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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

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Supplementary Table S1. The sequences of primer pairs used for real-time PCR.

Gene name	GenBank accession #	Forward primer	Reverse primer
TaZFP22	EU408222	5'-cgtgtgccggaagacgtt	5'-cgtcgtagtggcacctttg
TaZFP34	EU408224	5'-acggcgatcagtggtgt	5'-gacgaacagctcgagcaaga
TaZFP46	EU650398	5'-acaacgacagcagcgacaat	5'-gtgggtgttcaggtcgag
TaRR12B	AK336252	5'-aagctgcaggtggattcaa	5'-cacaccgaggtcatttcc
TaRR12D	CJ612635	5'-gcaggtgtgggttcaattcc	5'-ctctcatatgcaggaccaagt
TaRR1L1	CK206029	5'-cattggaagcagtgagacatc	5'-tccagggtattaccctactacaatc
TaRR1L2	JF951927	5'-cagccagtgtccagtctgcta	5'-aagcacccttccaacaatc
TaIAA7	AJ575098	5'-caaggccaaggaagatacaa	5'-caccatcctgtggcatatag
TaIAA8	GH723801	5'-tgacagctggaatagctcaa	5'-ctgcgacatcatcgcaact
TaIAA27	HX049647	5'-gcccgccgagaagataagaa	5'-cgatcgaggaaaggaaaca
TaSHY2	HX177892	5'-ggatcatgaaggatccgaag	5'-catggcatgttgcgtgttct
TaGA20-ox1	FR716525	5'-ggaagtcgctggccttctt	5'-cacgtgaagtcgggtagg
TaGID1	FR668556	5'-caagtactctgcacgctccag	5'-ggcaggtacgccttccagta
TaRht1	KC767924	5'-ccaggtcatctccgaggtga	5'-ggccaccacgttgcagat
TaARF1	AK331474	5'-tcagactaccagcctcgtttg	5'-cccgaatgatcaaaaggaagaa
TaARF2a	AK334169	5'-ggaaggttcagtgatggtgac	5'-tggttagaatttaaatcgtttag
TaARF2b	AK335756	5'-tcaacaaggaattggcagattg	5'-cttgcgcaactatgtgcagAAC
TaARF6	BJ13449	5'-gcagcagcggaaactgttctat	5'-ggcaaatcttccactataacc
TaETTINa	AK330400	5'-attggcatttctctggctgaa	5'-atagttggcaccacatcatca
TaETTINb	AY376128	5'-cgactccacaatggatgga	5'-caaatcaagaatcacaacagatga
TATIR1L1	AK330774	5'-gccctaatctggtgttgaagtga	5'-tgccaatgatcgatacaggtaca
TATIR1L2	CJ589802	5'-ccgcttaccgtggagataat	5'-ggctatctccggaagtgactc
TaTIR1L3	HX160462	5'-aatgcctaatctggtgttgaag	5'-gccaacgatcgatacaggtaca
TaPIN1a	CK162375	5'-catacatgccagccaactgtg	5'-ggcagatgcacacaagtgtca
TaPIN1b	AY496058	5'-ctcagcagcgtctcatcttc	5'-aggccgagcaggtatgtagaga
TaPIN2	BT008949	5'-tgctctcatctgttccattgt	5'-tgctactcttggcgaacacaaa
TaPIN4	CA703452	5'-gagccaaggagaggaggacta	5'-cctgttcccgaagctgaagtc
TaExpB18	CD897610	5'-cctctgtggcagtagtactc	5'-accacaaccacgcctagt
TaCYCB1-2	AK334336	5'-tccatggagaagaatctctgaaca	5'-atctcggcgaagaagaatca
TaCYCB2-3	CJ847821	5'-atgtcttggacaagtctgtg	5'-ctgcagtcctatgagctgttct
TaCYCD4-1	AF512432	5'-ggatctccagcaactgctacc	5'-gatcggctatctgccaattctc
TaNCED3	LC077864	5'-ccaaggtggacctggtgac	5'-ccctcggctactcgaact
TaNCED9	LC077862	5'-gggagctccaagtctgag	5'-gtcgtgaacgaagtgagaatg
TaCYP707A1	LC077861	5'-gctggagatgctcgtctct	5'-tctggacttggaggtgac
TaABI1	AB238930	5'-gctgctgaatgctgtcga	5'-cctctgcgcttgggctca
TaNAC69-1	AY625682	5'-tgcttcccgaaccca	5'-ttgttcacgtagcctgtgtgt
TaWRAB1	AF139915	5'-aatgccaccaccaaggacac	5'-ggcaacgatcgcttagtga
TaWRAB18	AB115914	5'-aggacagcaccaccgagaag	5'-ggaagcaaatagcaagattgga
TaLEA1	AY148490	5'-cacaacaccaccaaggaca	5'-cgccaacacatgcgtctagt
TaAPX1	HX167147	5'-accctgctgactgaccctgt	5'-cctgaggtgtgcctcctgt
TaAPX2	HX177140	5'-gaccgacaagaccctcctga	5'-cactctatccgcagcatatt
TaFSD2	HX176689	5'-tcgtgtcattggaaatggtg	5'-gttctctctctgctgcttc
TaGA3ox2	AK330302	5'-gtcacgtgccccagtagat	5'-catcgttctcgttggagatg
TaGIP	EU095332	5'-tgccctgctacaacaactg	5'-tgccaccagaagaagaag
TaSLRL1	HG670306*	5'-acggcgaagataaccataccag	5'-cccgctacattaccgttgc
TaExpA4	AY543530	5'-gagcaggaactgggtgcta	5'-gtccgtcgtcgtctgtacc
TaExpB1	CJ803265	5'-ggcacgtctacagctctc	5'-gtcacaattgcagaaccaga
TaRP15	HX098404	5'-gcacagctgcttgcagataag	5'-gccctcaagctcaaccataact

The full-length sequences of some genes were obtained by extending partial EST sequences from the NCBI wheat EST database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) 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. aestivum* 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 GAGTGA	N8	GG GAGTGA	100%
High	AC	N8	AC	71-90%
Medium	TT AB	N5-11	TT AB	41-70%
Low	HC	N5-11	HC	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 genes					
TaRR12B	Two-component response regulator	0.017 ± 0.004*	0.018 ± 0.001*	0.033 ± 0.009	0.53
TaRR12D	Two-component response regulator	0.041 ± 0.013*	0.051 ± 0.017*	0.099 ± 0.008	0.46
TaRR1L1	Two-component response regulator 1-like	0.870 ± 0.073	0.768 ± 0.033*	1.089 ± 0.210	0.75
TaRR1L2	Two-component response regulator 1-like	0.244 ± 0.029	0.241 ± 0.044	0.257 ± 0.032	0.94
TaIAA7	Aux/IAA protein	2.62 ± 0.42*	2.71 ± 0.19*	3.59 ± 0.33	0.74
TaIAA8	Aux/IAA protein	1.43 ± 0.15	1.27 ± 0.07*	1.47 ± 0.04	0.92
TaIAA27	Aux/IAA protein	0.114 ± 0.006*	0.116 ± 0.005*	0.143 ± 0.013	0.80
TaSHY2	Aux/IAA protein	0.977 ± 0.061*	0.933 ± 0.031*	1.322 ± 0.203	0.72
TaGA20-ox1	Gibberellin 20-oxidase	0.030 ± 0.008*	0.040 ± 0.004*	0.062 ± 0.011	0.56
TaGID1	Gibberellin receptor	0.933 ± 0.148*	0.934 ± 0.110*	0.632 ± 0.067	1.48
TaRht1	DELLA protein	0.239 ± 0.027	0.213 ± 0.024	0.286 ± 0.049	0.79
TaARF1	Auxin response factor	0.187 ± 0.010	0.198 ± 0.016	0.193 ± 0.018	1.00
TaARF2a	Auxin response factor	0.061 ± 0.003	0.065 ± 0.004	0.062 ± 0.003	1.02
TaARF2b	Auxin response factor	0.160 ± 0.005	0.151 ± 0.010	0.140 ± 0.022	1.11
TaARF6	Auxin response factor	0.013 ± 0.004	0.017 ± 0.003	0.017 ± 0.004	0.88
TaETTINa	ETTIN-like auxin response factor	0.038 ± 0.004	0.034 ± 0.005	0.038 ± 0.001	0.95
TaETTINb	ETTIN auxin response factor	0.051 ± 0.006	0.047 ± 0.004	0.048 ± 0.004	1.02
TaTIR1L1	Transport inhibitor response 1-like	0.313 ± 0.035	0.266 ± 0.028	0.308 ± 0.038	0.94
TaTIR1L2	Transport inhibitor response 1-like	0.674 ± 0.052	0.558 ± 0.024	0.593 ± 0.007	1.04
TaTIR1L3	Transport inhibitor response 1-like	0.259 ± 0.012	0.181 ± 0.018	0.208 ± 0.038	1.06
TaPIN1a	Auxin efflux carrier protein	0.0017 ± 0.0002	0.0013 ± 0.0001	0.0013 ± 0.0004	1.15
TaPIN1b	Auxin efflux carrier protein	0.155 ± 0.008	0.175 ± 0.005	0.215 ± 0.042	0.77
TaPIN2	Auxin efflux carrier protein	0.282 ± 0.019	0.295 ± 0.030	0.293 ± 0.041	0.99
TaPIN4	Auxin efflux carrier protein	0.117 ± 0.028	0.082 ± 0.021	0.083 ± 0.023	1.20
TaExpB18	Expansin B	0.009 ± 0.003*	0.012 ± 0.002	0.017 ± 0.003	0.62
TaCYCB1-2	Cyclin B1	0.073 ± 0.025	0.087 ± 0.021	0.089 ± 0.022	0.90
TaCYCB2-3	Cyclin B2	0.050 ± 0.015	0.054 ± 0.016	0.058 ± 0.011	0.90
TaCYCD4-1	Cyclin D4	0.171 ± 0.012	0.174 ± 0.015	0.175 ± 0.030	0.99
ABA metabolic, signalling or drought-upregulated genes					
TaNCED3	9-cis-epoxycarotenoid dioxygenase 3	0.009 ± 0.001	0.014 ± 0.002	0.009 ± 0.003	1.28
TaNCED9	9-cis-epoxycarotenoid dioxygenase 9	0.015 ± 0.001	0.019 ± 0.001	0.017 ± 0.001	1.00
TaCYP707A1	ABA 8'-hydroxylase 1	0.375 ± 0.027	0.401 ± 0.040	0.359 ± 0.056	1.08
TaABI1	Protein phosphatase 2C	0.209 ± 0.005	0.228 ± 0.012	0.260 ± 0.037	0.84
TaNAC69-1	ABA-inducible NAC transcription factor	0.084 ± 0.005	0.055 ± 0.005	0.070 ± 0.013	0.99
TaWRAB1	Late embryogenesis abundant protein	0.161 ± 0.028	0.121 ± 0.043	0.180 ± 0.025	0.78
TaWRAB18	Late embryogenesis abundant protein	0.291 ± 0.193	0.499 ± 0.054	0.488 ± 0.072	0.81
TaLEA1	Late embryogenesis abundant protein	0.027 ± 0.006	0.019 ± 0.009	0.021 ± 0.002	1.10
Antioxidant enzyme genes					
TaAPX1	L-ascorbate peroxidase 1	8.96 ± 0.17*	10.11 ± 0.65	13.35 ± 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 ± 0.001	0.104 ± 0.008	0.108 ± 0.020	0.86

The root samples were from 3-week-old hydroponically grown wild type and T₂ transgenic (ZFP34-2 and ZFF34-13) plants. Values are means ± 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; ** $P < 0.01$;

† Ratio of the mean values of two transgenic lines to that of the wild type control.

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1
TaZFP22  MAVEAVLEAAAMVPSPPSKEMEASSSTSEEASALLGQAEGWSKRKRSRRPRALAPSEEEYLALCLLMLAHGHRDSAPAAA
TaZFP34  MGAAVKRAREEEEPVSLALALTTDSASSTTSADSAGAAPARKRARRGRVVATS
TaZFP46  MTKRFAFEEKEEMARVLLLV-----SQEQAMPMPMPMAVRGDR----A

81
TaZFP22  SEQQHGCSVCGKVFASYQALGGHKASHRKPTAAPAGAEDLKPQAAVAAAASSSGSGEAAVGAGGGKLHECNVCRKTFPT
TaZFP34  GEGEFVCKTCSRAFATFQALGGHRTSHLRGRH----GLEL----GVGVARAIKERKKQEE-----KQHECHICGLGFEM
TaZFP46  PERVFVCKTCDRVFPSFQALGGHRASHKKPRLDD--GGDLKP-----KLHGCSVCGLEFAI

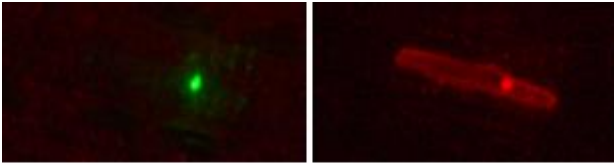
161
TaZFP22  GQALGGHKRCHYDGTIGSAAAGPA----HKLAAKATAASATAASRG-----FDLNLPALPDIPERCAVTEDEGEEVLSPV
TaZFP34  GQALGGHMRRHREEMALRGGDDGDQVWVRGVGLPDQEAVAHQAAANYEPPVLLELFV
TaZFP46  GQALGGHMRRHRA-MVAGGGSGVMAMTPRTAAIKKHNDSSDNAVVGMKRGLWLDLNHPPCDEYGASCEGDDECGHDAAAA

241
TaZFP22  SLKKPRLMLTA
TaZFP34
TaZFP46  GYTFHQFLDTGTMEVDCV
258

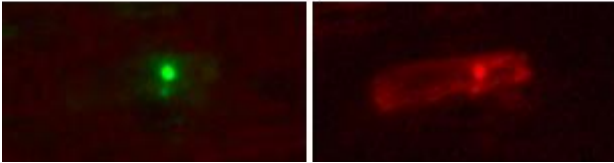
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Supplementary Figure S1. Amino acid sequence alignment of TaZFP22, TaZFP34 and TaZFP46. C₂H₂ residues and QALGGH motifs are in bold. EAR motifs are underlined.

Ubi1GFPZFP22



Ubi1GFPZFP46



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.

A

ZFP46S1	CAGTAAGCTGACAGTGACGGAACTGCCTA
ZFP46S2	CT GAGTGAGAAGGAGTG CAATCACAAATGGC
ZFP46S3	GCACAT GAGTG GATGTTA CAGTG CTTGAAT
ZFP46S4	CTGAG GAGTG ATTCAAT CACTCC ATCCAAGC
ZFP46S5	CACTC ATATA CAGTAT GCGAT TACTC CTGA
ZFP46S6	ATTCCAG CAGTGT GAATGT CAGTG CGATTGT
ZFP46S7	GGAGTG CTGAACT GAGTG CGTACACAAGCAT
ZFP46S8	CACTC TAGCACAC CAGTAT CAAAATGG CAGTATC
ZFP46S9	CACTC AAGATT GAGTGT GCGTATAG CACTCCA
ZFP46S10	GCACCATGG GAGTG GATTTG CAGTG GAATTGT
ZFP46S11	CCAAGCTCTGT GACAGTG ACATTAG GAGTG CTCG
ZFP46S12	CGCTGAG GAGTG ATTTCACAC CAGTG CCCCATAA
Consensus	SAGTR

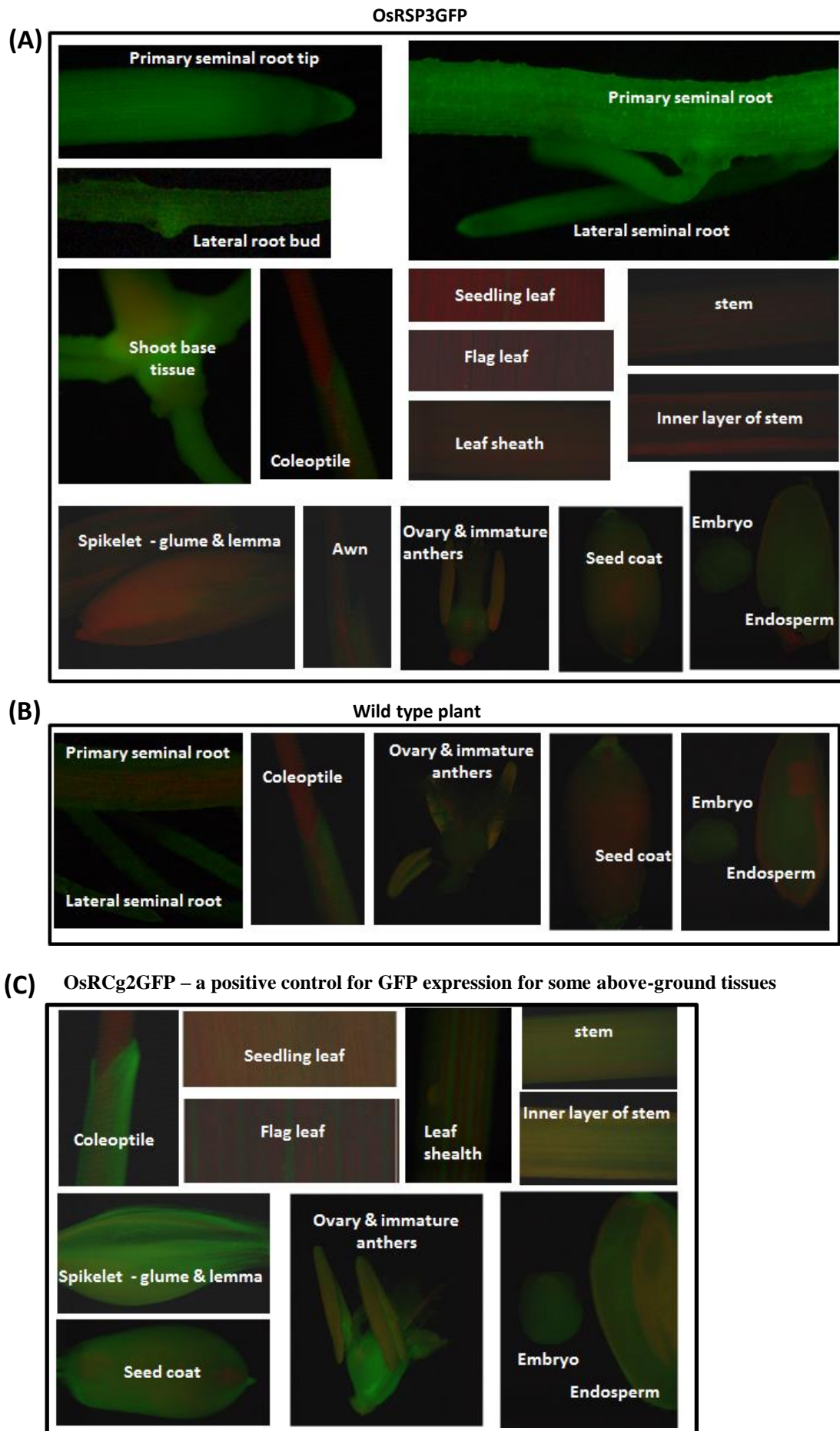
B

Scanning core region		RBA
EP1	GGTTG CAGTGT CACATG CAGTGT CATT	1.00 ± 0.07
EP1m1	GGT aGACAGTGT CACA aGACAGTGT CATT	0.97 ± 0.11
EP1m2	GGT TaACAGTGT CACAT aACAGTGT CATT	0.46 ± 0.12
EP1m3	GGTTG cCAGTGT CACAT CcCAGTGT CATT	0.52 ± 0.04
EP1m4	GGTTG AaAGTGT CACATG AaAGTGT CATT	0.17
EP1m5	GGTTG AcGTGT CACATG AcGTGT CATT	0
EP1m6	GGTTG CaTG TACATG CaTG TACATT	0
EP1m7	GGTTG CAGaGT CACATG CAGaGT CATT	0
EP1m8	GGTTG CAGT aTCACATG CAGT aTCATT	0.30 ± 0.05
EP1m9	GGTTG CAGTGT GaCACATG CAGTGT GaCATT	1.91 ± 0.17
EP1m10	GGTTG CAGTGT TtACATG CAGTGT TtATT	0.76 ± 0.15
EP1m11	GGTTG CAGTGT aaACATG CAGTGT aaATT	1.01 ± 0.09
EP1m12	GGTTG ATAGT GaCACATG ATAGT GaCATT	1.23 ± 0.16
EP1m13	GGTTG AgAGT GaCACATG AgAGT GaCATT	2.43 ± 0.23
EP1m14	GGTTG CAGaGa CACATG CAGaGa CATT	0
EP1m15	GGTTG CAGcGa CACATG CAGcGa CATT	0.09 ± 0.02
Defining sequences flanking the core		
EP1m13	GGTTG AgAGT GaCACATG AgAGT GaCATT	2.43 ± 0.23
EP1m16	GGTTG AgAGT aaCACATG AgAGT aaCATT	0.80 ± 0.17
EP1m17	GGTTG AgAGT caCACATG AgAGT caCATT	0.05 ± 0.01
EP1m18	GGTTG AgAGT taCACATG AgAGT taCATT	0.22 ± 0.03
EP1m19	GGTTG AgAGTG cCACATG AgAGTG cCATT	1.13 ± 0.13
EP1m20	GGTTG AgAGTG gCACATG AgAGTG gCATT	1.41 ± 0.15
EP1m21	GGTTG AgAGTGT CACATG AgAGTGT CATT	1.27 ± 0.08
EP1m22	GGTTG GcAGT GaCACAT CcAGT GaCATT	0.92 ± 0.06
EP1m23	GGTTG GgAGT GaCACAT GgAGT GaCATT	3.07 ± 0.28
EP1m24	GGTTG GtAGT GaCACAT GtAGT GaCATT	1.71 ± 0.16
EP1m25	GGTT AaAgAGT GaCACAT aAgAGT GaCATT	0.58 ± 0.08
EP1m26	GGTT TcAgAGT GaCACAT cAgAGT GaCATT	0.79 ± 0.10
EP1m27	GGTT TtAgAGT GaCACAT tAgAGT GaCATT	1.05 ± 0.03
Defining spacer length		
EP1m23	GGTTG GgAGT GaCACATG GgAGT GaCATT	3.07 ± 0.28
EP1m28	GGTTG GgAGT GaCACaATG GgAGT GaCATT	1.91 ± 0.02
EP1m29	GGTTG GgAGT GaCACaaATG GgAGT GaCATT	1.37 ± 0.07
EP1m30	GGTTG GgAGT GaCACaaaATG GgAGT GaCATT	1.33 ± 0.09
EP1m31	GGTTG GgAGT GaCACaaaaATG GgAGT GaCATT	0.52 ± 0.03
EP1m32	GGTTG GgAGT GaCACaaaaaATG GgAGT GaCATT	0.49 ± 0.08
EP1m33	GGTTG GgAGT GaCAATG GgAGT GaCATT	1.53 ± 0.04
EP1m34	GGTTG GgAGT GaCATG GgAGT GaCATT	1.68 ± 0.09
EP1m35	GGTTG GgAGT GaCTG GgAGT GaCATT	1.92 ± 0.10
EP1m36	GGTTG GgAGT GaCG GgAGT GaCATT	0.49 ± 0.04
EP1m37	GGTTG GgAGT GaG GgAGT GaCATT	0.52 ± 0.10

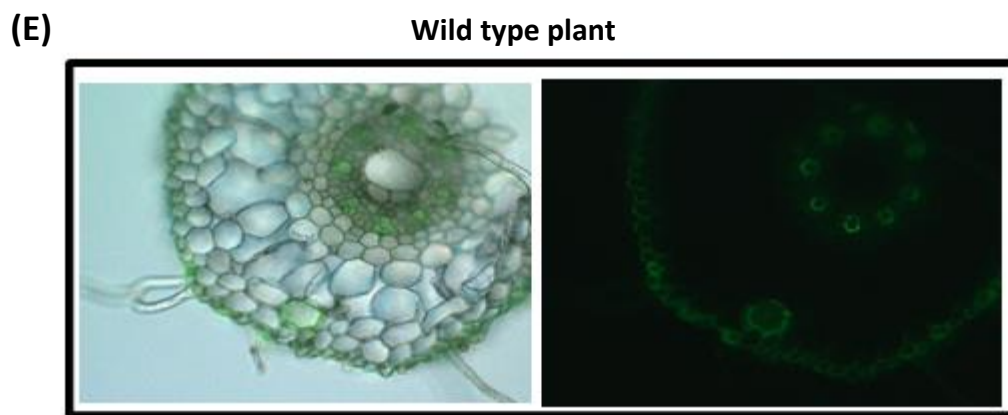
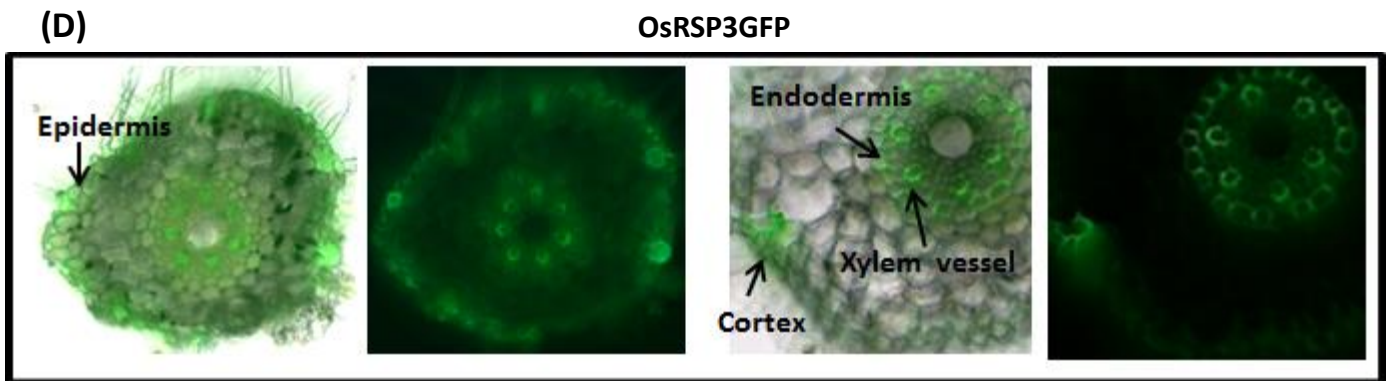
Supplementary Figure S3. The DNA-binding specificity of TaZFP46

(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 ± 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.

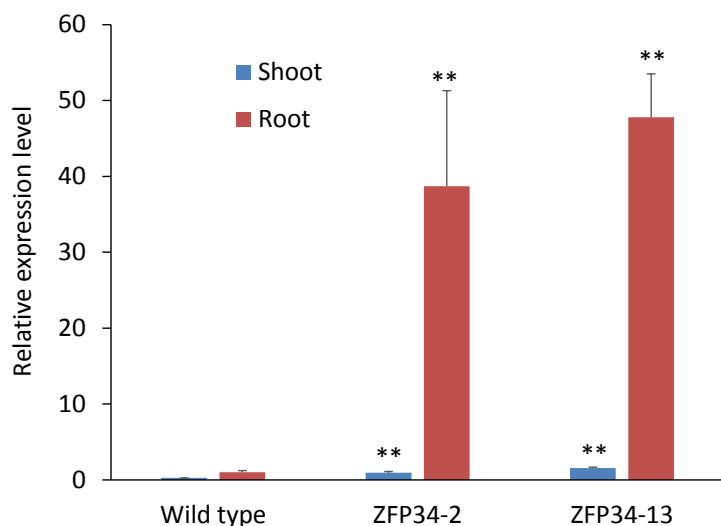


Supplementary Figure S4. Root specificity of an *OsRSP3* promoter-driven *GFP* reporter gene (*OsRSP3GFP*) in transgenic wheat plants. Root specificity of *OsRSP3GFP* reporter in transgenic wheat plants is illustrated in (A) with wild type control in (B) and *OsRCg2GFP* (Xue et al., 2016, Reference #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

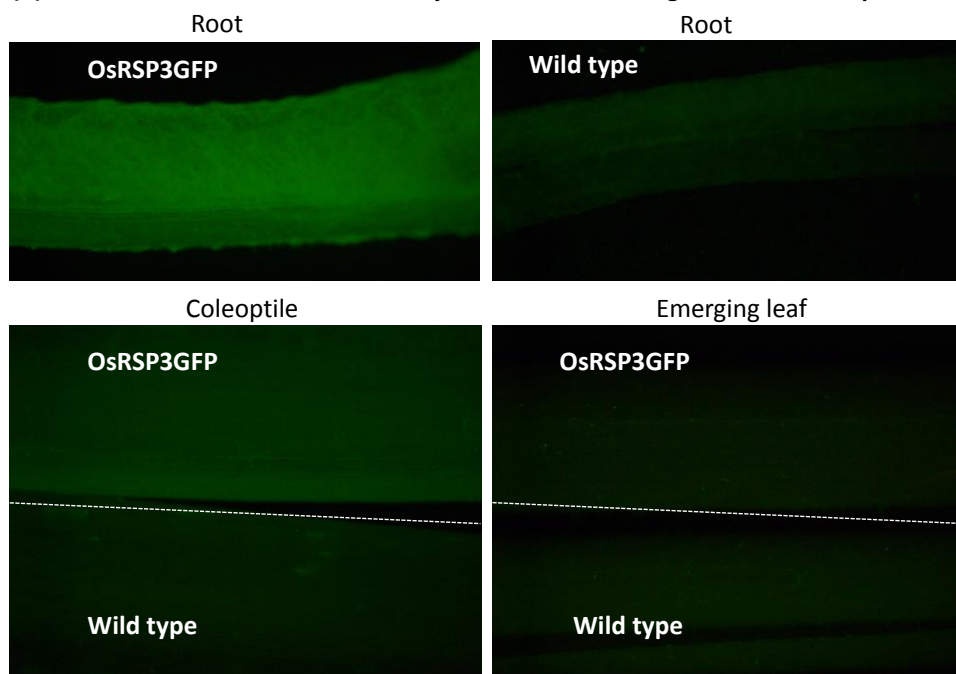


Supplementary Figure S4-continue. Cell specificity of OsRSP3GFP reporter expression is shown in (D) with wild type control in (E). Cross sections of wheat primary seminal roots (D & E) are shown as merged images of a bright-field image and a GFP fluorescence image with corresponding GFP fluorescence 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

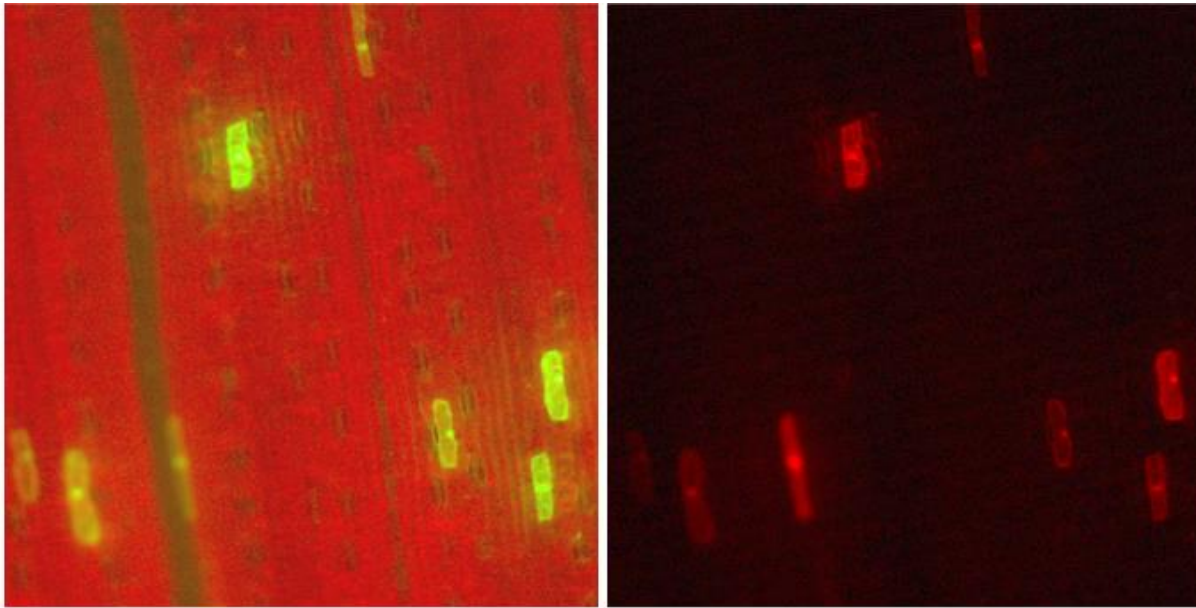


Supplementary Figure S5. Relative *TaZFP34* expression levels in the roots and shoots of two high *TaZFP34*-expressing transgenic lines and relative GFP fluorescence intensity in the etiolated seedlings of *OsRSP3GFP* plants.

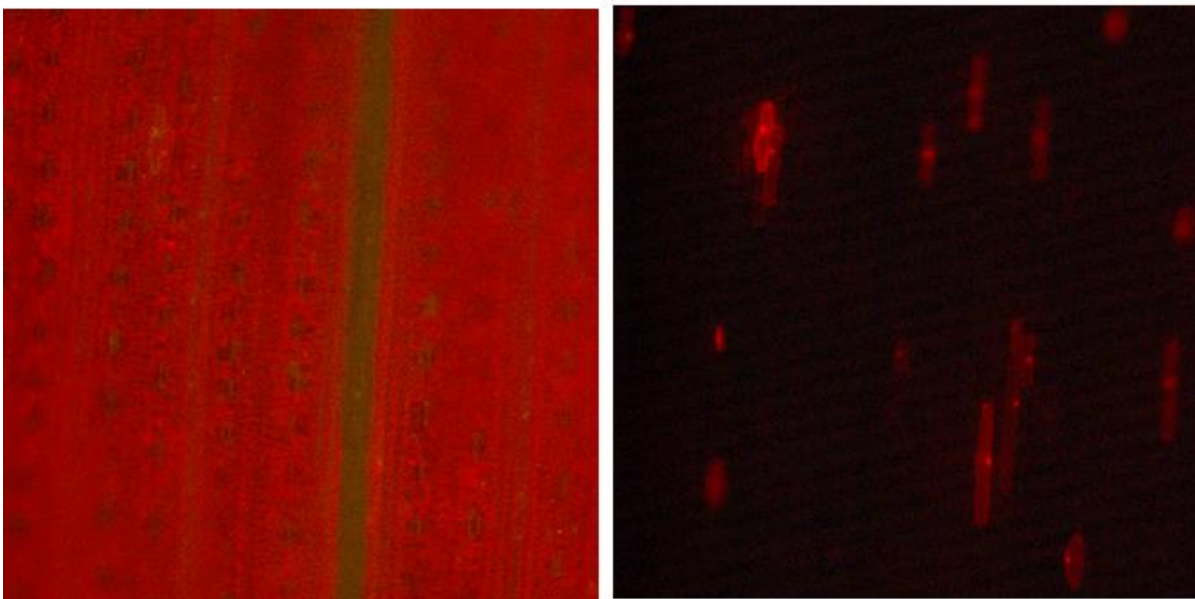
(A) Relative *TaZFP34* expression levels in the roots and shoots of two high *TaZFP34*-expressing transgenic lines (ZFP34-2 and ZFP34-13). Three-week-old seedlings were used for expression analysis. Values are means + SD of 3-4 biological replicates and expression levels are expressed as relative to 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.

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.