

Supplementary Materials:

Discovery and genome characterization of a closterovirus from wheat plants with yellowing leaf symptoms in Japan

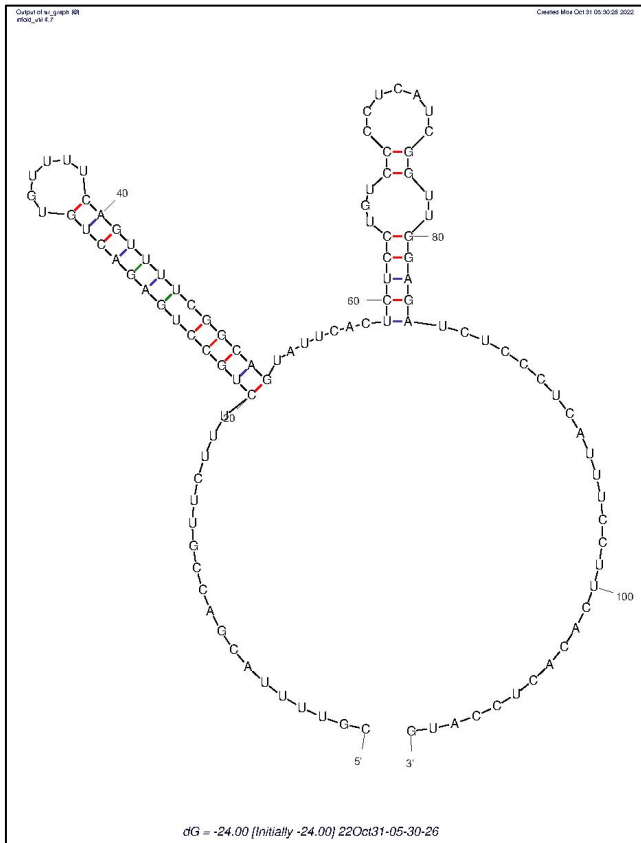
Hideki Kondo^{1,*}, Hitomi Sugahara¹, Miki Fujita¹, Kiwamu Hyodo¹, Ida Bagus Andika², Hiroshi Hisano¹, and Nobuhiro Suzuki¹

¹ Institute of Plant Science and Resources (IPSR), Okayama University, Kurashiki 710-0046, Japan

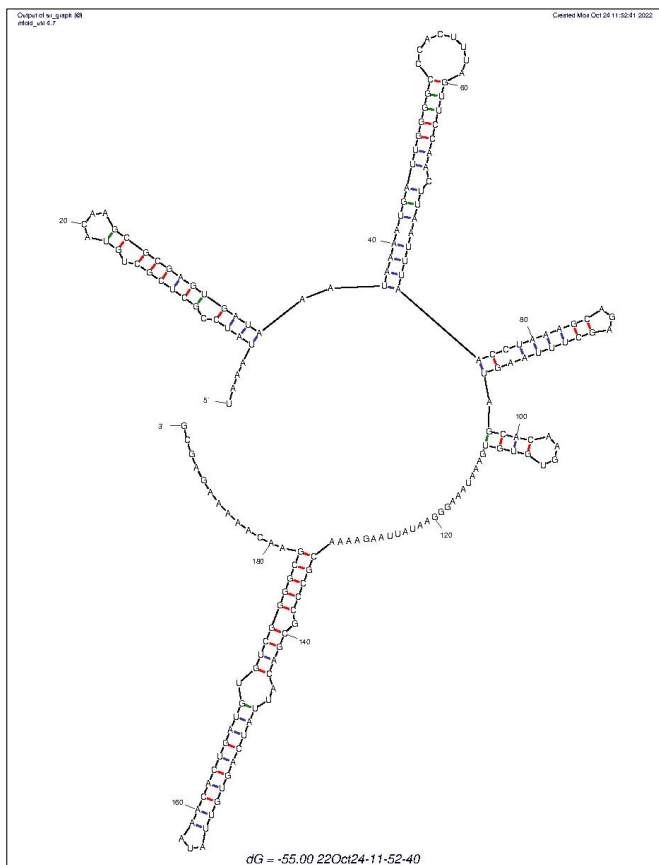
² College of Plant Health and Medicine, Qingdao Agricultural University, Qingdao 266109, China.

* Correspondence: **author Hideki Kondo** Institute of Plant Science and Resources (IPSR), Okayama University, Kurashiki 710-0046, Japan E-mail: hkondo@okayama-u.ac.jp Tel. +81(86) 434-1232 / Fax. +81(86) 434-1232

Supplemental Tables, 1; Supplemental Figures, 4



5' UTR ($\Delta G = -24.00$ kcal/mol)



3' UTR ($\Delta G = -55.00$ kcal/mol)

Figure S1. The potential secondary structures of the 5'- and 3'-UTRs of the WhCV1-WL19a genome RNA as predicted by Mfold under default settings.

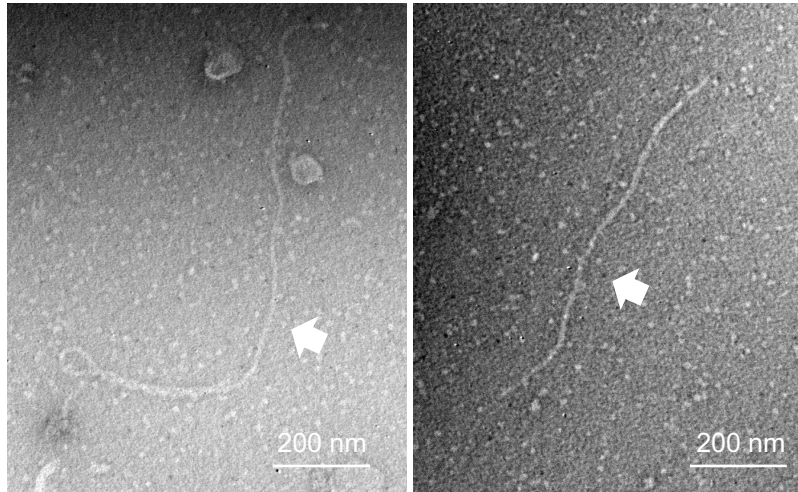


Figure S2. The representative virus-like particles observed in the wheat seedling infected with WhCV1-WL20 showing mild yellowing leaf symptoms after aphid feeding inoculation (see Figure 3A and B). The scale bar is 200 nm.

A

Percent Identity Matrix - created by Clustal Omega

virus name	HSP70h accession no.	1	2	3	4	5	6	7	8	9	10	11	12	13	13	14	16	17	18
1 blackcurrant closterovirus 1	YP_009553662	100																	
2 citrus tristeza virus	NP_042864	39	100																
3 raspberry leaf mottle virus	YP_874188	39	45	100															
4 rose leaf rosette-associated virus	YP_009058932	41	48	52	100														
5 strawberry chlorotic fleck-associated virus	YP_762625	38	47	53	52	100													
6 arracacha virus 1	YP_009551996	38	39	40	39	38	100												
7 WhCV1_WL19a (CSL19_contig 5)		39	42	41	39	40	42	100											
8 WhCV1-WL20 (CSL20_contig 8_12)		39	42	41	39	40	42	98	100										
9 TSA_Triticum polonicum closterovirus	DAZ91134	39	42	41	39	40	42	98	100	100									
10 mint virus 1	YP_224093	37	38	41	42	39	39	46	46	46	100								
11 rehmannia virus 1	YP_009552006	38	40	43	41	39	39	44	44	44	62	100							
12 tobacco virus 1	YP_009162624	37	39	43	41	38	38	43	44	44	66	74	100						
13 carnation necrotic fleck virus	YP_009506334	44	45	49	48	45	43	52	52	52	57	57	56	100					
14 carrot yellow leaf virus	YP_003075968	42	45	45	43	44	43	50	51	51	49	50	50	63	100				
15 carrot closterovirus 1	AHA85412	42	45	45	44	44	42	51	52	52	51	50	48	61	69	100			
16 grapevine leafroll-associated virus 2	YP_337914	39	39	39	37	38	40	42	42	42	40	41	40	46	44	44	100		
17 beet yellows virus	NP_041872	41	40	45	42	40	44	45	45	45	42	47	45	50	48	47	47	100	
18 beet yellow stunt virus	YP_009664807	41	41	40	39	41	43	45	45	45	42	43	42	48	48	48	47	53	100

B

Percent Identity Matrix - created by Clustal Omega

virus name	RdRP accession no.	1	2	3	4	5	6	7	8	9	10	11	12	13	13	14	16	17	18
1 carrot yellow leaf virus	YP_003075965	100																	
2 carrot closterovirus 1	AHA85410	84	100																
3 carnation necrotic fleck virus	YP_009506332	61	60	100															
4 mint virus 1	YP_224091	64	63	69	100														
5 rehmannia virus 1	YP_009552003*	65	66	65	82	100													
6 tobacco virus 1	YP_009162622	65	64	67	83	89	100												
7 WhCV1_WL19a (CSL19_contig 5)		56	54	56	59	59	59	100											
8 WhCV1-WL20 (CSL20_contig 8_12)		56	55	57	58	60	60	88	100										
9 TSA_Triticum polonicum closterovirus	DAZ91132*	55	55	57	58	59	59	89	99	100									
10 rose leaf rosette-associated virus	YP_009058929	54	54	54	55	54	55	55	55	55	100								
11 citrus tristeza virus	NP_042860	55	54	56	58	56	58	58	58	58	57	100							
12 raspberry leaf mottle virus	YP_874185	55	54	53	56	55	55	58	59	58	62	64	100						
13 strawberry chlorotic fleck-associated virus	YP_762622	56	57	56	56	58	57	58	58	58	61	65	65	100					
14 blackcurrant closterovirus 1	YP_009553660*	52	50	52	52	52	52	52	52	50	53	54	52	100					
15 grapevine leafroll-associated virus 2	YP_337912	55	54	56	56	57	56	55	54	54	52	55	55	55	56	100			
16 arracacha virus 1	YP_009551994	55	55	55	58	57	59	55	55	55	51	56	55	56	58	61	100		
17 beet yellows virus	NP_041870*	58	56	57	58	56	57	57	55	56	55	58	57	59	55	60	64	100	
18 beet yellow stunt virus	YP_009664804	56	55	53	56	56	57	58	57	57	53	58	57	59	56	62	65	66	100

C

Percent Identity Matrix - created by Clustal Omega

virus name	CP accession no.	1	2	3	4	5	6	7	8	9	10	11	12	13	13	14	16	17
1 citrus tristeza virus	NP_042867	100																
2 strawberry chlorotic fleck-associated virus	YP_762628	29	100															
3 rose leaf rosette-associated virus	YP_009058935	25	31	100														
4 raspberry leaf mottle virus	YP_874191	34	31	40	100													
5 blackcurrant closterovirus 1	YP_009553664	24	26	21	24	100												
6 mint virus 1	YP_224096	27	22	21	26	19.58	100											
7 rehmannia virus 1	YP_009552009	24	20	23	28	22.22	48.26	100										
8 tobacco virus 1	YP_009162627	23	21	23	26	22.22	48	63	100									
9 carrot yellow leaf virus	YP_003075971	27	23	27	25	26.29	35.2	35	34	100								
10 carrot closterovirus 1	AHA85415	26	24	24	29	21.65	36.92	39	36	46	100							
11 WhCV1_WL19a (CSL19_contig 5)		19	20	20	24	25.81	26.04	25	24	25	27	100						
12 WhCV1-WL20 (CSL20_contig 8_12)		19	21	21	24	24.73	26.04	26	24	24	26	95	100					
13 TSA_Triticum polonicum closterovirus	DAZ91137	19	21	21	24	24.73	26.04	26	24	24	26	95	99	100				
14 arracacha virus 1	YP_009551999	22	19	20	25	15.05	27.75	25	29	22	31	28	27	28	100			
15 grapevine leafroll-associated virus 2	YP_337917	23	23	22	23	22.92	23.71	28	29	26	28	29	29	29	35	100		
16 beet yellows virus	NP_041875	23	21	24	25	17.95	28.5	27	27	24	31	25	26	26	31	36	100	
17 beet yellow stunt virus	YP_009664810	23	25	27	28	20	32.99	32	30	27	29	28	28	28	31	34	36	100

Figure S3. Pairwise comparison of the HSP70h (A), RdRP (B) and CP (C) encoded by closteroviruses.

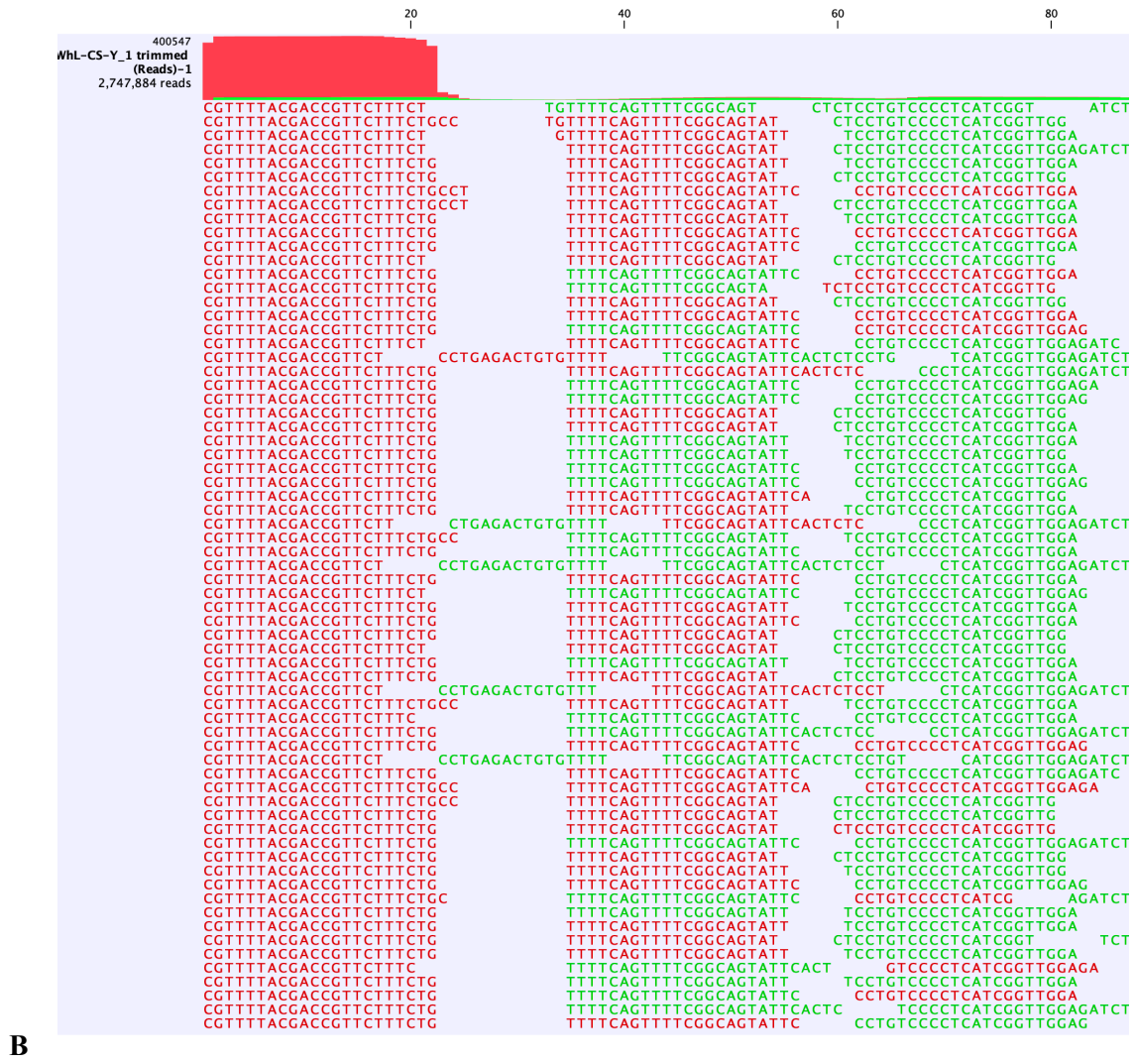
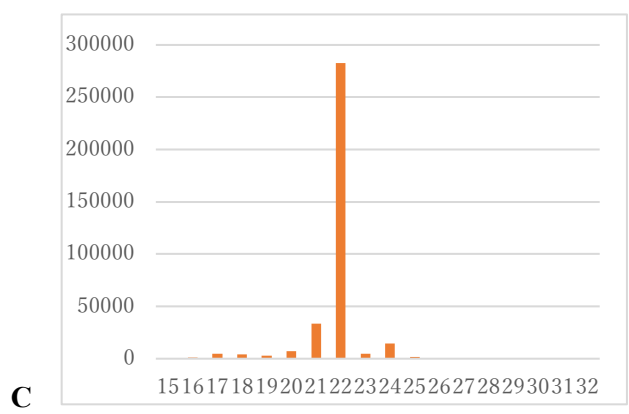
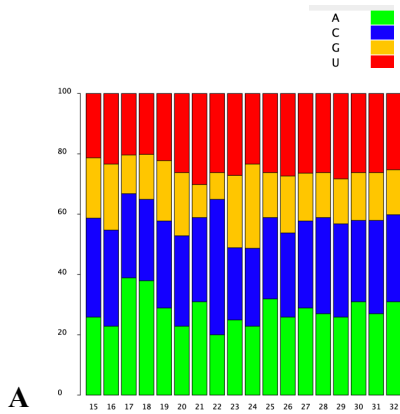


Figure S4. Profiles of WhCV1-WL19a small RNAs. **(A)** Proportions of 5'-terminal nucleotide sequences of WhCV1-WL19a-derived small RNAs accumulated in the wheat leaves. **(B)** The partial mapping result of small RNAs at the position around the end of the 5'-terminal region of the WhCV1-WL19a genome. A high number of minus-strand small RNAs were mapped around the nucleotide positions 1–22 of the genome (red text). Small RNA reads were mapped to the WhCV1-WL19a isolate using the Read Mapping algorithm in the CLC Genomics Workbench with stringent mapping parameters (length fraction=1.0; similarity fraction=1.0). **(C)** Size distribution of small RNAs mapped to the nucleotide positions 1–22 of the genome (mostly minus-strand molecules).

Table S1. List of primer used in this study.

Name	oligo nucleotide sequence (5' → 3')
detection (WhCV1 variant; WL19a, CSL19_contig 5)	
clost3_c11_F	TGTTGATCCTATACCTAGCCATGCT
clost3_c11_R	GCTGCATAAACACTCTTAAGTGGCC
detection (WhCV1 variant; WL20, CSL20_contig 8_12)	
clost2_c9_F	AACCGATCCCCAGCCACGCCGAAC
clost2_c9_R	TACCCTCTAAGAGGAATGATCCTCG
detection (BYDV-PAV; WL19b, CS19L contig 1289)	
BYDV-PAV_2939F	CACACGAAGGCGGCGCCGTCGAGC
BYDV-PAV_3358R	GCTGCGCGGAAGGACTTTGAGGCG
3' RLM-RACE (5' terminal)	
Clo3_CS19L-5RaR1	GGGACCGGCGCAAGTGGTGCGGTG
Clo3_CS19L-5RaR3	AAGCGGTCAGGAGCGGTGTGACGAG
3' RLM-RACE (3' terminal)	
Clos2a-Ra3F1	GTTGCGCTGATACGGCGGTCTGAG
Clos2a-Ra3F3	CGCTCGCTGTATAATCGCGAGCG
3' RLM-RACE (adapter and reverse primers)	
5'-p-Adapter	CAATACCTTCTGACCATGCAGTGACAGTCAGCATG
1st-3Race	ATGCTGACTGACACTGCAT
2nd-3Race	CACTGCATGGTCAGAAGGTATT