 1030 1040 1050 1060 1070 1080
 GCGATTATTA GAAAGTTTAA TCGTGTAAAT AATTATGTAG TTTATAATCA CCAGGAAGCT
 1090 1100 1110 1120 1130 1140
 GGAAGATATG AGAACCATAC TGAAAATGCT TTGTGTTTAT ATATGGCATG TACTCATGCC
 1150 1160 1170 1180 1190 1200
 TCTAACCCCTG TGTATGCTAC TTTGAAAGTC AGGAGTTATT TCTATGACTC AGTGACGAAT
 1210 1220 1230 1240 1250 1260
 TCTATAAATAT TAAATTTTAT ATCATGTTCT TCAATTACAT CAATTGTTCC TCTTAATACA
 1270 1280 1290 1300 1310 1320
 TGTGACAATA CATGAGATAT TGCCCTAATT ACATTGTTTA TACTAATCAC GCCTAATCTA
 1330 1340 1350 1360 1370 1380
 TTTAAATATT TATTACATTG ATATTAAAT ACTCTTAAGA AACGCGAGGT CTGAGGATGT
 1390 1400 1410 1420 1430 1440
 AAATGAGTCC AGATTTTGCA GGCTAGAAAA CATTGTGTGA TCCCAACGCG TTTCCTCAGG
 1450 1460 1470 1480 1490 1500
 TTGTAGTTGA ACTGGATTGG TAACGTGATT ATGTCGTGGT TCCTCTGGAA TGGTCTCTCT
 1510 1520 1530 1540 1550 1560
 AGGTGCTTGG TTATCTTGAA ATATAGGGGA TTTTGGACCG TCCAGATATA TACGCCACTC
 1570 1580 1590 1600 1610 1620
 TCTGATTGAG TTGCAGTGAG TAGTTCCTCG GTGCGTGAAT CCAATTATTG GACAAATATG
 1630 1640 1650 1660 1670 1680
 GACAGCATATG CACAAGTACG AACATCCACA AGGTGATGCA ACTCTCCGTC GTCTGGTTGT
 1690 1700 1710 1720 1730 1740
 CCTCTTGGCT ATTCTGGTGT GCACCTTGAT AGGTACCTGA GTACAGTGGG CCTTCGAGGG
 1750 1760 1770 1780 1790 1800
 TGACGAAGAT CGCATTTTTT ATAGCCCACT TTCTAAGTGC GGAGTTCTTT TCCTCTTCCA
 1810 1820 1830 1840 1850 1860
 AGTACTCTTT ATAGCTGGAG TTTGGTCCAG GATTGCAGAG GAAGATAGTG GGAATTTCCG
 1870 1880 1890 1900 1910 1920
 CTITAAATTTG AACTGGTTTG CCGTATTGGT TGTGTCTTTG CCACTCCCTT TGGGCCCTCA
 1930 1940 1950 1960 1970 1980
 TGAACCTTTT AAAGTGTITT AGATAATGCG GATCGACGTC ATCGATGAGC TTGAACCAAG
 1990 2000 2010 2020 2030 2040
 CATCATTTAT ATACACTTTT GGACTTAAAT CTAAATGGCC ACACAGATAA TTATGTGGTC
 2050 2060 2070 2080 2090 2100
 CCAATGACCT AGCCCACTAT GTCTTCCCTG TTCTGCTATC ACCCTCAAATG ACTATAGTTA
 2110 2120 2130 2140 2150 2160
 TTGGTCTCAA TGGCCCGCGA CGACTGAGAA CATTATTACA AGCCCACTCT TCAAGTTCTT
 2170 2180 2190 2200 2210 2220
 CGGGAACCTG ATTGAAGAAA GAAGAAGAAA AGGGAGAAAT ATATTCTCTT ATTGGAGGAG
 2230 2240 2250 2260 2270 2280
 TAAAAATCCT ATCTAAATTA GAATTTAAAT TGTGAAATTT TAAAAAATA TCTTTAGGGG
 2290 2300 2310 2320 2330 2340
 CCTTTTCCCT CAGTATATTG AGGGCCGAAG CCTTGGACCC TGAATTGATT GCCTCGGCAT
 2350 2360 2370 2380 2390 2400
 ATGCGTGGTT GGCAGATTGG CAACCTCCTC TAGCCGATCT TCCATGATT TGGAAAAATC
 2410 2420 2430 2440 2450 2460
 CATGATCAAG CAGTCTCCG TCTTTTCCA TGTATGCTTT AACATCTGTT GAGCTTTTAG
 2470 2480 2490 2500 2510 2520
 CTCCTCGAAT GTTCGGATGG AAATGTGCTG ACCTGGTTGG GGATGTGAGA TCGAAGAAATC
 2530 2540 2550 2560 2570 2580
 GGTTATTTTG GCATTTGAAT TTTCTTCGA ATTGGATGAG GAGATGCAGC TGAGGAGTCC
 2590 2600 2610 2620 2630 2640
 CATCTTCATG GAGTTCCTCG CAGATTCTGA TGATTAATTT ATTAGTTGGA GTTGATAGGT
 2650 2660 2670 2680 2690 2700
 TTAATATTGG GGAGAGTGCT TCTTCTCTT TGGTGAGTGA GCAGTGTTGG TATGTGAGGA
 2710 2720 2730 2740 2750 2760
 AATAATCTT GGCATTATT AGAAATTTCT TTGAAGGAGG CATATTGACT T 3'
 C1 start

TYLCV-B SEQ.

	10	20	30	40	50	60
5'	GGTCAATCGG	TGTCTCTCAA	ACTTGGCTAT	GCAATCGGGT	GCTGGTGTCT	TATTTATACC
	70	80	90	100	110	120
	TGGACACCAA	ATGGCATAAT	TGTAATTTAG	TAAATGTAAAT	TCAAAAATCA	AAATCCAATC
	130	140	150	160	170	180
	GTGGCCATCC	GTATAATATT	ACCGGATGGC	CGCGATTTTT	TTCAAGTGGT	CCCACCACCTG
	190	200	210	220	230	240
	ACATCTCAGC	CCCACCTGGG	AAATCATAGC	CGTTGAATCG	TAACACGCGT	TACATATCCA
	250	260	270	280	290	300
	ATTGATTTCG	AGTGATTTTA	TTCTGACTAT	AAATGTGATG	TTATGGCGTC	ATTTGTTGTT
	310	320	330	340	350	360
	GTGAAATAGC	ATGAGAGTTC	CTATTAGACG	ACCTTTTGCT	TAAACATGAA	CCCCCTCTCC
	370	380	390	400	410	420
	GGCGGTTTCT	ATGTCGGTTA	TAATTATCCC	TACGCCAATT	ACTTTGGCAG	GAGGGTTGGC
	430	440	450	460	470	480
	AACCGTGAT	ATGGAATGCC	CTTTGGTAGT	ACCACCTCTG	TCCGGCGACC	AATTAGAAGT
	490	500	510	520	530	540
	ACAGTTCGGA	GGAATTTATT	TTCCGACCAG	TCTTCTTCAG	GTAACAAGAG	CGGTAAATCT
	550	560	570	580	590	600
	ATTGAGGAAG	TGCATGATGG	TTCTGACTAT	CTTCTTGTTA	ATAACACTTC	GAAGGTGTCTG
	610	620	630	640	650	660
	TATATTAGTT	ATCTCTCTCT	TAGTCGGTCG	GAAATTTGGTA	ACCGTCTTGA	CGCATTTGTC
	670	680	690	700	710	720
	AAGATTTTGG	GATTTAATGT	TTCTGGTTCA	GTTGCTGTGA	GACATCTGGA	ACAGCGTGCC
	730	740	750	760	770	780
	ACTGGAGCAA	GTCAAGGTAT	CCATGGCATA	TATTGCACAG	CTGTTGTACG	TGACAAGCGA
	790	800	810	820	830	840
	CCATGTCAGT	TCTCCGCTGT	GGAGCCTATT	GTGCCATTTC	CGGAGTTTAT	TGGTCTGGAG
	850	860	870	880	890	900
	AAGATGGCAT	GCTCCTCATT	ACGTGTTAGG	GATATTCATA	GGAGTAGGTT	TAGTCCAGTT
	910	920	930	940	950	960
	TACCAGAAGA	AAGTTGTTGT	TAACAGCTCC	CTTCCGACCC	ATGTTTTTAA	GTTTAAATATC
	970	980	990	1000	1010	1020
	CTGTTAAGTT	TAATAGGTTT	CCGTTCTGGG	TGTCATTTAA	AGATACCTCT	GATTCTGAGC
	1030	1040	1050	1060	1070	1080
	CAAGTGGCCG	GTACAGCAAC	GTATCTAAGA	ATGCTCTCAT	AGTTTATTAT	TTGGCTTTGT
	1090	1100	1110	1120	1130	1140
	GACACAAATG	TAACCTGAGA	TTCGTATGTA	AAGTACGATT	TGAACATCAT	TGGATAAATA
	1150	1160	1170	1180	1190	1200
	AAATTAATAT	TGCTTTGCAT	TTGAAGATAC	ATTACTCCCT	TCCATACATA	GTTCAACTGT
	1210	1220	1230	1240	1250	1260
	TTTTTTAATA	ATATTAATT	CATCGTCATT	TACTTTGTTG	CTTCCTGCAA	CAACCTCCGA
	1270	1280	1290	1300	1310	1320
	CGCCGAAGGG	CCTGGATCGA	GAGTTGCATC	TTGTAGCTGA	TGCAAAATGTC	TATATGGGTA
	1330	1340	1350	1360	1370	1380
	TTCTCGTTTC	AATGCGTTCA	CGACTGTGCT	TGCGGAAGCC	CAAGTATGCG	CTTGTTGGAAT
	1390	1400	1410	1420	1430	1440
	GGCATCTGTA	GTGTATCTGG	AAGACTGTGA	CCTCATGTTT	GATAGGGCTT	GGACTGGTTT
	1450	1460	1470	1480	1490	1500
	CCTTGATACC	TTGGATTGAG	GCACATGCCA	GAACTCTATG	TGGGTCAATG	TATACGCTTT
	1510	1520	1530	1540	1550	1560
	TGACAGTATT	TCAATCTTGG	GGGATTTAAA	TGTTATCTCC	GAGGACTGTT	TTGCAGATGA
	1570	1580	1590	1600	1610	1620
	CATCTTTAAT	TTTCTTTCGA	TCCTGCAGAA	GTGAACTCCA	TTTACCACGT	TAGTGTCTGC
	1630	1640	1650	1660	1670	1680
	CACTCGGTAC	AGTACTCTCC	ATGGATTGGG	GTCTTTGGGG	GAGAAATAGG	AAGAGGAGTA
	1690	1700	1710	1720	1730	1740
	GTAGTGATAT	TTGCAGTTGC	ACCCTATGGG	TATAGTGAAC	TCAGCTGTCT	TCGAGTCACC
	1750	1760	1770	1780	1790	1800
	TTCTGTGTAAC	CTTGTGTCGT	GCATTTCTAT	GACCACATGA	CCAGTTGCAT	TTACAGGGAC
	1810	1820	1830	1840	1850	1860
	TTGGTTCCTG	TATTTGGAGGA	TGACGTGGTC	AATTCGTAGA	CACCTTTCCCA	AAAGCAATGA
	1870	1880	1890	1900	1910	1920
	CAATTTTGA	TGACCGTGG	AGGGAAACAA	CAGCGTAACT	TCTGTTTTTT	CATTGTACAG
	1930	1940	1950	1960	1970	1980
	CTGAAATCCA	GTCTATCCG	ACGTCCTGAT	ATGCAATATT	GTTAGTCTCT	GACTCCATTA
	1990	2000	2010	2020	2030	2040
	ATTGTTGATG	GCCAATAAAA	TAATGATAAT	TCCATGTTTT	TATAGACTTT	TGAAGAGGCT
	2050	2060	2070	2080	2090	2100
	GTCAACATTAG	TGGAATGTGA	CTAGTCGAGT	TTTTGTCATT	AAAATGTACT	ATCTCAAAGG
	2110	2120	2130	2140	2150	2160
	ACCAATGAGA	TACGTCCACG	TATGTGCTAG	ACCTGACCAAG	AGATAAGGTT	AAATACTTGA
	2170	2180	2190	2200	2210	2220
	ATGTGGTTCA	GCCACGTGCG	CAATATGATA	ATGGAATAAC	GCTGTGTCCA	CTAGCAGGTC
	2230	2240	2250	2260	2270	2280
	TTGTTGTTTT	AAGTTGTTAA	ATATAGCATT	TTGAGATATT	TGTCCAATAT	TAATAATATA
	2290	2300	2310	2320	2330	2340
	ATTAAAAATT	AAAGGAAGTA	ATAGAAGTTA	TGTAAGTGGT	ACTTTAGTAC	CACAAATTTAA
	2350	2360	2370	2380	2390	2400
	ACCCAAATTG	AGAAGATTCC	AGCCTCAATT	AAAGAACGAT	TTGTAATTCG	TTATGAGTTA
	2410	2420	2430	2440	2450	2460
	TCGTGAAATG	GACAACCTTAG	ACCAATTGTT	AACCTGATCCA	ACAATCTAAT	TACGAGAAAA
	2470	2480	2490	2500	2510	2520
	CAGAAAAAC	ATACCTGAAC	ATCTTGAGAA	AACCCGCGCG	CAGCGGCTGT	GTGCCGAAAT
	2530	2540	2550	2560	2570	2580
	ATATTAGAA	TTCCAGAACT	GTAAATCGCT	AATTATCATA	TCTAATTTTA	AAGTGAAGTA
	2590	2600	2610	2620	2630	2640
	TCGTAAATCA	GTGTCATGCA	AATGGTATGT	ATCTCCTGCA	GCTAACATAA	TTAAAGTTAA
	2650	2660	2670	2680	2690	2700
	CAACATTAAT	GCAATATAAT	TATTGCATAT	TTACAGAAAA	TACTAAAAAT	ACATGTTTAG
	2710	2720	2730			
	AGAGAGAAAG	TCTAGAGAGA	AGGAAGAGCG	ATTGACT	3'	

Figure 1 Nucleotide sequence of TYLCV-THL component A and B. The veral strand sequence is given for both components. The first base at 5' end of the 200 base-common region is designed as nucleotide 1.

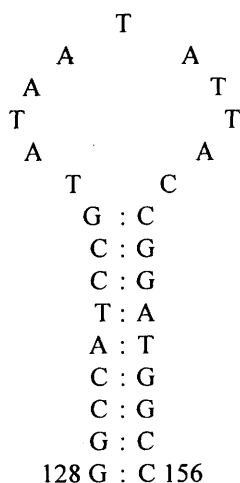


Figure 2 Nucleotide sequence of 29 base hairpin structure within TYLCV-THL component A and B.

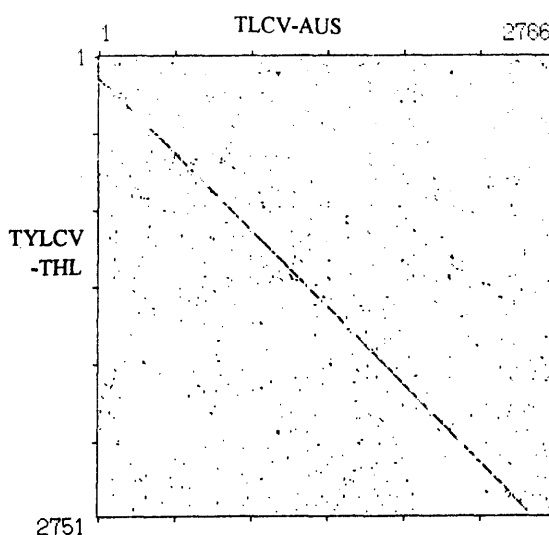


Figure 3 Computer plot of a comparison of the nucleotide sequences of TYLCV-THL component A and TLCV-AUS genomes (Check size :15, Match base no.: 11).

Table 1 Open reading frame in TYLCV-THL DNA, and size of putative translated products.

ORF*	Frame	Nucleotide		Amino acid	Protein M.W. (Da)
		Start	Stop		
Component A					
V1	1	433	1201	256	29,698
V2	3	327	609	94	10,730
C1	3	2743	1656	361	40,847
C2	2	1755	1347	136	15,567
C3	2	1604	1202	134	15,906
C4	1	2589	2292	99	11,342
C5	1	741	408	111	12,409
Component B					
V1	1	347	1061	238	26,555
V2	2	371	698	121	12,238
C1	1	1857	1146	237	26,680

*V and C denote viral strand and complementary strand ORFs, respectively.

by using Seq. Ed computer software. The nucleotide sequences of TYLCV-DNA components A and B were compared with other reported geminivirus DNA sequences using the DNASIS program (Hitachi, Co. Ltd.).

RESULTS AND DISCUSSION

Nucleotide sequence of TYLCV-THL genomes

The sequence of both viral genomes in pTYLCA and pTYLCB are shown in Figure 1. The

component A comprises 2751 nucleotides and component B comprises 2737 nucleotides. The first base of the 5' end of the intergenic non-coding region (Lazarowitz, 1987) was designed as nucleotide 1. This region occupied 326 nucleotides, in which a conserved 29 base-hairpin structure was detected (Figure 2). The results of Harr Plot construction showed that TYLCV-THL component A is most similar to the component A-DNA of tomato leaf curl virus -Australia isolate (TLCV-AUS) (Dry *et al.*, 1993) and tomato yellow leaf curl virus-Israel isolate (TYLCV-ISR) (Navot *et*

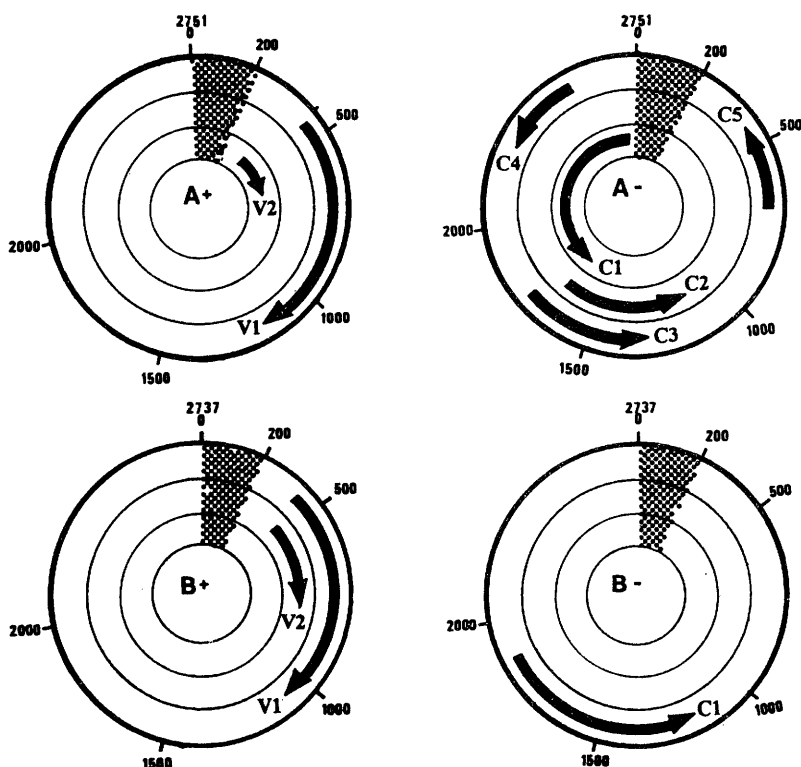


Figure 4 Potential coding regions in TYLCV-THL component A and B. All ORFs start with an ATG codon and coding for proteins with molecular weight more than 10,000 Da are shown. The common region is shaded.

TYLCV-THL-40		1-LLGKCLRTHUULQYRN	19
CLV	1 MDTSUPUISSDYIHSARTEYKLTNDESPITLQFPSTLERTR	IRINGCHKUDHUUEYRN	60
20	DUPNH-TG-UUIEMHETRU-EGISKQAEFTIPIGNCNIHYVSSSYFSPKIPK-PARAL		79
61	DUAFNQGGS-UUTITRDRISLEQDQAGFTFPIGNDLHYFSASVFSIDI-KUPLQLL		120
80	VRUDDTNILINGLHFCRNGGKLMHAAGSSETTFK-SKTIETLSKAYNMTH-IDFWUPQ		139
121	VKUEDSNKNGITRAQIKKIKLSAH-HSTDIKEKPT-IKILSKDYG-EDCUDFMSUGK		180
140	SKUS-RKPUQ--ALSNMRSGSSPK-T-T-DEIPQG--HT--WAS--E-S-TU-UNG		199
181	PK-EIPRLIGNEPGIYDI-GPKIRFITUQ-GETWATKSTIGRYTSMRYIRNPDIID		240
200	L---N---E-EVRYH-LHQLGDTLDPGPS-N-SEUUGSGSKNDDOIN-IKKTUELCYE		259
241	SSSKCYTSEAEPLRGLHQLPEPSLIPEDSISCTGSSSKKE-IES-ITEQ---TUNKCLI		300
260	GSNUSSNAK-DT.....		319
301	CHR-SS-SHKDL.....		360

Figure 5 Amino acid homology between translated products coded for by the complementary strand ORFs of component B of TYLCV-THL and CLV DNA 2.

Table 2 Number of amino acids and sequence homology percentage between putative polypeptide products of TYLCV-Thailand isolate, TYLCV-THL, and selected whitefly-transmitted geminiviruses. TLCV-AUS: tomato leaf curl virus-Australia isolate, TYLCV-ISR : tomato yellow leaf curl virus-Israel isolate, TGMV: tomato golden mosaic virus, SqLCV: squash leaf curl virus, MYMV:mungbean yellow mosaic virus, CLV:cassava latent virus, BGMV: bean golden mosaic virus, AbMV:Abutilon mosaic virus.

ORF of TYLCV-THL (no.of amino acid)	% Amino acid homology (no.of amino acid)							
	TLCV -AUS	TYLCV -ISR	TGMV	SqLCV	MYMV	CLV	BGMV	AbMV
Component A								
V1(256)	87(256)	84(260)	85(247)	85(251)	84(244)	84(258)	84(261)	82(241)
V2(94)	72(115)	84(116)	-	46(98)	-	86(113)	43(114)	-
C1(361)	89(362)	88(357)	39(156)	67(316)	83(361)	82(358)	41(158)	39(115)
C2(136)	79(135)	82(134)	65(132)	65(134)	65(134)	76(134)	66(132)	79(135)
C3(134)	74(135)	75(135)	69(129)	65(131)	48(99)	70(135)	59(172)	63(129)
Component B								
V1(238)	-	-	52(256)	52(283)	52(256)	51(256)	53(256)	55(257)
C1(237)	-	-	84(184)	64(293)	59(298)	66(298)	64(293)	61(292)

al., 1991) with 74% and 69% total nucleotide similarity, respectively (Figure 3), despite that both latter geminiviruses had been reported to possess only one component which correspond to the component A of TYLCV-THL.

Alignment of the sequence of TYLCV-THL component B with those of bipartite geminiviruses showed that the B component of TYLCV-THL and tomato golden mosaic virus (TGMV) (Hamilton *et al.*, 1984) as well as squash leaf curl virus (SqLCV-e) (Lazarowitz and Lazdins, 1991) were about 55% nucleotide homology. In addition, the conserved hairpin loop structure of component B of the latter showed only two bases differences from that of TYLCV-THL as following ;

GGCCATCCGTA-TAATATTACCGGATGGCC.

C-A

Coding region and putative translation products

The viral strands of both components A and B of TYLCV-THL have two open reading frames (ORFs) with coding potential for proteins of molecular weight more than 10,000 daltons (Da). All ORFs are described in Table 1 and their organization along the genomes are depicted in Figure 4.

Direct comparison of individual ORFs of TYLCV-THL with those of other mono- and bi-partite geminivirus genomes showed a striking high degree of homology to TLCV-AUS and TYLCV-ISR, especially an ORF V1 which is the viral coat protein (Table 2). The only exception is that an ORF V2 of 10,730 Da of TYLCV-THL is more closely related (86% homology) to an ORF V2 of 13,101 Da of cassava latent virus (CLV) (Stanley and Gay, 1983) rather than those of tomato-infected geminiviruses.

The presence of four ORFs in the complementary strand of component A is very similar to those observed for whitefly transmitted bipartite geminiviruses except for bean golden mosaic virus, BGMV (Howarth *et al.*, 1985), mungbean yellow mosaic virus, MYMV (Ikegami, 1988), and TGMV. The largest ORF (C1) encoded for protein molecular weight of 40,789 Da which located on the complementary strand of the A component showed 89% and 88% amino acid homology with C1 ORF of TLCV-AUS and TYLCV-ISR, respectively. This C1 polypeptide is believed to be a protein involving the replication of each viral genomic components (Elmer *et al.*, 1988).

Based on the above comparison, both on the nucleic acid sequences and the predicted amino acid

sequences, TYLCV-THL showed greatest homology with TLCV-AUS and TYLCV-ISR when compared their component A sequences.

The component B of TYLCV-THL has two ORFs in the viral strand and one ORF in the complementary strand. Unlike component A, the viral strand ORF showed comparatively low amino acid homology with those of other geminiviruses, ranging from 51-55% for CLV, SqLCV, MYMV, TGMV, BGMV and Abutilon mosaic virus, AbMV (Frischmuth *et al.*, 1990), descendantly. It is also very interesting that the ORF of complementary strand exhibited high amino acid homology with those of CLV (66%), SqLCV (64%), BGMV (64%) and AbMV (61%). A significant homology is found with TGMV (84%), although this translated TGMV peptide (184 amino acids) is shorter than TYLCV-THL (237 amino acids). Extensive amino acid homology between translated products coded for by C1 of TYLCV-THL and CLV-DNA2 is shown in Figure 5.

Considering the biological properties, especially host range, with other tomato infected geminiviruses according to the data previously published (Thongrit *et al.*, 1986; Mansour and Al-Musa, 1992; Dry *et al.*, 1993), TYLCV-THL is more similar to TLCV-AUS than TYLCV-ISR. Unlike TYLCV-ISR, TYLCV-THL could not infect legume and cotton plants. As it is proposed that the component B is needed for systemic spread (Elmer *et al.*, 1988), it will be very useful to study gene function of the component B of TYLCV-THL and the difference between this bipartite tomato-infecting geminivirus and other monopartite ones which infected tomato and some other hosts as well.

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