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Article

Wheat miR408 and miR159 weaken the virulence of *Parastagonospora nodorum* and induce the defense response in the plants (*Triticum aestivum* L.) against the pathogen

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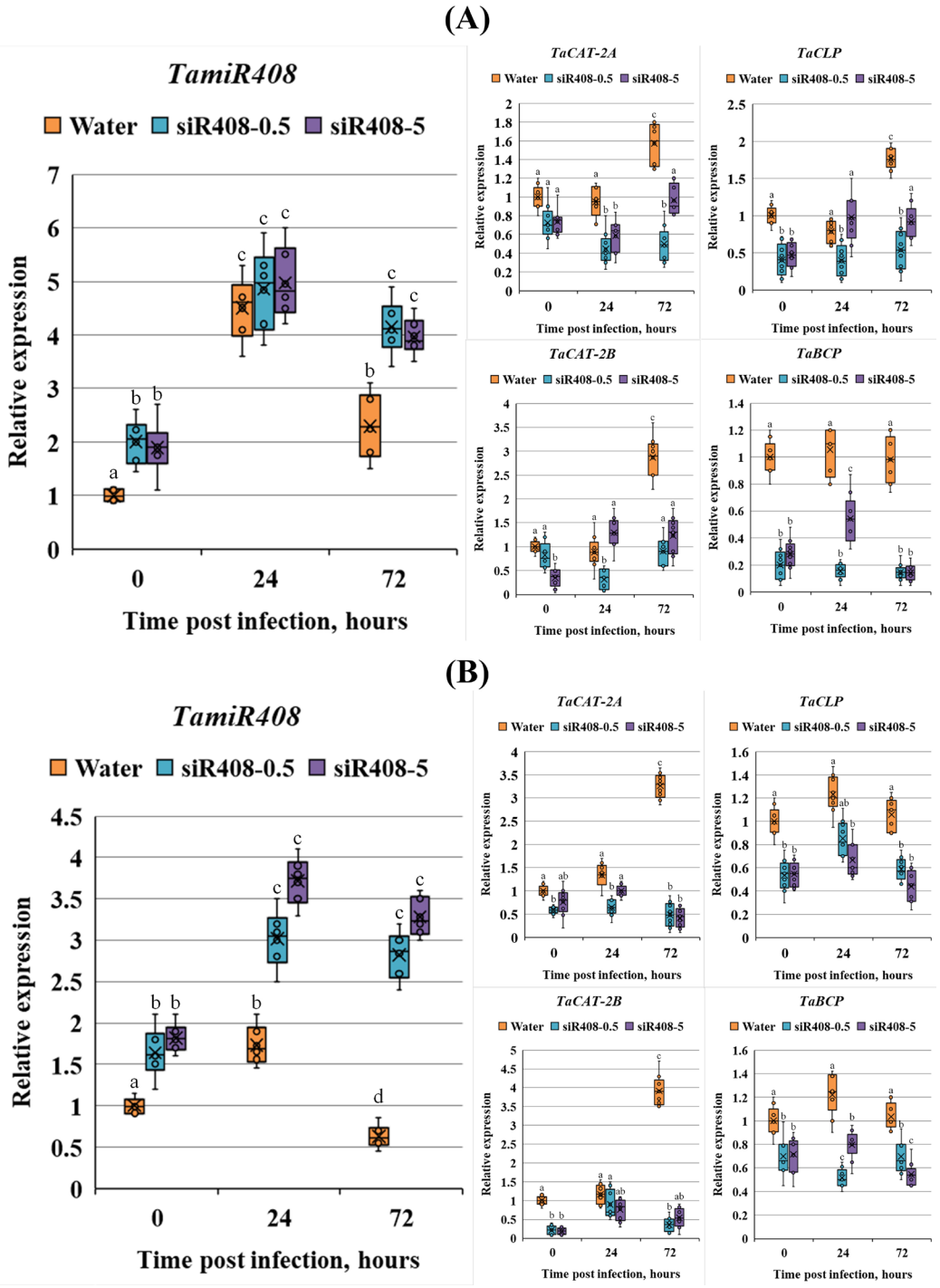
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		Kazahstanskaya 10																			
		24 hours of infection										72 hours of infection									
		Control	SnB	siR408-0.5	siR408-0.5(SnB)	siR408-5	siR408-5(SnB)	siR408-50	siR408-50(SnB)	SnB+siR408-50		Control	SnB	siR408-0.5	siR408-0.5(SnB)	siR408-5	siR408-5(SnB)	siR408-50	siR408-50(SnB)	SnB+siR408-50	
SA-signaling	*TaWRKY45*	1	1.3	1.1	2.9	1	1.5	1.5	2.5	1.2	0.2	1	1.5	1.1	1.1	1	3.7	1.5	5	3.3	0.2
	TaWRKY13	1	1.1	1.2	1.7	1.2	1.7	1.2	2.5	2	0.7	1	1.3	1.2	1.7	1.2	2.7	1.2	3.3	3.6	0.7
	TaPR1	1	1.5	1.2	2.8	0.5	5.4	0.5	11	7	1	1	3.3	1.2	16	0.5	8.1	0.5	8.3	8	1
											1.5										1.5
ET-signaling	*TaEIN3*	1	6.1	0.8	1.1	1.1	1.2	0.5	0.9	0.5	3.5	1	1.1	0.8	0.4	1.1	0.4	0.5	0.4	0.7	4
	TaWRKY53b	1	2.6	1.1	3.8	1.2	1.2	1.1	1.3	0.4	6	1	2.8	1.1	3.2	1.2	4.4	1.1	4.6	0.5	8
	TaPR3	1	2.6	0.6	0.4	0.5	1.8	0.4	1	0.6	11	1	9.9	0.6	2.4	0.5	6.1	0.4	5.6	3.1	16

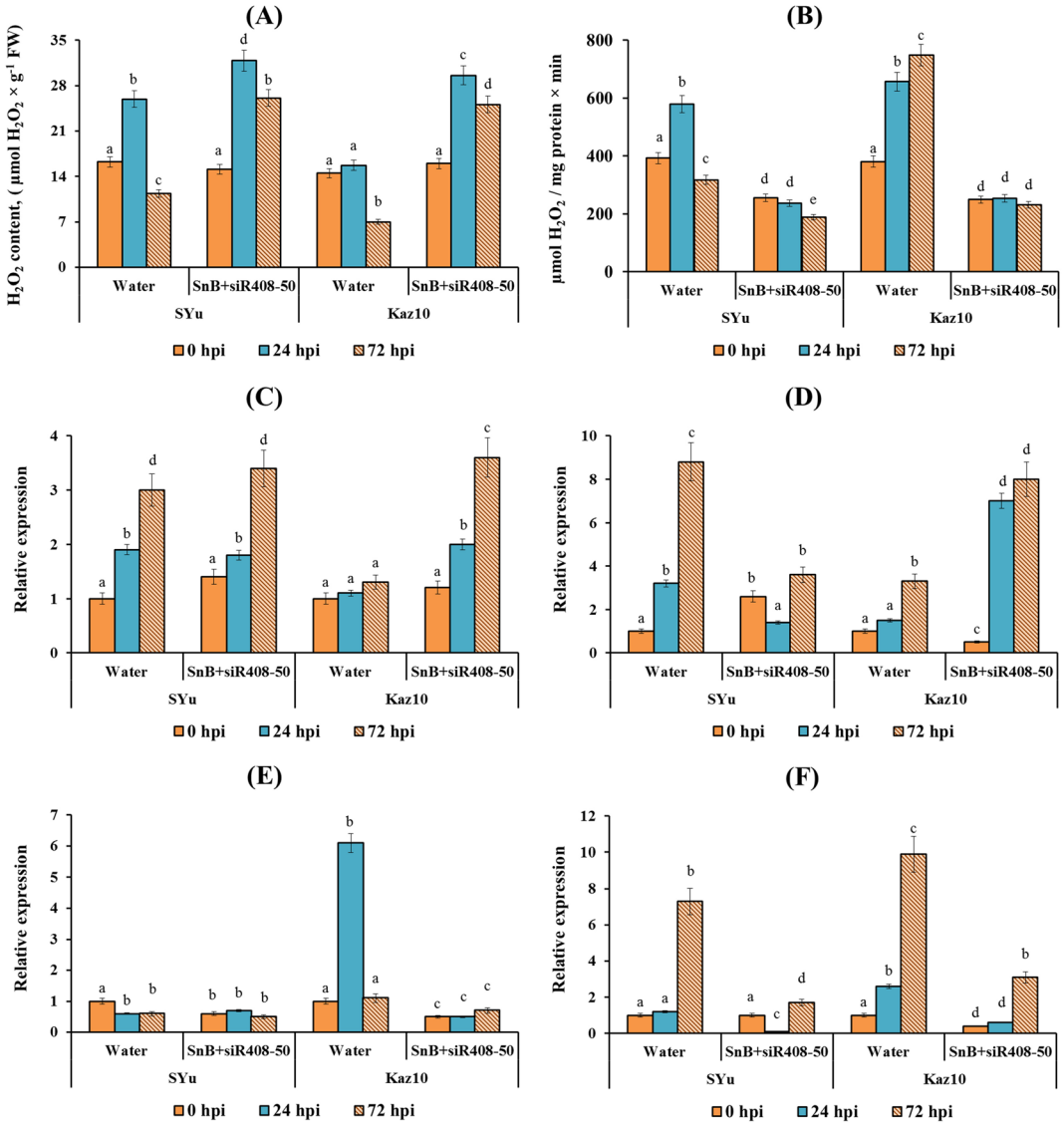
		Salavat Yulaev																			
		24 hours of infection										72 hours of infection									
		Control	SnB	siR408-0.5	siR408-0.5(SnB)	siR408-5	siR408-5(SnB)	siR408-50	siR408-50(SnB)	SnB+siR408-50		Control	SnB	siR408-0.5	siR408-0.5(SnB)	siR408-5	siR408-5(SnB)	siR408-50	siR408-50(SnB)	SnB+siR408-50	
SA-signaling	*TaWRKY45*	1	1.5	1	1	1.3	2.5	1.3	2.2	1.7	0.2	1	2.2	1	0.8	1.3	1.3	1.3	1.5	1.1	0.2
	TaWRKY13	1	1.9	0.9	2.1	1.8	5.6	1.4	5.4	1.8	0.6	1	3	0.9	3	1.8	4.3	1.4	2.6	3.4	0.7
	TaPR1	1	3.2	1.8	2.1	2.6	16	2.6	8.9	1.4	0.9	1	8.8	1.8	3.4	2.6	3.5	2.6	9.9	3.6	1
											1										1.5
ET-signaling	*TaEIN3*	1	0.6	0.4	0.5	0.6	0.3	0.6	0.6	0.7	6	1	0.6	0.4	0.8	0.6	1.2	0.6	0.5	0.5	3.5
	TaWRKY53b	1	0.8	0.7	0.7	1	1.3	0.7	0.7	0.9	8	1	1.1	0.7	0.7	1	1	0.7	2.3	0.5	7
	TaPR3	1	1.2	0.9	0.6	1	3.4	1	1.7	0.1	16	1	7.3	0.9	3.7	1	3.4	1	3.6	1.7	10

		Kazahstanskaya 10																			
		24 hours of infection										72 hours of infection									
		Control	SnB	siR159-0.5	siR159-0.5(SnB)	siR159-5	siR159-5(SnB)	siR159-50	siR159-50(SnB)	SnB+siR159-50		Control	SnB	siR159-0.5	siR159-0.5(SnB)	siR159-5	siR159-5(SnB)	siR159-50	siR159-50(SnB)	SnB+siR159-50	
SA-signaling	*TaWRKY45*	1	1.3	1.4	0.5	1.5	1.4	2	1.1	1.8	0.2	1	1.5	1.4	1.6	1.5	3	2	2.1	1.9	0.2
	TaWRKY13	1	1.1	1.8	0.8	1.6	2	1.3	1.9	2.4	0.7	1	1.3	1.8	1.1	1.6	1.4	1.3	1	1.2	0.7
	TaPR1	1	1.5	3.3	3.2	4.8	4.9	1.6	9.2	2.4	1	1	3.3	3.3	3	4.8	5	1.6	8.5	9.2	1
											1.5										1.5
ET-signaling	*TaEIN3*	1	6.1	1.2	0.9	0.8	1.3	0.9	1.1	1.3	3.5	1	1.1	1.2	0.9	0.8	1.2	0.9	0.8	0.9	3.5
	TaWRKY53b	1	2.6	0.6	0.5	1	0.3	0.6	0.4	0.6	6	1	2.8	0.6	0.6	1	0.3	0.6	0.1	0.4	6
	TaPR3	1	2.7	1	0.4	0.4	1.3	0.4	0.9	1	10	1	10	1	3.9	0.4	6	0.4	2.5	0.3	10

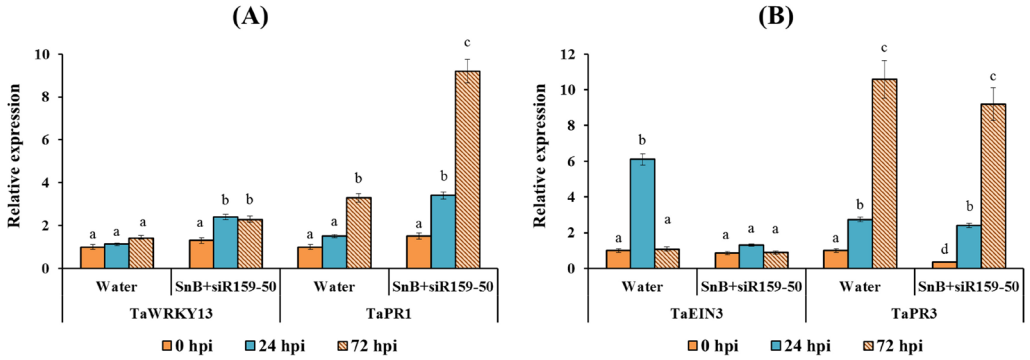
Supplementary Figure 1. Heat map of the relative expressions of the SA signaling pathway genes and ET signaling pathway genes in two cultivars of bread wheat Salavat Yulaev (SYu) and Kazahstanskaya10 (Kaz10) 24 and 72 hours post infection (hpi) with the *P. nodorum* SnB isolate under the tffect of spraying in vitro-synthesized siR408 and siR159 duplexes. Symbols are the same as in Figures 7, 8 and S5. Average gene expression was calculated from nine biological samples in five technical replicates (n = 9). Values are presented as means.



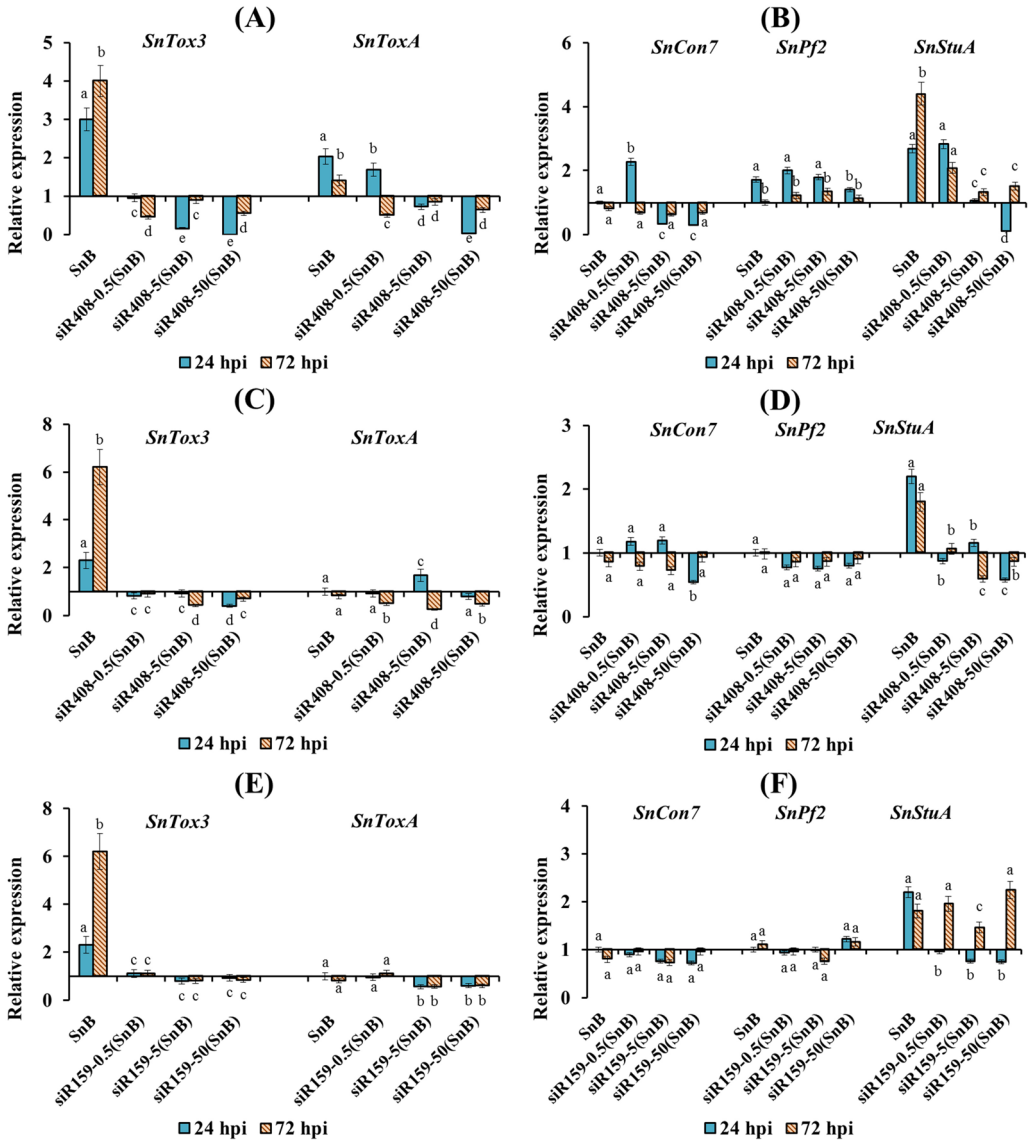
Supplementary Figure 2. Effect of foliar spraying with in vitro-synthesized siR408 duplex (0.5 and 5 pmol/µl) on the relative expressions of the miR408 and four target genes (*TaCAT-2A*, *TaCAT-2B*, *TaCLP1* and *TaBCP*) in the two cultivars of bread wheat Salavat Yulaev (SYu) (**A**) and Kazahstanskaya10 (Kaz10) (**B**) 24 and 72 hours post infection (hpi) with the *P. nodorum* SnB isolate. Designations: 0 hpi—untreated and uninfected wheat plants; other symbols are the same as in Figure 1. Average gene expression was calculated from nine biological samples in five technical replicates (n = 9). Values are expressed as mean SE (ANOVA with Duncan's test; comparison between untreated and treated plants are presented; significant differences are marked with different letters at $p \leq 0.05$).



Supplementary Figure 3. Effect of infection wheat leaves with a suspension spores of the *P. nodorum* SnB isolate co-incubated with in vitro-synthesized siR408 duplex (50 pmol/µl) on the changes in H_2O_2 content (**A**) and activity of catalase (**B**), relative expressions of the SA signaling pathway genes *TaWRKY13* (**C**), *TaPR1* (**D**) and ET signaling pathway genes *TaEIN3* (**E**), *TaPR3* (**F**) in two cultivars of bread wheat Salavat Yulaev (SYu) and Kazahstanskaya10 (Kaz10) 24 and 72 hours post infection (hpi). Designations: 0 hpi— wheat plants uninfected with *P. nodorum* isolate SnB; Water — untreated wheat plants; SnB+siR408-50 (0 hpi) — wheat plants treated with 50 pmol/µl siR408 and uninfected with *P. nodorum* isolate SnB; SnB+siR408-50 (24 hpi and 72 hpi) — wheat plants infected with suspension spores of the *P. nodorum* SnB isolate co-incubated with in vitro-synthesized siR408 (50 pmol/µl). Average gene expression was calculated from nine biological samples in five technical replicates (n = 9). Values are expressed as mean SE (ANOVA with Duncan's test; comparison between untreated and treated plants are presented; significant differences are marked with different letters at $p \leq 0.05$).



Supplementary Figure 4. Effect of infection wheat leaves with a suspension spores of the *P. nodorum* SnB isolate co-incubated with in vitro-synthesized siR159 duplex (50 pmol/µl) on the relative expressions of the SA signaling pathway genes *TaWRKY13* and *TaPR1* (**A**) and ET signaling pathway genes *TaEIN3* and *TaPR3* (**B**) in the cultivar of bread wheat Kazahstanskaya10 (Kaz10) 24 and 72 hours post infection (hpi).). Symbols are the same as in Figure S3. Average gene expression was calculated from nine biological samples in five technical replicates (n = 9). Values are expressed as mean SE (ANOVA with Duncan's test; comparison between untreated and treated plants are presented; significant differences are marked with different letters at $p \leq 0.05$).



Supplementary Figure 5. Effect of foliar spraying with in vitro-synthesized siR408 and siR159 duplexes (0.5, 5 and 50 pmol/µl) on the relative expressions of the genes encoding virulence factors, NEs (*SnToxA* and *SnTox3*) (**A**, **C**, **E**) and TFs (*SnCon7*, *SnPf2*, and *SnStuA*) (**B**, **D**, **F**) in the two cultivars of bread wheat Salavat Yulaev (SYu) (**A, B**) and Kazahstanskaya10 (Kaz10) (**C-F**) 24 and 72 hours post infection (hpi). Designations: SnB — wheat plants infected with *P. nodorum* SnB isolate; siR408-0.5(SnB) or siR159-0.5(SnB) — wheat plants treated with 0.5 pmol/µl siR408 or siR159 and then infected with *P. nodorum* SnB isolate; siR408-5(SnB) or siR159-5(SnB) — wheat plants treated with 5 pmol/µl siR408 or siR159 and then infected with *P. nodorum* SnB isolate; siR408-50(SnB) or siR159-50(SnB) — wheat plants treated with 50 pmol/µl siR408 or siR159 and then infected with *P. nodorum* SnB isolate. Average gene expression was calculated from nine biological samples in five technical replicates (n = 9). Values are expressed as mean SE (ANOVA with Duncan's test; comparison between untreated and treated plants are presented; significant differences are marked with different letters at $p \leq 0.05$).

Supplementary Table 1. In vitro-synthesized single-stranded RNA oligonucleotides

	ssRNA	5′-3′ RNA oligonucleotides	Modification	
			3'	5′
1	tae-miR159-MF	uuu-gga-uug-aag-gga-gcu-cug	2'-*O*-methyl	phosphate
2	tae-miR159-MR	gag-cuc-ccu-uca-auc-caa-auu	2'-*O*-methyl	phosphate
3	tae-miR159-T	uuu-gga-uug-aag-gga-gcu-cug	2'-*O*-methyl	TAMRA
4	tae-miR408-MF	cug-cac-ugc-cuc-uuc-ccu-ggc	2'-*O*-methyl	phosphate
5	tae-miR408-MR	cag-gga-aga-ggc-agu-gca-guu	2'-*O*-methyl	phosphate
6	tae-miR408-T	cug-cac-ugc-cuc-uuc-ccu-ggc	2'-*O*-methyl	TAMRA

Supplementary Table 6. The sequences of primers for miRNAs genes used for qRT-PCR

Genes	**Strand**	**5′ to 3′ Primer Sequences**	**GenBank accession number**
TamiR159	Forward	ttt gga ttg aag gga gct ctg	MF540051
TamiR408	Forward	ctg cac tgc ctc ttc cct g	MF540069
RTQAdp	Reverse	ctc tga cga gaa ctt gac ttc act atg act	
Ta5S-rRNA	Forward	gga tgc gat cat acc agc act	XR006485435

Supplementary Table 7. The sequences of primers for wheat genes used for qRT-PCR

Genes	Strand	5′ to 3′ Primer Sequences	GenBank accession number	Primer efficiency, %
Ta PR1	Reverse	ggg acc gag cga gta aat aaa	KF196278	98.0
	Forward	gac tgc cat aga gaa gct cat t		
Ta PR3	Reverse	acc tcc ttg gcg tca gct	AB029936	99.5
	Forward	tcg cac cat tat tcc ctt		
Ta EIN3	Reverse	ggc tca aca acc tgg att tt	KU03083	97.5
	Forward	ccc tgt cca aag aaa ccc t		
Ta WRKY13	Reverse	gag gag gaa cat gag cag aag	EF397614	96.5
	Forward	gaa cca ccc gaa gtc gaa g		
Ta WRKY45	Forward	att gcg cgt cgt agg ttt ag	EF397613	95.0
	Reverse	gac gga tgg atg agt agc aaa g		
Ta WRKY53	Forward	ctc tct ggc ttc tcc ttt cac	EF368364	97.0
	Reverse	ctc ctg ctg ctc ctt gaa taa		
Ta CAT2-A	Forward	aag acc ggc tcg agc gag ct	XM044551419	99.0
	Reverse	gca ggg atc cat cct tcc tct ct		
Ta CAT2-B	Forward	cag caa taa gag acc atg cca g	XM044554759	98.5
	Reverse	agg atc acc cca aaa aaa aca		
Ta MYB65	Forward	tcc agc agg atg tca gtg ag	XM044594981	96.5
	Reverse	ggt gcc aaa gtt ttg gtg at		
Ta BCP	Forward	gct gta cgt tag gtt gca tgt c	AK333616	99.0
	Reverse	tgc caa cca caa caa aag cc		
Ta CLP	Forward	cgt gct tcg ttc ttt caa gtc c	KC852069	99.0
	Reverse	gca cca tac aac agc aag tac g		
Ta GAPDH	Forward	gtg ttc cca ctg ttg atg ttt c	EU022331	100.0
	Reverse	cct cct tga tag cag cct taa t		
Ta RLI	Forward	ttg agc aac tca tgg acc ag	AY059462	100.0
	Reverse	gct ttc caa ggc aca aac at		

Supplementary Table 8. The sequences of primers for fungal genes used for qRT-PCR

Genes	Strand	5′ to 3′ Primer Sequences	GenBank accession number	Primer efficiency, %
Sn ToxA	Forward	aac gcc aat aca gtg cga gt	JX997419	95.5
	Reverse	gct gca ttc aat ttt cac g		
Sn Tox3	Forward	cga gct gat atc ccg ttt ga	FJ823644	96.5
	Reverse	ggg aca gtg aca ata ggt aag g		
Sn StuA	Forward	acc acc gtc tag caa tct tta c	SNOG14941	98.0
	Reverse	ggt atc ctt ggc tcg cat att		
Sn Con7	Forward	ctc acg agc ttt gac gac tt	SNOG08362	97.0
	Reverse	cat gac cca tcc gta aag aga g		
Sn Pf2	Forward	cat tca tca gtc tct gga acc g	SNOG00649	98.5
	Reverse	cga atc tcg acg cct tgg g		
Snβ-tubulin	Forward	aca cca gga aca act gct aac agc	S56922	100.0
	Reverse	tat gcg cgc gtg ctg caa att cga		