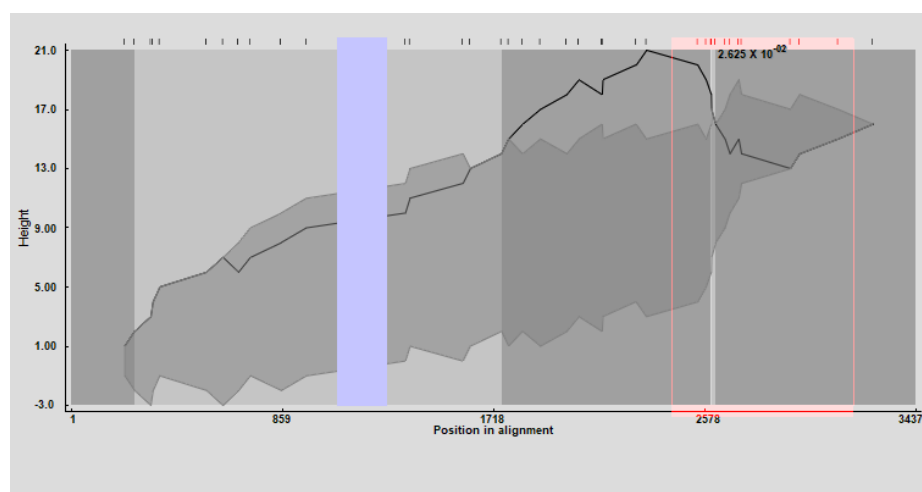


**Figure S1.** The second recombination event detected among 38 full-length genomic sequences of STV.



**Figure S2.** The third recombination event detected in 03 full-length sequences of STV.

**Table S1.** Tomato samples collected from different regions of China and Pakistan were employed in this study for the detection of STV and confirmation of its infection dynamics under single and mixed infection conditions with its variable rates of infections.

Sample locality	GPS (Latitude, Longitude)	Sample designation	Type of sample (Production)	Symptoms	Suspected viral infections	Rate of ToYSD infection (%) <sup>1</sup>		Detection and confirmation of STV		Confirmed rate of STV infection (%) <sup>1</sup>	Percentage of STV single infection	Percentage of STV mixed infection in different combinations of viruses				
						ToYSD Incidence <sup>a</sup>	ToYSD Prevalence <sup>b</sup>	RT-PCR	Sanger			STV	STV+TYLCV	STV+TYLCV+ToCV	STV+TYLCV+ToCV+TSWV	STV+TYLCV+ToCV+ToMV
Beijing, China	Fangshan	FS-08122017-1	Leaf (Greenhouse 1)	Typical yellowing, leaf curling, mild crinkling, and deformations	TYLCV, STV, ToCV	75.00	80.00	STV, TY-LCV	STV, TY-LCV	58.33	25.00	16.66	08.33	00.00	08.33	
		FS-08122017-2		Leaf yellowing, chlorosis, mild crinkling, and	TYLCV, STV, ToCV, TICV			TY-LCV, STV, ToCV	TY-LCV, STV, ToCV							

[illegible]

[illegible]

				ToMV, PVY		
TZ- 12042018 -3			Typical yellowing, necrotic patches, and dark green leaves	TYLCV, STV, ToCV, TSWV, CMV, ToMV, TICV	STV, ToCV, TSWV, TY- LCV, ToMV, TICV*	STV, TSWV, TY- LCV, ToMV, TICV*
TZ- 12042018 -4			Leaf yellowing, deformed, and reduce fruit size	TYLCV, STV, ToCV	STV	STV
TZ- 12042018 -5	Leaf (Green-house 2)		Leaf chlorosis, yellowing, mosaic, and mottling	TYLCV, STV, ToCV	STV, TY- LCV, ToCV	STV, TY- LCV, ToCV
TZ- 12042018 -6			No obvious symptoms look healthy sample	STV	STV	STV
TZ- 12042018 -7			Stunted plant growth having alternate patches on leaves	STV, ToMV, CMV	STV	STV
TZ- 12042018 -1			Leaf yellowing, crinkling, deformation	STV, ToMV, ToCV, TYLCV, TICV	STV, ToCV, TY- LCV	STV, ToCV, TY- LCV
TZ- 12042018 -2			Leaf chlorosis, mosaic, and mottling	TYLCV, ToCV, CMV, ToMV, PVY	-	-
TZ- 12042018 -3	Leaf (Green-house 3)		Leaf chlorosis and deformation	TYLCV, STV, ToCV, CMV, ToMV	STV	STV
TZ- 12042018 -4			Leaf yellowing, chlorosis, mild crinkling, and fruit deformations	TYLCV, STV, ToCV, TICV	STV, TY- LCV, ToCV	STV, TY- LCV, ToCV
TZ- 12042018 -5			Mild crinkling, and fruit deformations	TYLCV, ToCV, ToMV, CMV	-	-
TZ- 18122019 -1				TYLCV	-	-
TZ- 18122019 -2	Seed, F1 generation (Seed source 1)	Directly obtained from the seed source		TYLCV, STV	STV, TY- LCV	STV, TY- LCV
TZ- 18122019 -3				STV, TYLCV	STV	STV
TZ- 18122019 -4				TYLCV	TY- LCV	TY- LCV

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	SY- 15062018 -7	Leaf curling infested with whiteflies	STV, ToCV TYLCV	-	-										
	SY- 15062018 -8	Healthy sam- ple	Control	-	-										
Miyun	MY- 15062018 -1	Typical yel- lowing, chlo- rosis between veins, and having no crinkle	TYLCV, STV, ToCV, CMV			TY- LCV, STV	TY- LCV, STV								
	MY- 15062018 -2	Leaf (Green- house 1)	Typical yel- lowing, chlo- rosis between veins, and having no crinkle	TYLCV, STV, ToCV, CMV		TY- LCV, STV, ToCV	TY- LCV, STV, ToCV								
	MY- 15062018 -3		Mild mosaic symptoms	ToMV, CMV, PVY		-	-								
	MY- 15062018 -4		No obvious symptoms look healthy sample	Control		-	-								
	MY- 15062018 -1		Mosaic, green island, and mild crinkle	ToCV CMV, PVY		-	-								
	40°22'36.9 5"N,		Leaf chlorosis	TYLCV, STV, ToCV, CMV	58.33	60.00	TY- LCV	TY- LCV	33.33	08.33	16.66	08.33	00.00	00.00	
	116°50'35. 04"E		between veins and having no crinkle	TYLCV, STV, ToCV, CMV											
	MY- 15062018 -3		Leaf yellow- ing, chlorosis, and having no crinkle	TYLCV, STV, ToCV, CMV			STV	STV							
	MY- 15062018 -4	Leaf (Green- house 2/3)	Leaf yellow- ing, chlorosis, and having no crinkle	TYLCV, STV, ToCV, CMV			-	-							
	MY- 15062018 -5		Leaf yellow- ing, chlorosis, and having no crinkle	TYLCV, STV, ToCV, CMV			TY- LCV, STV	TY- LCV, STV							
	MY- 15062018 -6		Leaf curling and crinkling	ToMV, ToCV, TYLCV			TY- LCV	TY- LCV							
	MY- 15062018 -7		Leaf yellow- ing, crinkling, and defor- mation	STV, ToMV, ToCV, TYLCV			-	-							
	MY- 15062018 -8		Leaf crinkling and defor- mation	ToMV, ToCV, TYLCV			TY- LCV	TY- LCV							
Pinggu	PG- 08112018 -1	Leaf (Green- house 1)	Leaf crinkling and defor- mation with whiteflies in- festations	TYLCV, ToCV, TICV, TSWV	57.14	60.00	-	-	35.71	14.28	14.28		07.14	00.00	
	PG- 08112018 -2		Leaf yellow- ing and fruit deformation	STV, TSWV			STV	STV							
	PG- 08112018 -3		Leaf yellow- ing and fruit deformation	STV, TSWV			-	-							

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			CMV, PVY											
			TYLCV, STV, ToCV, CMV ToMV, PVY											
			YQ- 27062019 -7	Leaf (Green- house 3)	No symptoms (health sam- ple)			-	-					
<u>Beijing, China</u>														
[Total harvested samples= 109, Negative= 45 samples, STV infection= 21 samples, TYLCV infection= 13 samples, TSWV infection= 1 sample, STV+TYLCV infection= 14 samples, STV+ToCV infection= 1 sample, STV+TYLCV+ToCV infection= 8 samples, STV+TYLCV+ToCV+TSWV infection= 2 samples, STV+TYLCV+ToCV+TSWV+ToMV infection= 2 samples, STV+TYLCV+ToCV+ToMV infection= 1 sample, TYLCV+ STV+ ToMV infection= 1 sample]														
*Samples, FS-08122017-8 and TZ-12042018-3, were subjected to the Next generation sequencing technology and STV was detected with multiple viruses (STV, TSWV, TYLCV, ToCV, ToMV, and TICV).														
19.26 12.84 07.33 01.83 01.83														
<u>Rate of infections</u>														
Negative= 41.28 %														
STV single infection= 19.26 %														
STV mixed infection= 26.60%														
STV+TSWV+TYLCV+ToCV+ToMV+TICV= 01.83%														
			FSD- 13022019 -Nagina	Seed (ARRI, Green- house 1)	Pronounce chlorotic, yel- lowing, and leaf curling	TYLCV, STV		STV, STV, TY- TY- LCV LCV						
			FSD- 13022019 -Nagina		Pronounce chlorotic, yel- lowing and leaf curling	STV, TYLCV		TY- TY- LCV LCV						
			FSD- 13022019 -Rio Grande	Seed/ Leaf (ARRI, Green- house 2)	Emerging leaves becom- ing yellow, mild mosaic and crinkle	STV, TYLCV, ToCV, ToMV, CMV		STV, STV, ToCV, ToCV, TY- TY- LCV, LCV, ToMV ToMV *						
			FSD- 13022019 -Thorgal	Seed/ Leaf (ARRI, East green- house)	Leaves are be- coming smaller and darker in- fested with whiteflies and mites.	TYLCV, ToCV, ToMV, CMV		- -						
Pun- jab, Paki- stan	Faisala- bad	31°27'1.32"N, 73°8'5.86"E	FSD- 13022019 -Morgal		Typical chlo- rosis, yellow- ing and leaf curling	STV, TYLCV, ToCV, ToMV, CMV, PVY		TY- TY- LCV LCV						
			FSD- 13022019 -SBS-292	Seed (PPRI, South green- house)	Typical chlo- rosis, yellow- ing and leaf curling	STV, TYLCV,		STV, STV, TY- TY- LCV LCV						
			FSD- 13022019 -GSL- 198	Seed/ Leaf (PPRI, South green- house)	Leaf yellow- ing, mild mo- saic mottling	STV, TYLCV, ToCV, ToMV, CMV, PVY		STV, STV, TY- TY- LCV LCV, ToCV ToCV						
			FSD- 13022019		Leaf yellow- ing, mild mo- saic mottling	STV, TYLCV, ToCV,		STV, STV, TY- TY- LCV LCV						

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emerging leaves					
LHR-09062020-Rutgers			STV, TYLCV	-	-
LHR-09062020-Hybrid	Seed (South-ern	Directly ob- tained from	STV, TYLCV	STV, TY- LCV	STV, TY- LCV
LHR-09062020-Early stone	Army vegetable Farm)	the seed source	STV, TYLCV	STV	STV
LHR-09062020-Rio Grande			STV, TYLCV	TY- LCV	TY- LCV
LHR-11062020-1		Leaf curling and deformation with severe infestation of white-flies and mites	ToCV, ToMV, TYLCV, PVY, CMV	TY- LCV	TY- LCV
LHR-11062020-2	Leaf (Western vegetable garden 1)	Leaf curling and deformation with severe infestation of white-flies and mites	STV, ToCV, ToMV, TYLCV, PVY, CMV	-	-
LHR-11062020-3		Plant stunting, leaf curling and yellowing, and reduced fruit size	STV, ToCV, ToMV, TYLCV	STV	STV
LHR-11062020-4		No obvious symptoms	STV	-	-
LHR-11062020-5	Leaf (Western vegetable garden 2)	Typical yellowing, leaf curling, crinkling, and fruit deformations	TYLCV, STV, ToMV, CMV, ToCV	TY- LCV, STV, ToCV	TY- LCV, STV, ToCV

Punjab, Pakistan

[Total harvested samples= 44, Negative= 18 samples, STV infection= 5 samples, TYLCV infection= 6 samples, STV+TYLCV infection= 7 samples, STV+TYLCV+ToCV infection= 6 samples, STV+TYLCV+ToCV+ToMV infection= 2 sample]

\* FSD-13022019-Rio Grande sample was also subjected to the Next generation sequencing technology to identify the STV virus from Pakistan.

11.36 15.90 13.63 00.00 00.00

#### Rate of infections

Negative samples= 40.90%

STV single infection= 11.36%

STV mixed infection= 34.09%

STV+TYLCV+ToCV+ToMV infection= 04.54%

**Abbreviations:** AARI, Ayub agricultural research institute; CMV, cucumber mosaic cucumovirus; GPS, global positioning system; PPRI, plant protection research institute; PVY, Potato potyvirus Y; STV, southern tomato amalgavirus; ToCV, tomato chlorosis crinivirus; TICV, tomato infectious chlorosis crinivirus; ToMV, tomato mosaic tobamovirus, TSWV, tomato spotted wilt orthotospovirus; TYLCV, tomato yellow leaf curl begomovirus; ToYSD, tomato yellow stunt disease; VRI, vegetable research institute. <sup>1</sup> The rate of ToYSD infection (prevalence and incidence) was calculated by the following equations: <sup>a</sup>ToYSD prevalence =  $\frac{x}{y} \times 100$ , where X is the number of sample production localities (greenhouses/fields) with visible ToYSD symptoms; Y is the total number of sample production localities observed in a region [26]. <sup>b</sup> ToYSD incidence =  $\frac{(N-n)}{N} \times 100$ , where N is the total

number of samples under observation, and n is the total number of healthy samples without ToYSD symptoms and viral infections [26].

**Table S2.** Primer sequences used in this study for the detection of different plant viruses and amplification as well as molecular cloning of STV.

Primers used for the detection of multiple viruses						
Primer name	Detection of virus	Primer Sequence (5'-3')	Amplicon length	Annealing temperature	Remarks	Reference
STV-F STV-R	Southern tomato amalgavirus	CGTTATCTTAGGCGTCAGCT GGAGTTTGATTGCATCAGCG	448bp	53°C	A fragment covering a conserved region of p42 and RdRp at the position of ribosomal frame shifting.	[8]
ToCV-16-F ToCV-481-R	Tomato chlorosis crinivirus	GGTTTGGATTTTGGTACTA-CATTCACT AAACTGCCTG-CATGAAAAGTCTC	466bp	54 °C	An amplicon covering Heat shock protein 70 homolog (HSP70h).	[28,34]
TICV-32-F TICV-532-F	Tomato infectious chlorosis crinivirus	TCAGTGCCTACGTTAATGGG CACAGTATACAGCAGCGGCA	501bp	55 °C	A fragment corresponding to part of the coding sequence of the HSP70h, excluding the phosphate 1 and 2 motif.	[31]
ToMV_527-F ToMV_843-F	Tomato mosaic tobamovirus	CGAGAGGGGCAACAAACAT ACCTGTCTCCATCTCTTTGG TAATATTACCKGWKGVCSC	318bp	56 °C	An amplicon covering a portion within the replicase gene.	[29]
TYLCV/IR-F TYLCV/AV1-R	Tomato yellow leaf curl begomovirus	TGGACYTTRCAWGG-BCCTTCACA (B=C, T or G, K=G or T, R=A or G, S=C or G, V=A, C or G, W=A or T, Y=C or T)	~500bp	54 °C	Adopted from universal degenerate primers PA and PB corresponding to portion of the intergenic region (IR) and the AV1 gene of the DNA-A.	[30,32,33]
Tospovirus_RdRp-F Tospovirus_RdRp-R	Tomato spotted wilt orthotospovirus	CCTTTAACAGT(A/T/G)GAAACAT CAT(A/T/G)GC(A/G)CAAGA(A/G)TG(A/G)TA(A/G)ACAGA	~800bp	50 °C	Adopted from degenerate primers gL3637 and gL4435c, covering a part of RdRP from L-RNA of orthotospovirus.	[27]
Primers designed for the amplification and molecular cloning of STV genome						
Primer name	Amplification	Primer Sequence (5'-3')	Amplicon length	Annealing temperature	Remarks	
STV-F1 STV-R1	Amplification of Southern tomato amalgavirus insert part A	ggaagttcatttcattggagaggGA-TAAATTTAGTAAGCTACCTAGC CTTGATCTCTCCGCAG-TATATTA	1880bp	62 °C	Overlapping pairs of primers, homologous to the vector plasmid (pCB301-2-HDV), were designed for the amplification of STV genome.	
STV-F2 STV-R2	Amplification of Southern tomato amalgavirus insert part B	GTGAGGCGGTTAA-GAAGTTTACAG gaggtggagatgccatgccaccGAA-GACGCGCTACTCTAATAACAG	1681bp	65 °C		
pCB301 backbone-F pCB301 backbone-R	Amplification of plasmid pCB301-2μ-HDV	CTGTTATTAGAG-TAGCGCGTCTTCgggtcggcatggcatctccactc GCTAGGTAGCTTACTA-AATTTATCctctccaaatgaaatgaacttc	7838bp	66 °C	A pair of primers, homologous to STV, were designed to linearize the plasmid (pCB301-2μ-HDV) between the CaMV 35S promoter and the HDRz sequence.	

**Table S3.** STV reference isolates used in this study with a detailed description, including accession number, name of isolate, sequence length, country, and host.

No.	Accession	Isolate description	Sequence length	Country	Host
1	OK309707	STV, isolate Turkey_Antalya_1	3315	Turkey	<i>Solanum lycopersicum</i>
2	OK309708	STV, isolate Turkey_Antalya_6	3315	Turkey	<i>S. lycopersicum</i>
3	OK309709	STV, isolate Turkey_Balikesir_2	3315	Turkey	<i>S. lycopersicum</i>
4	OK309710	STV, isolate Turkey_Balikesir_5	3315	Turkey	<i>S. lycopersicum</i>
5	OK309711	STV, isolate Turkey_Canakkale_42	3315	Turkey	<i>S. lycopersicum</i>
6	OK309712	STV, isolate Turkey_Manisa_12	3315	Turkey	<i>S. lycopersicum</i>
7	OK309713	STV, isolate Turkey_Manisa_8	3315	Turkey	<i>S. lycopersicum</i>

8	OK309714	STV, isolate Turkey_Canakkale_16	3315	Turkey	<i>S. lycopersicum</i>
9	OK309715	STV, isolate Turkey_Edirne_32	3315	Turkey	<i>S. lycopersicum</i>
10	OK309716	STV, isolate Turkey_Tekirdag_29	3315	Turkey	<i>S. lycopersicum</i>
11	OK309717	STV, isolate Turkey_Izmir_60	3315	Turkey	<i>S. lycopersicum</i>
12	OK309718	STV, isolate Turkey_Izmir_63	3315	Turkey	<i>S. lycopersicum</i>
13	OK309719	STV, isolate Turkey_Izmir_65	3315	Turkey	<i>S. lycopersicum</i>
14	OK309720	STV, isolate Turkey_Bursa_52	3315	Turkey	<i>S. lycopersicum</i>
15	OK309721	STV, isolate Turkey_Bursa_56	3315	Turkey	<i>S. lycopersicum</i>
16	LC429302	STV, M82 genomic RNA	3376	Japan	<i>S. lycopersicum</i>
17	MW26606 2	STV, isolate 21807453	3393	United Kingdom	<i>S. lycopersicum</i>
18	MW01241 3	STV, isolate DDT	3398	Vietnam	<i>S. lycopersicum</i>
19	MW01241 2	STV, isolate DCT	3411	Vietnam	<i>S. lycopersicum</i>
20	MK61025 7	STV, isolate Canada	3414	Canada	<i>S. lycopersicum</i>
21	MW01241 1	STV, isolate DTT	3415	Vietnam	<i>S. lycopersicum</i>
22	MG80838 3	STV, isolate STV-MG	3416	Brazil	<i>S. lycopersicum</i>
23	MW01241 0	STV, isolate GLT	3425	Vietnam	<i>S. lycopersicum</i>
24	KJ174690	STV, isolate GCN06	3426	Spain	<i>S. lycopersicum</i>
25	KT438549	STV, isolate CN-12	3430	China	<i>S. lycopersicum</i>
26	EF442780	STV, isolate Mexico-1	3433	Mexico	<i>S. lycopersicum</i>
27	EU413670	STV, isolate MS-7	3433	USA	N/A
28	KT634055	STV, isolate BD-13	3433	Bangladesh	<i>S. lycopersicum</i>
29	KT852573	STV, isolate NC12-03-08	3433	USA	<i>S. lycopersicum</i>
30	KX949574	STV, isolate Florida	3433	USA	<i>S. lycopersicum</i>
31	KY228384	STV, isolate XJ-p	3433	China	<i>S. lycopersicum</i>
32	KY810783	STV, strain FERA_160205	3433	United Kingdom	<i>S. lycopersicum</i>
33	LC270272	STV, genomic RNA, isolate: Gimcheon	3433	South Korea	<i>S. lycopersicum</i>
34	LC487710	STV, Thailand genomic RNA	3433	Thailand	<i>Capsicum annuum</i>
35	MF422617	STV, isolate CH_bpo161	3433	Switzerland	<i>S. lycopersicum</i>
36	MF422618	STV, isolate CH_bpo163	3433	Switzerland	<i>S. lycopersicum</i>
37	MN09571 6	STV, isolate Antioquia/May5	3433	Colombia	<i>S. lycopersicum</i>
38	MT051992	STV, isolate STV_Panama	3433	Panama	<i>S. lycopersicum</i>
39	MT066231	STV, isolate Pk	3437	Pakistan	<i>S. lycopersicum</i>
40	MT269808	STV, isolate 232-11	3433	Serbia	<i>S. lycopersicum</i>
41	MT269809	STV, isolate 323-12	3433	Serbia	<i>S. lycopersicum</i>
42	MW52086 0	STV, isolate RE-RE1375-Grand Tampon-2018	3433	Reunion	<i>S. lycopersicum</i>
43	OP548652	STV, isolate Tongzhou, Beijing	3437	China	<i>S. lycopersicum</i>
44	OP548653	STV, Isolate Fangshan, Beijing	3437	China	<i>S. lycopersicum</i>

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