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## Abiotic stress upregulated TaZFP34 represses the expression of type-B response regulator and SHY2 genes and enhances root to shoot ratio in wheat

Chang, Hongping; Chen, Dandan; Kam, Jason; Richardson, Terese; Drenth, Janneke; Guo, Xinhong; McIntyre, C. Lynne; Chai, Shoucheng; Rae, Anne L.; Xue, Gang-Ping

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tel: +44 1970 62 2400 email: is@aber.ac.uk

## Appendix A. Supplementary data

# Abiotic stress upregulated TaZFP34 represses expression of type-B response regulator and *SHY2* genes and enhances root to shoot ratio in wheat

Hongping Chang, Dandan Chen, Jason Kam, Terese Richardson, Janneke Drenth, Xinhong Guo, C. Lynne McIntyre, Shoucheng Chai, Anne L. Rae and Gang-Ping Xue

accession #	Gene name	GenBank	Forward primer	Reverse primer
TaZPP22EU4082245' -cgtcgganagacgtt5' -cgtcgtagtggangacacgtcggangacTaZPP34EU4082245' -acacgganagacgggangacTaZPP34EU6503985' -acacgganagacgggangacTaZPP34EU6503985' -acatgganagacgggangacTaRP12AK3362525' -acatggangcagtggangatta5' -catcgangatggangattaTaRP12Cl0162515' -gengggtggangattatte5' -tactgangatggangattatTaRP11CK2060295' -cattggangcagtggangatta5' -tactatggangcacctattecTaRP112JF951275' -cancggantacgtcgangattattatte5' -cattggantacttattecanattetTaIAA7A15750985' -cancggantacgtcgangattattatte5' -cattggantacttattegantattetTaIAA8GH7230015' -tggangtggangctcgang5' -cattggangtattattattegangTaGA20-x0FR7165255' -ggantafgangggtg15' -catggangtcggggangTaGA20-x0FR7165255' -canggantattegtacggangg5' -cgtcgangtangganganTaARF1AK314745' -canggantattegtacganggtg15' -tggcantagganganganganganganganganganganganganga		accession #		
TaZPF34EU4082245' -acgegatacgtgggtat5' -acgegatacgtgggtatTaZFF46EU4503985' -acaacgatacgtaggatacat5' -gtgggtggttaggtaggatTaRR12AK336255' -acaacgatgggggtataa5' -catacaccgtgggtataggatTaRR11CK006295' -cattggatggtggatasta5' -catcacgatggatacattacTaRR11CK006295' -cattggatggtggatastac5' -catcaggattaccatatagTaRA17AJ5750985' -catggatggtggatagata5' -cattggatggtatactatagTaLAA7AJ5750985' -catggatggtggatagatagatagatagatagatagatag	TaZFP22	EU408222	5' -cgtgtgccggaagacgtt	5' -cgtcgtagtggcaccttttg
TaZPP46EUG50385' -acacegacageacgeaceat5' etgeggetteraggetgagTaRR12BAK3362525' -acacegacgacgattera5' -ecaceccgacgateattutecTaRR12DCJ6126355' -caregagteattutec5' -ecteccattutecattutecattTaRR1LCK2060295' -caregagtearegtegacatt5' -tecacggettectecteattutecattTaRR12JP591275' -caregagtearegtegatageatt5' -caregacattectectectattutecattTaAA7AJ5750985' -caregagtegattagatageatt5' -caregagtatteggattageatTaIAA27HX0496475' -gacegetegattagtattagaa5' -caregtegatteggattageatTaGD1FR6685565' -caregteattegattageattagaa5' -caregtegatteggttttTaGD20-A0FR7165255' -caregteattegatteggetttt5' -geattegetteggattageattagaatTaGA20-A0FR7165255' -caregteattegatteggetttt5' -geattegetteggttttTaGA21-A0FR765255' -caregteatteggtttt5' -geattegetteggttttTaGA21-A0FR7165255' -caregteatteggattegttt5' -geattegetteggttttTaARF2AAK331495' -geattegtegactegtttt5' -geattegteagttattTaARF2AAK331495' -geattegtegatt5' -geattegteagtattTaAFF2AAK3304005' -attegtegattetttttggetgaa5' -deattagteggatteaTATF1NAY307185' -geattegteggattegttttttt5' -geattettttttttttttttttttttttttttttttttt	TaZFP34	EU408224	5' -acggcgatcagtgggtgt	5' -gacgaacagctcgagcaaga
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$ \begin{array}{llllllllllllllllllllllllllllllllllll$	TaARF2a	AK334169	5' -ggaaggttcagtggatggtgac	5' -tggctagaatttaaatcgcttgatg
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TaSLRL1HG670306*5' -acggcgaagataaccataccag5' -cccgctacacttaccgttgcTaExpA4AY5435305' -gagcaggaactggggtgcta5' -gtccgtcgtcgtcgtcgtccTaExpB1CJ8032655' -ggcacgtcctacagctcctc5' -gctcacaattgcagaaccagaTaRP15HX0984045' -gccaccgtgctttgcagataag5' -gccctcaagctcaaccataact	TaGIP	EU095332	5' -tgcccctgctacaacaactg	5' -tggcaccccagaagaagaag
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TaRP15 HX098404 5'-gcacacgtgctttgcagataag 5'-gccctcaagctcaaccataact	TaExpB1	CJ803265	5' -ggcacgtcctacagctcctc	5' -gctcacaaattgcagaaccaga
	TaRP15	HX098404	5'-gcacacgtgctttgcagataag	5' -gccctcaagctcaaccataact

Sup	olementary	v Table S1.	The sec	uences o	of 1	primer	pairs	used	for	real-	time	PCR
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The full-length sequences of some genes were obtained by extending partial EST sequences from the NCBI wheat EST database (<u>https://blast.ncbi.nlm.nih.gov/Blast.cgi</u>) and wheat genome sequence databases

(http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/search\_reads.php and https://urgi.versailles.inra.fr/blast/blast.php).

Some primers match with three homoeologous genes, as *T. aetivum* is a hexaploid species.

\* Sequence position in HG670306: 425714967-425716472

Supplementary Table S2. Summary of the DNA-binding specificity of TaZFP46.

Binding affinity	Left sequence	Spacer length between two SAGTR motifs	Right sequence	Relative binding activity to preferred base			
Preferred	GG <b>GAGTG</b> A	N8	GG <b>GAGTG</b> A	100%			
High	AC	N8	AC	71-90%			
Medium	TT AB	N5-11	TT AB	41-70%			
Low	HC	N5-11	НС	20-40%			

Data are based on Figure 2. The preferred binding sequence of TaZFP46 is GGGAGTGAN5GGGAGTGA. H = A, C or T; B = C, G or T; S = C or G; R = A or G.

**Supplementary Table S3.** Expression levels of *TaZFP34* and potential root growth-related, ABA signaling pathway or antioxidant enzyme genes in the roots of wild-type and transgenic plants expressing high-level *TaZFP34*.

Gene name	Gene description	ZFP34-2	ZFP34 -13	Wild type	Ratio T/W†
TaZFP34		0.042 + 0.014**	0.049 + 0.006**	0.001 + 0.000	46
Root growth-related gene	28				
TaRR12B	Two-component response regulator	$0.017 \pm 0.004*$	$0.018 \pm 0.001*$	$0.033 \pm 0.009$	0.53
TaRR12D	Two-component response regulator	0.041 ± 0.013*	$0.051 \pm 0.017*$	$0.099 \pm 0.008$	0.46
TaRR1L1	Two-component response regulator 1-like	$0.870 \pm 0.073$	0.768 ± 0.033*	$1.089 \pm 0.210$	0.75
TaRR1L2	Two-component response regulator 1-like	$0.244 \pm 0.029$	$0.241 \pm 0.044$	$0.257 \pm 0.032$	0.94
TaIAA7	Aux/IAA protein	$2.62 \pm 0.42*$	2.71 ± 0.19*	3.59 ± 0.33	0.74
TaIAA8	Aux/IAA protein	$1.43 \pm 0.15$	$1.27 \pm 0.07*$	$1.47 \pm 0.04$	0.92
TaIAA27	Aux/IAA protein	$0.114 \pm 0.006*$	$0.116 \pm 0.005*$	$0.143 \pm 0.013$	0.80
TaSHY2	Aux/IAA protein	0.977 ± 0.061*	0.933 ± 0.031*	$1.322 \pm 0.203$	0.72
TaGA20-ox1	Gibberellin 20-oxidase	$0.030 \pm 0.008*$	$0.040 \pm 0.004*$	$0.062 \pm 0.011$	0.56
TaGID1	Gibberellin receptor	0.933 ± 0.148*	0.934 ± 0.110*	$0.632 \pm 0.067$	1.48
TaRht1	DELLA protein	$0.239 \pm 0.027$	$0.213 \pm 0.024$	$0.286 \pm 0.049$	0.79
TaARF1	Auxin response factor	$0.187 \pm 0.010$	$0.198 \pm 0.016$	$0.193 \pm 0.018$	1.00
TaARF2a	Auxin response factor	$0.061 \pm 0.003$	$0.065 \pm 0.004$	$0.062 \pm 0.003$	1.02
TaARF2b	Auxin response factor	$0.160 \pm 0.005$	$0.151 \pm 0.010$	$0.140 \pm 0.022$	1.11
TaARF6	Auxin response factor	$0.013 \pm 0.004$	$0.017 \pm 0.003$	$0.017 \pm 0.004$	0.88
TaETTINa	ETTIN-like auxin response factor	$0.038 \pm 0.004$	$0.034 \pm 0.005$	$0.038 \pm 0.001$	0.95
TaETTINb	ETTIN auxin response factor	$0.051 \pm 0.006$	$0.047 \pm 0.004$	$0.048 \pm 0.004$	1.02
TaTIR1L1	Transport inhibitor response 1-like	$0.313 \pm 0.035$	$0.266 \pm 0.028$	$0.308 \pm 0.038$	0.94
TaTIR1L2	Transport inhibitor response 1-like	$0.674 \pm 0.052$	$0.558 \pm 0.024$	$0.593 \pm 0.007$	1.04
TaTIR1L3	Transport inhibitor response 1-like	$0.259 \pm 0.012$	$0.181 \pm 0.018$	$0.208 \pm 0.038$	1.06
TaPIN1a	Auxin efflux carrier protein	$0.0017 \pm 0.0002$	$0.0013 \pm 0.0001$	$0.0013 \pm 0.0004$	1.15
TaPIN1b	Auxin efflux carrier protein	$0.155 \pm 0.008$	$0.175 \pm 0.005$	$0.215 \pm 0.042$	0.77
TaPIN2	Auxin efflux carrier protein	$0.282 \pm 0.019$	$0.295 \pm 0.030$	$0.293 \pm 0.041$	0.99
TaPIN4	Auxin efflux carrier protein	$0.117 \pm 0.028$	$0.082 \pm 0.021$	$0.083 \pm 0.023$	1.20
TaExpB18	Expansin B	0.009 ± 0.003*	$0.012 \pm 0.002$	$0.017 \pm 0.003$	0.62
TaCYCB1-2	Cyclin B1	$0.073 \pm 0.025$	$0.087 \pm 0.021$	$0.089 \pm 0.022$	0.90
TaCYCB2-3	Cyclin B2	$0.050 \pm 0.015$	$0.054 \pm 0.016$	$0.058 \pm 0.011$	0.90
TaCYCD4-1	Cyclin D4	$0.171 \pm 0.012$	$0.174 \pm 0.015$	$0.175 \pm 0.030$	0.99
ABA metabolic, signallin	ig or drought-upregulated genes				
TaNCED3	9-cis-epoxycarotenoid dioxygenase 3	$0.009 \pm 0.001$	$0.014 \pm 0.002$	$0.009 \pm 0.003$	1.28
TaNCED9	9-cis-epoxycarotenoid dioxygenase 9	$0.015 \pm 0.001$	$0.019 \pm 0.001$	$0.017 \pm 0.001$	1.00
TaCYP707A1	ABA 8'-hydroxylase 1	$0.375 \pm 0.027$	$0.401 \pm 0.040$	$0.359 \pm 0.056$	1.08
TaABI1	Protein phosphatase 2C	$0.209 \pm 0.005$	$0.228 \pm 0.012$	$0.260 \pm 0.037$	0.84
TaNAC69-1	ABA-inducible NAC transcription factor	$0.084 \pm 0.005$	$0.055 \pm 0.005$	$0.070 \pm 0.013$	0.99
TaWRAB1	Late embryogenesis abundant protein	$0.161 \pm 0.028$	$0.121 \pm 0.043$	$0.180 \pm 0.025$	0.78
TaWRAB18	Late embryogenesis abundant protein	0.291 ± 0.193	$0.499 \pm 0.054$	$0.488 \pm 0.072$	0.81
TaLEA1	Late embryogenesis abundant protein	$0.027 \pm 0.006$	$0.019 \pm 0.009$	$0.021 \pm 0.002$	1.10
Antioxidant enzyme gene	es i		•	ı	
TaAPX1	L-ascorbate peroxidase 1	$8.96 \pm 0.17*$	$10.11 \pm 0.65$	$13.35 \pm 2.41$	0.71
TaAPX2	L-ascorbate peroxidase 2	17.27 ± 0.41*	23.65 ± 1.11*	29.90 ± 1.42	0.68
TaFSD2	Fe-superoxide dismutase 2	$0.081 \pm 0.001$	$0.104 \pm 0.008$	$0.108 \pm 0.020$	0.86

The root samples were from 3-week-old hydroponically grown wild type and T<sub>2</sub> transgenic (ZFP34-2 and ZFF34-13) plants. Values are means  $\pm$  SD of 3 biological replicates and expression levels are relative to an internal reference gene, *TaRP15*. GenBank accession numbers of these genes are shown in Supplementary Table S1. Values in bold are those with a significant difference at least in one of the two transgenic lines. \* *P* < 0.05 using Student's *t*-test; \*\* *P* < 0.01;

<sup>†</sup> Ratio of the mean values of two transgenic lines to that of the wild type control.

	1			80
TaZFP22	MAVEAVLEAAAMVPS	3PPSKEMEASSSTSEEASALLG <mark>Q</mark> AEG <sup>i</sup>	W <mark>S</mark> KRKRSRRPR <mark>A</mark> LAPSEEEYI	L <mark>A</mark> LCLLMLAHGHRDSAPAAA
TaZFP34		MGAAVKRAREEEP	V <mark>SLALALTTDSA</mark> ASSTT <mark>S</mark> ADS	S <mark>A</mark> GAAPARKRARRGRVVATS
TaZFP46		MTKRFAFEEKEI	MARVLLLVSQEQ	AMPMPMPMAVRGDRA
	81			160
TaZFP22	SEOOHGCSVCGKVF	ASYOALGGHKASHRKPTAAPAGAEDU	KPOAAVAAAAASSSGSGEAAU	/GAGGGKLHECNVCRKTFPT
TaZFP34	GEGEEVCKTCSRAF	ATF <b>OALGGH</b> RTSHLRGRHGLEL	GVGVARAIKERKKOEE-	KOHECHICGIGFEM
TaZFP46	PERVEVCKTCDRVF	PSFOALGGHRASHKKPRLDDGGDL	КР	KLHGCSVCGLEFAT
	161			240
TaZFP22	G <b>QALGGH</b> KRC <b>H</b> YDG	IIGSAAAGPAHKLAAKATAASA	TAASRGF <mark>DLNLP</mark> ALI	PDIPERCAVTEDGEEVLSPV
TaZFP34	G <b>QALGGH</b> MRRHREEN	ALRGGDDGDQWVWRGVGLPDQEAVA	HQAAANYEPPVL <mark>LEL</mark> FV	
TaZFP46	GQALGGHMRRHRA-N	VAGGGSGVMAMTPRTAAIKKHNDSS	DN <mark>AVVG</mark> MKRGLWL <mark>DLN</mark> HPPCI	DEYGASCEGDDECGHDAAAA
	241	258		
TaZFP22	SLKKPRLMLTA			
TaZFP34				
TaZFP46	GYTFHQFLDTGTME	JDCV		

**Supplementary Figure S1**. Amino acid sequence alignment of TaZFP22, TaZFP34 and TaZFP46. C<sub>2</sub>H<sub>2</sub> residues and QALGGH motifs are in bold. EAR motifs are underlined.

Ubi1GFPZFP22



**Supplementary Figure S2.** Subcellular localisation of TaZFP22-GFP and TaZFP46-GFP fusion proteins in wheat leaves. Each TaZFP-GFP construct (Ubi1GFPZFP22 or Ubi1GFPZFP46) was co-bombarded with an Act1RFP construct. The RFP red fluorescence illustrates the shape of transformed leaf epidermal cells (shown at the right) in the RFP channel. These N-terminal GFP-fused TaZFP22 and TaZFP46 proteins were localised in the nucleus.

Α	ZFP46S1	<b>CAGTA</b> AGCTGA <b>CAGTG</b> ACGGAA <b>CACTG</b> CCTA
	ZFP46S2	CT <b>GAGTG</b> AGAAG <b>GAGTG</b> CAATCACAAATGGC
	ZFP46S3	GCACATG <b>GAGTG</b> GATGTTA <b>CAGTG</b> CTTGAAT
	ZFP46S4	CTGAG <b>GAGTG</b> ATTCAAT <b>CACTC</b> CCATCCAAGC
	ZFP46S5	CACTCATATACAGTATGCGATTACTCCTGA
	ZFP46S6	ATTCCAG <b>CAGTG</b> TGAATGT <b>CAGTG</b> CGATTGT
	ZFP46S7	G <b>GAGTG</b> CTGAACT <b>GAGTG</b> CGTACACAAGCAT
	ZFP46S8	CACTCTAGCACACAGTATCAAATGGCAGTATC
	ZFP46S9	CACTCAAGATTGAGTGTGCGTATAGCACTCCA
	ZFP46S10	GCACCATGG <b>GAGTG</b> GATTTGA <b>CAGTG</b> GAATTGT
	ZFP46S11	CCAAGCTCTGTGA <b>CAGTG</b> ACATTAG <b>GAGTG</b> CTCG
	ZFP46S12	CGCTGAG <b>GAGTG</b> ATTCACA <b>CAGTG</b> CCCCATAA
	Consensus	SAGTR

В							
	Scanning	cor	e	region	RBA		
	EP1		GG	TTGA <b>CAGTG</b> TCACATGA <b>CAGTG</b> TCATT	1.00	±	0.07
	EP1m1		GG	TaGA <b>CAGTG</b> TCACAaGA <b>CAGTG</b> TCATT	0.97	±	0.11
	EP1m2		GG	TTaA <b>CAGTG</b> TCACATaA <b>CAGTG</b> TCATT	0.46	±	0.12
	EP1m3		GG	TTGC <b>CAGTG</b> TCACATCC <b>CAGTG</b> TCATT	0.52	±	0.04
	EP1m4		GG	TTGAa <b>AGTG</b> TCACATGAa <b>AGTG</b> TCATT	0.17		
	EP1m5		GG	TTGA <b>C</b> C <b>GTG</b> TCACATGA <b>C</b> C <b>GTG</b> TCATT	0		
	EP1m6		GG	TTGA <b>CA</b> a <b>TG</b> TCACATGA <b>CA</b> a <b>TG</b> TCATT	0		
	EP1m7		GG	TTGA <b>CAG</b> a <b>G</b> TCACATGA <b>CAG</b> a <b>G</b> TCATT	0		
	EP1m8		GG	TTGA <b>CAGT</b> ATCACATGA <b>CAGT</b> ATCATT	0.30	±	0.05
	EP1m9		GG	TTGA <b>CAGTG</b> aCACATGA <b>CAGTG</b> aCATT	1.91	±	0.17
	EP1m10		GG	TTGA <b>CAGTG</b> TtACATGA <b>CAGTG</b> TtATT	0.76	±	0.15
	EP1m11		GG	TTGA <b>CAGTG</b> aaACATGA <b>CAGTG</b> aaATT	1.01	±	0.09
	EP1m12		GG	TTGAt <b>AGTG</b> aCACATGAt <b>AGTG</b> aCATT	1.23	±	0.16
	EP1m13		GG	TTGAg <b>AGTG</b> aCACATGAg <b>AGTG</b> aCATT	2.43	±	0.23
	EP1m14		GG	TTGA <b>CAG</b> a <b>G</b> aCACATGA <b>CAG</b> aCATT	0		
	EP1m15		GG	TTGA <b>CAG</b> C <b>G</b> aCACATGA <b>CAG</b> C <b>G</b> aCATT	0.09	±	0.02
	Defining	seq	ue	nces flanking the core			
	EP1m13		GG	TTGAg <b>AGTG</b> aCACATGAg <b>AGTG</b> aCATT	2.43	±	0.23
	EP1m16		GG	TTGAg <b>AGT</b> aaCACATGAg <b>AGT</b> aaCATT	0.80	±	0.17
	EP1m17		GG	TTGAg <b>AGT</b> caCACATGAg <b>AGT</b> caCATT	0.05	±	0.01
	EP1m18		GG	TTGAg <b>AGT</b> taCACATGAg <b>AGT</b> taCATT	0.22	±	0.03
	EP1m19		GG	TTGAg <b>AGTG</b> CCACATGAg <b>AGTG</b> CCATT	1.13	±	0.13
	EP1m20		GG	TTGAg <b>AGTG</b> gCACATGAg <b>AGTG</b> gCATT	1.41	±	0.15
	EP1m21		GG	TTGAg <b>AGTG</b> TCACATGAg <b>AGTG</b> TCATT	1.27	±	0.08
	EP1m22		GG	TTGcg <b>AGTG</b> aCACATCcg <b>AGTG</b> aCATT	0.92	±	0.06
	EP1m23		GG	TTGgg <b>AGTG</b> aCACATGgg <b>AGTG</b> aCATT	3.07	±	0.28
	EP1m24		GG	TTGtg <b>AGTG</b> aCACATGtg <b>AGTG</b> aCATT	1.71	±	0.16
	EP1m25		GG	TTAAg <b>AGTG</b> aCACATaAg <b>AGTG</b> aCATT	0.58	±	0.08
	EP1m26		GG	TTcAg <b>AGTG</b> aCACATcAg <b>AGTG</b> aCATT	0.79	±	0.10
	EP1m27		GG	TTtAg <b>AGTG</b> aCACATtAg <b>AGTG</b> ACATT	1.05	±	0.03
	Defining	spa	ce	r length			
	EP1m23		GG	TTGgg <b>AGTG</b> aCACATGgg <b>AGTG</b> aCATT	3.07	±	0.28
	EP1m28		GG	TTGgg <b>AGTG</b> aCACaATGgg <b>AGTG</b> aCATT	1.91	±	0.02
	EP1m29		GG	TTGgg <b>AGTG</b> aCACaaATGgg <b>AGTG</b> aCATT	1.37	±	0.07
	EP1m30		GG	TTGgg <b>AGTG</b> aCACaaaATGgg <b>AGTG</b> aCATT	1.33	±	0.09
	EP1m31		GG	TTGgg <b>AGTG</b> aCACaaaaATGgg <b>AGTG</b> aCATT	0.52	±	0.03
	EP1m32		GG	TTGgg <b>AGTG</b> aCACaaaaaATGgg <b>AGTG</b> aCATT	0.49	±	0.08
	EP1m33		GG	TTGgg <b>AGTG</b> aCAATGgg <b>AGTG</b> aCATT	1.53	±	0.04
	EP1m34		GG	TTGgg <b>AGTG</b> aCATGgg <b>AGTG</b> aCATT	1.68	±	0.09
	EP1m35		GG	TTGgg <b>AGTG</b> aCTGgg <b>AGTG</b> aCATT	1.92	±	0.10
	EP1m36		GG	TTGgg <b>AGTG</b> aCGgg <b>AGTG</b> aCATT	0.49	±	0.04
	EP1m37		GG	TTGgg <b>AGTG</b> aGgg <b>AGTG</b> aCATT	0.52	±	0.10

Supplementary Figure S3. The DNA-binding specificity of TaZFP46

(A) In vitro selected DNA-binding sites of TaZFP46. Spacer length between SAGTR (or YACTS) and SAGTR is 5-9 nucleotides. S = C or G; R = A or G; Y = C or T.

(B) Systematic base substitution and insertion/deletion analysis of the TaZFP46 binding sequence using the EP1 element as a starting motif. Values are means  $\pm$  SD of 2-3 assays. Binding activity is expressed as relative to that of EP1. SAGTR in EP1 is typed in bold letters and substituted or inserted bases to EP1 in lower-case letters. Values with a marked increase in binding activity are in bold. RBA, relative binding activity of TaZFP46.

OsRSP3GFP



(C) OsRCg2GFP – a positive control for GFP expression for some above-ground tissues



**Supplementary Figure S4.** Root specificity of an *OsRSP3* promoter-driven *GFP* reporter gene (OsRSP3GFP) in transgenic wheat plants. Root specificity of OsRSP3GFP reporter in transgenic wheat plants is illustrated in (**A**) with wild type control in (**B**) and OsRCg2GFP (Xue et al., 2016, Reference # 38) as a positive control for some above –ground tissues (**C**).



**Supplementary Figure S4-continue**. Cell specificity of OsRSP3GFP reporter expression is shown in (**D**) with wild type control in (**E**). Cross sections of wheat primary seminal roots (**D** & **E**) are shown as merged images of a bright-field image and a GFP fluorescence image with corresponding GFP fluorescence images placed at the right side. Low green auto-fluorescence background is present in wild type root cells (**E**).

### (A) Relative TaZFP34 expression in roots and shoots



(B) Relative GFP fluorescence intensity in etiolated seedlings of OsRSP3GFP plants



**Supplementary Figure S5.** Relative TaZFP34 expression levels in the roots and shoots of two high *TaZFP34*-expressing transgenic lines and relative GFP fluorescence intensity in the etiolated seedlings of OsRSP3GFP plants.

(A) Relative *TaZFP34* expression levels in the roots and shoots of two high *TaZFP34*-expressing transgenic lines (ZFP34-2 and ZFP34-13). Three-week-old seedlings were used for expression analysis. Values are means + SD of 3-4 biological replicates and expression levels are expressed as relative to that in the roots of the wild type plants. Statistical significance of differences between control and transgenic lines is indicated by asterisks (\*\* P < 0.01).

(**B**) Relative GFP fluorescence intensity in the etiolated seedlings of OsRSP3GFP plants. One-week-old dark-grown seedlings were used for analysis.

#### TaRR12D promoter

CTTGTGGGGTTGAGGTTGTACTGCATGCACGGTGGAGGGGTGCAACGGCCAAGAGAACGAGCAGAACGCCGAATTCATACCTTAAGGATGGTACGCGCGAAGAG
ATGGGATCGCCCGCCGCCGCCGCCGATTCAGCCGGCGCAGATCCAGCCTTCTTCTTCTGCGTGACGGCGTGGGGGCTGGGGTTGGTT
ATGAATTTATTCCTCTCACCAGGCAGGTCGAGGACGGAGAAGGTCAGGAGCGGCGACGACGACGAATACATCGGAGGAGGATTCTGAACTGGATCGAGTAGACATGGA
TCTGGTCCTCAGGTGATCGGTTCAAGAAAAGATATTTTCCCCTGGACCATTATGCCCTTATCGCAATTAACATATTAAATTTAATCAATTAAAATTCCCCGCAAGATCG
GACGGTTAATAAAAATCAGACGTGATCAGA <mark>CATGTAAGTACTGCTAAGTACTCCAAGTACTA</mark> ACCCCAATCACCATAAAACAGCTAATTATATATATATGTGTTGAGCCCA
RR12DBS1
AAATAGCACTCGGCGAGCAGAGAGCACCTTTCAATGGTATACTACAAGACAATCTACAAAAATATTAAGAAATTGCTTCAAACGTTCATGGCTTTTTGTAGTTGAATT <mark>C</mark>
AAACTGCAGAAACTTCTTACGTTAGTTTCCAGAGGGAGTAGCATATATTCTCTCTC
RR12DBS3
TAAATGTGAAAAAGAAAAAAAAACAATGCTGCAGAAATCAAAATCACCAGGAATGGAGAAACAACCGGAGCAAGCA
TGGCAGGTGGGTCCCACGGGCTCCTCCTCCTGTAGCCTTGCACCATATGCAAACGCAAACGCAAACGCAGC <mark>GAGCACTTCCACTCTGATGACTGACC</mark> CCTATAAATCGG
RR12DBS2
CCACCAGCAGTCCAAAACTTTGCTTTCCATCGCCATCGAAACCTGCTCGCCCTTTCACCTCCCCAAACAACACCCTCCCCACCAAGCGTGCTGCTGCTGCTGCTGCGCG
GTGGTTTGGCTGCTTCCTCCTCCTCCTCCACCTCGTCGTCGTCGTCTTCTACCTTTGCCTGCGTGCTGCTGTGCTGGGCGGGGTGGGCTGGGCTGGGCTGCGAGCA
CGGGCAGGCAGGGCTTGCTTGCTCGCTTGGGCACCCGGAGTGTCGCTGTCTGT
CTTTTGCTTTCCCAACCTCTCCCGTCCCGGCCTCAATAATGATGGAGGGGCCGCGGGGTTCGTTC
CTCCACCGGTGGGCCGGAATACAAATCCACCGGCAGTTTTCCCCTCTCTGATCTTCTCTCTC
TGTTGTGTTCAGGTGGTGTTGGTGTCGAGCGCTCGATTAAAGTCCCCTTTCTTCTTCTTCTTAATTGCTAGCTGCCATTAGCTTCTCAGGGTTCTTGCTGGGGATTGGG
TCAATTGGGAGCTTTCGTCTTTAGGGTCAATTGGG <b>ATG</b>
TaSUV2 promotor
AAATCGCACTAGTTCAACCGTTGGGTTTTTTTACCATGAATTACATTGCATAAATGCACGAAAACTTTGTTTAATCTTGCAAAAAAGAGATTATTTACTAAATAATAA
ATAGTTTTTTTCCCAGGATTTCCTCAAGCTGAGCCCGGCATTCCTGATATCCCGTAGGCCAAATTCATCTTGTTTGATAATCTAGCTGAAGCTAGGGACCACCTTTGC
TCACCAGGAAAATGAATATACAAGCATCGGTTAGTTAAAGGAGCAG <mark>CACCATCACTGCAGTTCACAATACTTTGCAGTTTO</mark> CATAGAAAAGAAAAGGAGAGGTGAAGGTGAAG
SHIZES1 SHIZES2
CAGTGCACTTTGCAAGGGAACTTTTTGGACTAATTAAACAAGCTCCCCTACCACTAGGGCCTGCTTTGCATAGGCAAAAGAAGCAAGGGGCAGGGAAAAGAAGAAGAAGAA
GGTAGTGGAGAGAGAGAGAGATATATGATGAGGAGGCATGCAAATAGACAAGTGCCCATATACTCCATGTTACATTGTTGCATACATA
GAGCAGTTTTGTATTAGCCAAAGATGCTCCCCCAAGATCTCTAATGATAACATCCTAATCATGGCATTGGCGCCCTATTTATT
CATCAGTGCTGCTTTGAACCTTTCTAGATGACGGGTACCGGGTGCCGGGCCCAGATCCATGCCCGGGTTCAACCGGGTTATGGTAACTACATTTTGAGGATAATTGATATGG
GGTCCAATTAGTAATGTACACGTACAGTGATCACGGGCTATCGGGTCAAGTGCAGCAATCCAGCAACGACTCGACCCGGCACCCGAGAATCTCTAGTGGTA
CCCTAGTGGAGCCCCCGCGTGGTCCCCCGCGCCCCGCCC
TGGTAAGTGTGGGCCCGCACATGCGCGTGGGCCTGGGCCGTGCGCGCGTGCGT
acgcccgcctatctctctctctctctcctcGtcgcagccGgcctaccccggggggCtccgctctataaaacatttccatggAGAACCtCCACAGCtCACAAACCCtCA

**Supplementary Figure S6.** Promoter region sequences of *TaRR12D* and *TaSHY2*. The underlined ATG is a translation initiation codon. SAGTR (or YACTS)-like motifs containing sequences used for DNA-binding assays are highlighted.

TaRR12D and TaSHY2 promoter sequences were isolated from wheat genomic DNA (genotype: SB169) PCR-amplification, based on the wheat genome sequence database URGI using in (https://urgi.versailles.inra.fr/blast/blastresult.php?jobid=141834266111&opt=none). The following two sequence IDs used for promoter isolation are WGSC\_chr7DS\_ab\_k71\_contigs\_longerthan\_200\_3948850 (the TaRR12D promoter) and IWGSC\_chr5DS\_ab\_k71\_contigs\_longerthan\_200\_273208 (the TaSHY2 Primers promoter). used were: 5'-<u>cacagatct</u>cttgtggggttgaggttgtac (sense primer) and 5'-TaRR12D 5'ctgccatggcaattgaccctaaagacgaaag (antisense primer) for the promoter; ccggaattcaaatcgcactagttcaaccg (sense primer) and 5'-cgcggatccacgaggtgcgtgtctaccgact (antisense primer) for the TaSHY2 promoter. The underlined sequences are restriction enzyme sites used for cloning. The PCRamplified DNA fragments were cloned and sequenced.

TaSHY2GFP & Act1RFP



TaSHY2GFP & Act1RFP + Ubi1ZFP34



**Supplementary Figure S7**. Illustration of co-transformation efficiency of TaSHY2GFP and Act1RFP constructs in wheat leaves using particle bombardment-mediated transformation and repression of TaSHY2GFP expression by *ZmUbi1* promoter-driven TaZFP34 (Ubi1ZFP34). Left panels are GFP channel images and right panels are corresponding RFP channel images. This illustrates that the co-transformation efficiency of GFP and RFP constructs is quite high in this transient expression system.