

## Supplementary material

### CRISPR/Cas9-Mediated Resistance to Wheat Dwarf Virus in Hexaploid Wheat (*Triticum aestivum* L.)

Xiaoyu Yuan<sup>1,†</sup>, Keya Xu<sup>1,†</sup>, Fang Yan<sup>1</sup>, Zhiyuan Liu<sup>1</sup>, Carl Spetz<sup>2</sup>,  
Huanbin Zhou<sup>1</sup>, Xiaojie Wang<sup>3</sup>, Huaibing Jin<sup>1</sup>, Xifeng Wang<sup>1,\*</sup>, and Yan  
Liu<sup>1,\*</sup>

<sup>1</sup> State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection,  
Chinese Academy of Agricultural Sciences, Beijing 100193, China;

<sup>2</sup> Norwegian Institute of Bioeconomy Research, Hoegskoleveien 7, 1432 ÅS, Norway;

<sup>3</sup> State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest  
A&F University, Yangling 712100, China

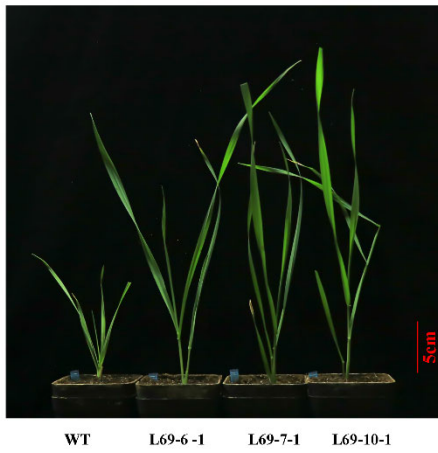
\* Correspondence: wangxifeng@caas.cn (X.W.); liuyan06@caas.cn (Y.L.)

† These authors contributed equally to this work.

**Table S1 PCR Primers used and their applications**

Primer name	Primer sequence (5'-3')	Application
gWDV-LIR-F1	actcgTATGCTCTACCCTGCGTGG	To clone WDV LIR-sgRNA
gWDV-LIR-R1	aaacCCACGCAGGGTAGAGCATAc	
gWDV-CP-F1	actcgAACAAGGACTCCCCGAGGTA	To clone WDV CP/MP-sgRNA
gWDV-CP-R1	aaacTACCTCGGGAGTCCTTGTTc	
TaCas9-F1	GGTCTGGAGGATGCCCTTCT	PCR detection for Cas9
TaCas9-R1	GGCTATCGTGGACCTCCTGT	
WDV-Rep-F	TGGTCTCATGGCAAAATGAGTG	PCR detection for Rep of WDV
WDV-Rep-R	TCCATCACCAATCCCAATGCC	
WDV-Rep-Q4-F	TGAGTGGGGAACATTCGTGG	RT-qPCR for WDV accumulation
WDV-Rep-Q4-R	GAAATAGATGGCGTGCCGTG	
LIR-Q1-F	AAAACCCACGCAGGGTAGAG	RT-qPCR for sgRNA-LIR expression
LIR-Q1-R	CATCGGCTAGCGAAAGGGAA	
CP-Q2-F	GCGCGTGGCTTATAAACTGG	RT-qPCR for sgRNA-CP/MP expression
CP-Q2-R	ACTATTTTGGGCCACACCGA	
GAPDH-Q-F	ACTTCCAGGGTGACAACAGG	RT-qPCR for GAPDH expression
GAPDH-Q-R	GTGCTGTATCCCCACTCGTT	
WDV-CP-Det-F	TGTCCGATGCATCAGCTCCGT	Amplifying WDV probe for northern blot
WDV-CP-Det-R	TAACACGCGTGCGTATAGGCA	
CP-Hi-F2	GGAGTGAGTACGGTGTGCAGGACCGAGG	Hi-TOM sequencing for CP/MP target site
CP-Hi-R2	AAATTGGTTACG GAGTTGGATGCTGGATGGCGGCCTTACCC TGAGCAAAA	
LIR-Hi-F1	GGAGTGAGTACGGTGTGCAACAGCAGCG	Hi-TOM sequencing for LIR target site
LIR-Hi-R1	ATGTAGAGG GAGTTGGATGCTGGATGGGGACACATTGC ATTTGCAGTG	
WDV-554-F	TGTGTTTGCATCGGAGTCAACC	Mutation analysis in LIR target site
WDV-554-R	GCCCACACGATTCGCTACG	
WDV-773-F	GTAGGCGTTGCTTGGCTTGC	Mutation analysis in CP/MP target site
WDV-773-R	TAATGTCGCCTATCTTGCCGTC	

(A)



(B)

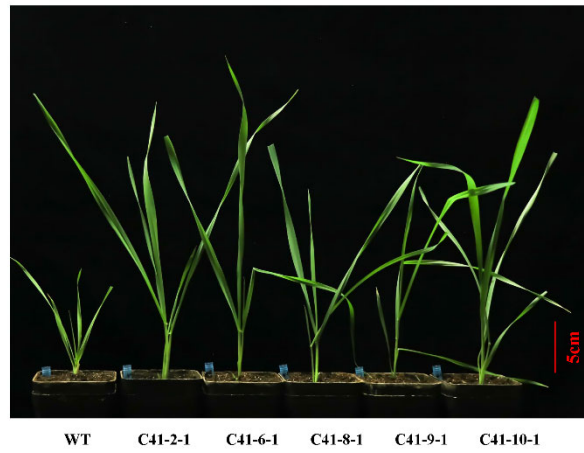


Figure S1. Symptoms in the transgenic T<sub>2</sub> lines and control plants at 30 dpi. WT, wild-type plant.

Line	Total ratio		Indels	Ratio
V <sub>CP</sub> -line41-6	1 48.34%	C A G A T T A A A T G G T G A C C A A C A A G G A C T C C C G A G G T A A G G T A A G C G G A A G A T G G A A G	WT	
		C A G A T T A A A T G G T G A C C A A C A A G G A C T C - - - - G T A A G G T A A G C G G A A G A T G G A A G	-5	41.08%
		C A G A T T A A A T G G T G A C C A A C A A G G A C - - - - A G G T A A G G T A A G C G G A A G A T G G A A G	-5	7.26%
	6 16.97%	C A G A T T A A A T G G T G A C C A A C A A G G A C T C C C G - - G T A A G G T A A G C G G A A G A T G G A A G	-2	9.91%
		C A G A T T A A A T G G T G A C C A A C A A G G A C T C C C G - G G T A A G G T A A G C G G A A G A T G G A A G	-1	2.75%
		C A G A T T A A A T G G T G A C C A A C A A G G A C T C C C G - G G T A A G G T A A G C G G A A G A T G G A A G	-1	2.23%
		C A G A T T A A A T G G T G A C C A A C A A G G A C T C C C G - G G T A A G G T A A G C G G A A G A T G G A A G	-3	2.08%
V <sub>LIR</sub> -line69-10	3 28.62%	G G T G T G C C A G A A A A C T C T A T G C T C T A C C T G C G T G G A G G T G T G A A T T C T G C A C A C T G	WT	
		G G T G T G C C A G A A A A C T C T A T G C T C T A C C T G C - T G G A G G T G T G A A T T C T G C A C A C T G	-1	28.62%
	5 39.33%	G G T G T G C C A G A A A A C T C T A T G C T C T A C C T G C - T G G A G G T G T G A A T T C T G C A C A C T G	-1	39.33%

**Figure S2.** Hi-TOM sequencing analysis of sgRNA-Cas9-induced mutations in T2 progenies of lines C41-6 and L69-10. WT, Wild-type control, PAM is in red and gRNAs in purple. Black dashes denote nucleotide deletions. -/+ indicates deletion/insertion of nucleotides.