Supplemental information

The wheat ABA receptor gene TaPYL1-1B contributes to drought tolerance and grain yield by increasing water-use efficiency

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Figure S1 Phylogeny and protein sequences of TaPYL1-1A, TaPYL1-1B and TaPYL1-1D. (a) Phylogenetic analysis of clade III PYLs from Arabidopsis, rice, maize, B. distachyon, and wheat. (b) Comparison of amino acid sequences between TaPYL1-1A, TaPYL1-1B and TaPYL1-1D. The gate and latch loop positions are indicated at the bottom. Black asterisks indicate residues that interact with ABA according to the structure of PYL2-ABA (Melcher et al., 2009). Asterisks in red and green indicate polar and non-polar residues, respectively.
Figure S2 Subcellular localization of TaPYL1 homeologs. (a) Wheat protoplasts were transformed with either 35S:GFP or 35S:TaPYL1-1A-GFP, 35S:TaPYL1-1B-GFP, and 35S:TaPYL1-1D-GFP to observe green fluorescent signal. Bars = 10 μm. (b) Tobacco protoplasts were transformed with either 35S:GFP or 35S:TaPYL1-1B-GFP to observe TaPYL1-1B subcellular localization. Bars = 10 μm.
Figure S3 Screening for ABRE and MYB cis-elements in the TaPYL1-1B promoter. The red boxes indicate the four putative abscisic acid-responsive elements (ABRE) and blue boxes indicate MYB binding sites in the 1.5-kb promoter region of TaPYL1-1B.
Figure S4 Drought tolerance assessment of WT and *Ubi:TaPYL1-1B* transgenic lines cultivated in 30% PEG solution. (a) *TaPYL1-1B* expression levels in WT and twelve *Ubi:TaPYL1-1B* transgenic wheat lines (OE1-OE12) grown under well-watered conditions. Data represent the mean ± SD of three replicates. Statistical significance was determined by a two-sided *t*-test, *P* < 0.05; **P** < 0.01. (b) Plant phenotypes of WT and *Ubi:TaPYL1-1B* transgenic lines cultivated in 30% PEG solution.
Figure S5 Comparison of grain-quality related traits between WT and transgenic lines under well-watered conditions. Data represent the mean ± SD of two replicates. Statistical significance was determined by a Student’s *t* test, *P* < 0.05; **P** < 0.01.
**Figure S6** Venn diagrams of up- or down-regulated genes in *TaPYL1-1B* transgenic OE4 and OE5 lines relative to WT plants under normal, drought and ABA conditions using a significance cutoff of \(P < 0.01\) and a fold change (FC) > 2.
Figure S7 qRT-PCR verification of increased expression of eight genes involved in drought and ABA response in TaPYL1-1B transgenic wheat lines.
Figure S8: Identification and molecular characterization of drought tolerance gene TaPYL1-1B in wheat. (a) The regional association signals for wheat drought tolerance are shown in the 372.5 - 375.0 Mb region on chromosome 1B (x-axis). (b) The locations of the 11 predicted genes in the region at 372.5 - 375.0 Mb region. The chromosomal positions of the predicted genes is based on the wheat genome IWGSC RefSeq v1.1. (c) Relative expression levels of the 11 predicted genes in drought-tolerant cv. Pubing202 and drought-sensitive cv. Chinese Spring seedlings grown in well-watered (control) and water deficit (drought) conditions.
Figure S9 Molecular marker development based on InDel-442 variant. (a) The DNA sequence of the genetic variant InDel-442 in the TaPYL1-1B promoter and the primers used for genotyping. (b) PCR genotyping of wheat varieties carrying a homozygous allele of either TaPYL1-1B_{In-442} or TaPYL1-1B_{Del-442}.
Figure S10 Phenotypic analysis of drought tolerance of CRISPR-Cas9 mutant lines and WT plants under well-watered, water-deficit and re-watering conditions.
Figure S11 Agronomic traits of WT and CRISPR-Cas9 edited C3 mutant under well-watered conditions. Data represent the mean ± SD. Statistical significance was determined by a Student’s $t$ test, * $P < 0.05$; ** $P < 0.01$. 
**Figure S12** The DNA sequence and structure of MITE insertions in the $TaPYL1-1B^{In-442}$ and $TaPYL1-1B^{Del-442}$ promoters. The target site duplications (TSDs) and the loop are indicated by the red boxes. The blue arrows indicate two terminal inverted repeats. The predicted hairpin structure of the MITE is illustrated at the bottom.
Figure S13 Identification of drought-responsive MYB genes in wheat and validate their binding ability to TaPYL1-1B promoter fragment. (a) Expression patterns of six wheat MYB genes in response to drought stress. (b) Yeast one-hybrid assay to confirm the binding of six MYB proteins to TaPYL1-1B promoter fragment. The empty vectors, pGAD EV and pLacZi EV, were used as negative controls.