Figure 1. Expression response of TaZFP22, TaZFP34 and TaZFP46 to abiotic stress in the roots of 3-week-old wheat seedlings and the nuclear localisation of TaZFP34-GFP fusion protein in wheat leaves and roots.

(A) Expression of TaZFP22, TaZFP34 and TaZFP46 in wheat roots in response to cold (4°C), H2O2, salt and PEG-mediated dehydration stresses. Values are means ± SD of 3-4 biological replicates. Statistical significance of differences between control and treated groups is indicated by an asterisk (* P < 0.05 using Student’s t-test).

(B) Subcellular localisation of TaZFP34-GFP fusion protein. The TaZFP34-GFP construct (Ubi1GFPZFP34) was co-bombarded with an Act1RFP construct. The RFP red fluorescence illustrates the shape of transformed leaf and root epidermal cells (shown at the right) in the RFP channel of a fluorescence microscope. Ubi1GFP was used as a control, which showed distribution of GFP green fluorescence in the whole leaf and root epidermal cells, whereas N-terminal GFP-fused TaZFP34 protein was localised in the nucleus.
Figure 2. TaZFP34 expression levels in the roots of transgenic wheat lines (ZFP34-2, 13, 14 and 25) with root overexpression of TaZFP34 and enhancement of root-to-shoot ratios.

(A) Relative TaZFP34 expression levels in wheat roots. T2 homozygous transgenic lines were used for analysis. Values are means + SD of 3-4 biological replicates.

(B-D) Root and shoot lengths measured at 15 and 19 days after seedlings were grown hydroponically. Values are means + standard errors of the means (SEM) of 15-20 seedlings.

(E & F) Root and shoot lengths measured at 14 days after planting in thin-soil-layer chambers. Values are means + SEM of 5 seedlings.

(G-I) Dry weights of roots and shoot measured at 20 days after germination. Seedlings were grown under hydroponic conditions. Values are mean + SD of 4-5 biological replicates and each replicate contained 4 seedlings.

Statistical significance of differences between control and transgenic lines is indicated by an asterisk (* P < 0.05).
Figure 3. The above-ground phenotypic changes of transgenic lines (ZFP34-2, 13, 14 and 25) with root overexpression of *TaZFP34*. T₂ homozygous transgenic lines were used for analysis. Transgenic lines showed short final plant height (A), short spikes (B), essentially no differences in grain number per spike (C), reduced straw weight at the maturity (D), no significant changes in tiller number at the maturity (E), reduced grain yield in highly expressing lines (F) and reduced hundred grain weight (G). Values are means ± SD of 6-8 plants. Statistical significance of differences between control and transgenic lines is indicated by an asterisk (* $P < 0.05$).
Figure 4. Expression of TaZFP34 target genes in the roots of three-week-old TaZFP34 transgenic lines (ZFP34-2, 13, 14 and 25 at the T2 stage) (A & B) and expression of TaZFP34 downregulated genes during salt (0.2 M NaCl) and dehydration (15% PEG) stresses in wheat roots (C). Values are means ± SD of 3-4 biological replicates. Statistical significance of differences between control and transgenic lines is indicated by an asterisk (* P < 0.05).
Figure 5. Expression levels of shoot growth-related genes in the shoots of two high TaZFP34-expressing T₃ transgenic lines and wild type plants. Shoots of three-week-old transgenic (ZFP34-2 and ZFP34-13) and wild type plants were used for expression analysis. Values are means ± SD of four biological replicates and expression levels are expressed as relative to those of the wild type plants. Statistical significance of differences between control and transgenic lines is indicated by asterisks (* P < 0.05 and ** P < 0.01).

TaGA3-ox2, GA 3-oxidase 2 (catalysing the conversion of inactive GA to active forms); TaGIP, GA-induced protein; TaSLRL1, slender 1-like (a repressor of the GA signalling, which negatively regulates plant height in rice); TaExpA4, expansin A4 (expansins are involved in plant growth); TaExpB1, expansin B.

TaZFP34  
TaGA3-ox2  
TaGA20-ox1  
TaGIP  
TaSLRL1  
TaGID1  
TaRht1  
TaExpA4  
TaExpB1
Figure 6. Binding of TaZFP34 to TaRR12D and TaSHY2 promoter elements and repression of TaRR12D and TaSHY2 promoter-driven GFP reporters by TaZFP34, TaZFP22 and TaZFP46.

(A) Binding of TaZFP34 to the SAGTR-like elements in the promoters of TaRR12D and TaSHY2 in comparison with the EP1 element. RBA, relative binding activity.

(B) Illustration of reporter and effector constructs.

(C) Transrepression of TaRR12D and TaSHY2 promoter-driven GFP reporters by TaZFP34, TaZFP22 and TaZFP46 in a transient expression system. The GFP foci in the leaf sections of wheat plants indicate the expression of reporter genes. Act1RFP was included in each transient expression assay to demonstrate that leaf cells were transformed. Each leaf section was examined in both GFP and RFP channels. The red background in the GFP channel is chlorophyll auto-fluorescence.
**Effector constructs** (Ubi1ZFP34, Ubi1ZFP46, Act1HvDRF1 & Ubi1HsfA6f)

Maize Ubi1 or rice Act1 promoter  \( \text{TaZFP34} \& 46, \text{HvDRF1} \& \text{TaHsfA6f} \)

Rice \( rbcS \) 3’

**Reporter constructs** (ZFP46E-MiniDhn6GFP, BDRF1E-MiniDhn6GFP & HSE90-MiniDhn6GFP)

鳗鱼Dhn6 promoter  gfp  Rice \( rbcS \) 3’

**ZFP46E-MiniDhn6**

\[
\text{GAATTCGGGAGTGAACACATGGGAGTGAATTTCTGGGAGTGAACACATGGGAGTGAACACATGGGAGTGAACACATGGGAGTGAACACATGGGAGGACCG}
\]

**BDRF1E-MiniDhn6**

\[
\text{GGCCACCTACCGCTTGCTCCTCGGTTTACCGCTTTACTGGTAGTTAACCGCCTT}
\]

**HSE90-MiniDhn6**

\[
\text{GGATCCA GAATGTTCTAGAACGAACATTCTTGAA}
\]

**Figure 7.** Transient expression analysis of GFP reporter genes driven by the minimal HvDhn6 promoter (MiniDhn6) with an addition of TaZFP46-binding motifs or other cis-acting elements in wheat leaves. Constructs with the addition of two TaZFP46-preferred binding motifs (TaZFP46E: GGGAGTGAN5GGGAGTGA) in direct repeats, three barley DRF1 binding motifs (BDRF1E) or three heat shock elements from the promoter of wheat HSP90 (HSE90) to the minimal HvDhn6 promoter are shown at the top. The GFP foci in wheat leaf sections indicate the expression of reporter genes. Act1RFP was included in each transient expression assay to demonstrate that leaf cells were transformed. Each leaf section was examined in both GFP and RFP channels.