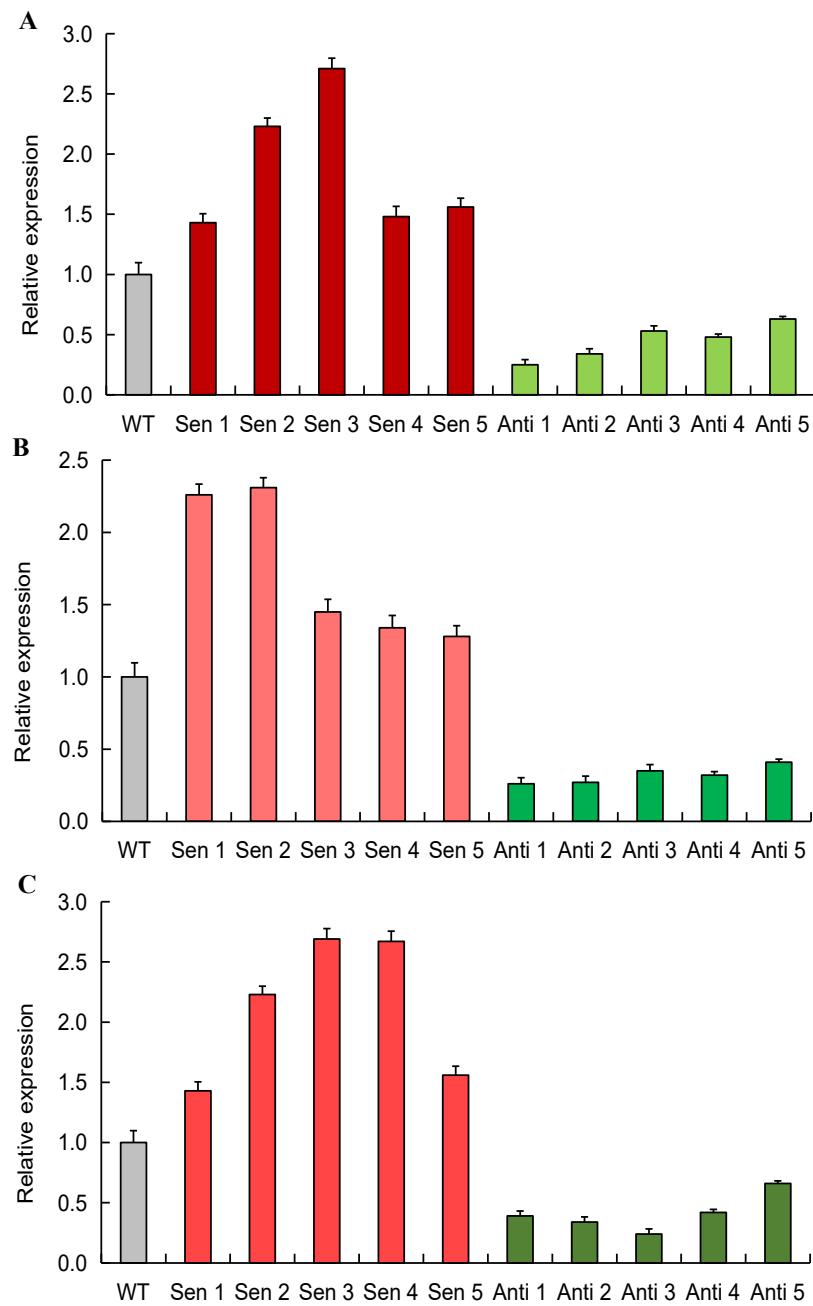
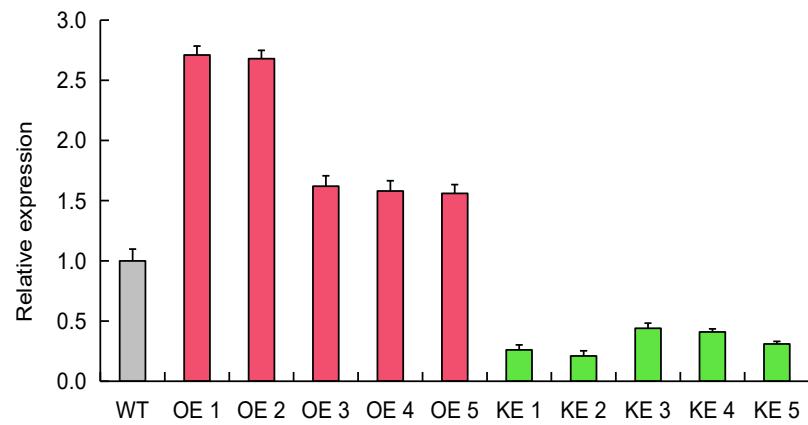


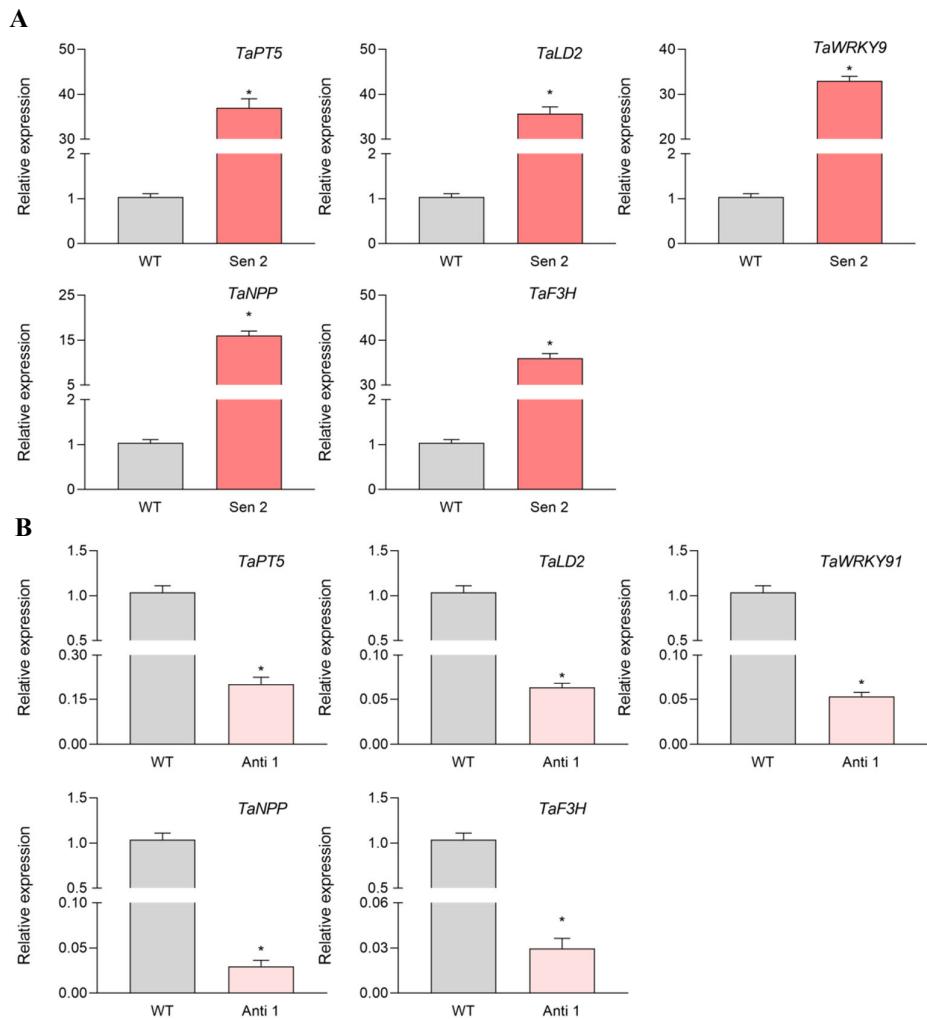
Supplementary Figure S1. Phylogenetic relations among *TaPYL5* and its homologous genes distributed in various plant species.



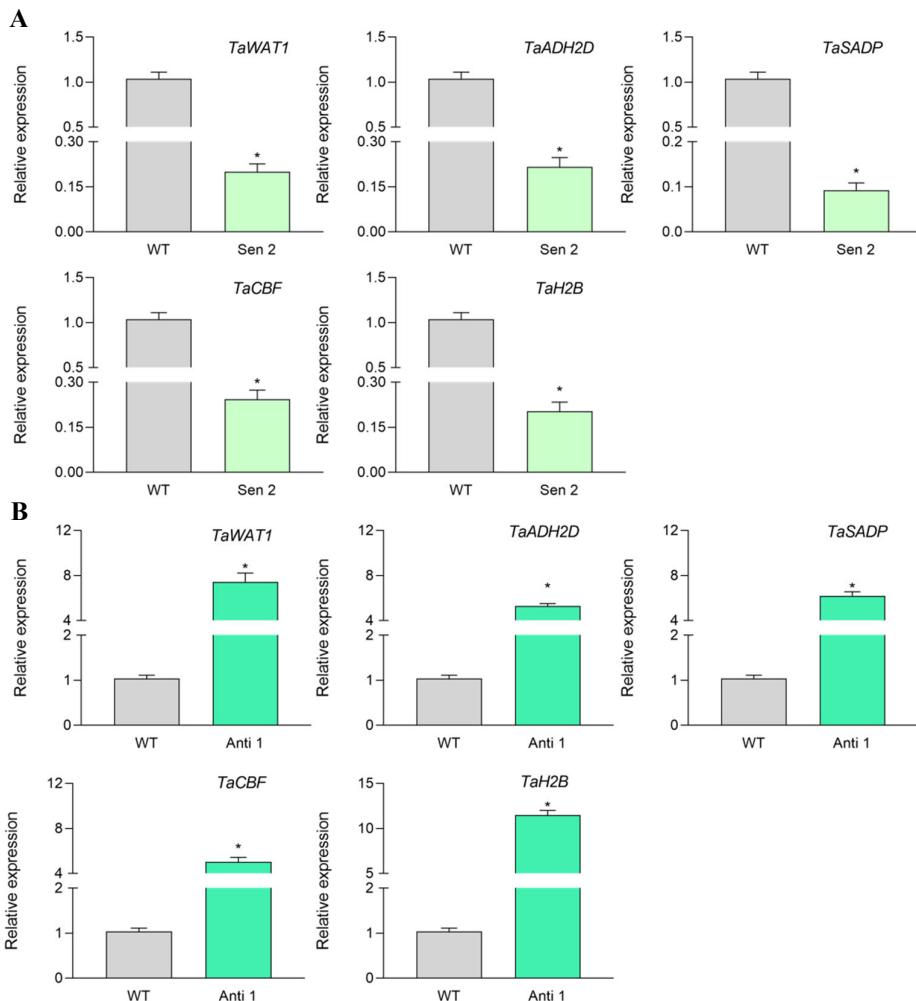
Supplementary Figure S2. Expression levels of the target gene in *TaPYL5*, *TaPPP2C53* and *TaSnRK2.1* transgenic wheat lines. **(A)** Expression levels of *TaPYL5* in transgenic lines. **(B)** Expression levels of *TaPPP2C53* in transgenic lines. **(C)** Expression levels of *TaSnRK2.1* in transgenic lines. WT, wild type. Data are shown by average from triplicate results plus standard error. The expression values were normalized by *Tatubulin*, a constitutive gene in *T. aestivum* species whose expression level was set as 1.



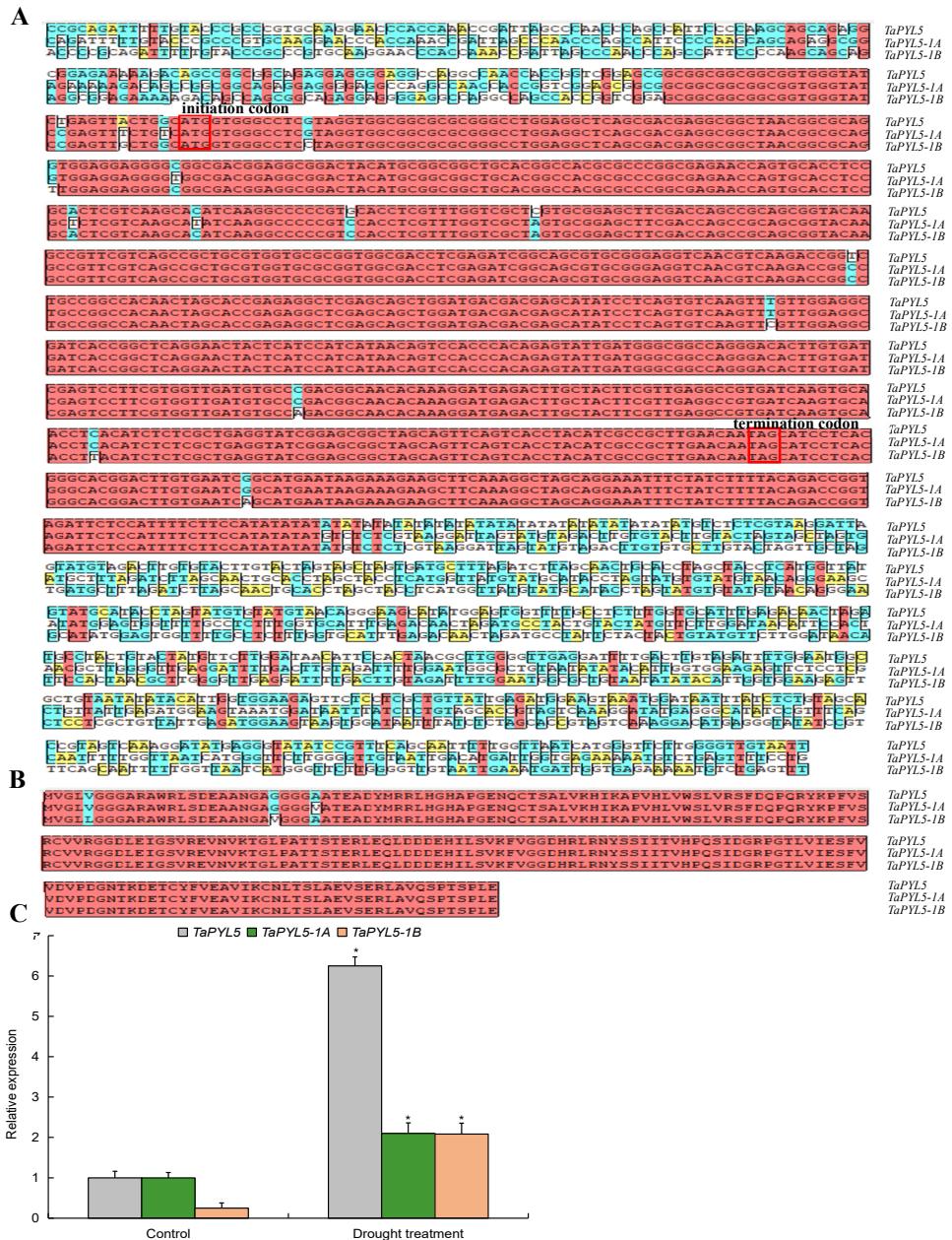
Supplementary Figure S3. Expression levels of the target gene in *TaABII* transgenic wheat lines. Data are shown by average from triplicate results plus standard error. The expression values of target gene were normalized by *Tatubulin*, a constitutive gene in *T. aestivum* species whose expression level was set as 1.



Supplemental Figure S4. qRT-PCR results in roots of *TaPYL5* transgenic wheat lines for the differentially expressed genes with upregulated expression pattern identified based on RNA-seq analysis. **(A)** Expression levels in Sen 2. **(B)** Expression levels in Anti 1. In **(A,B)**: *TaPT5*, potassium transporter 5-like gene (TraesCS3D02G439300), *TaLD2*, leucoanthocyanidin dioxygenase 2-like gene (TraesCS6D02G004300), *TaWRKY9*, transcription factor WRKY9 gene (TraesCS6A02G326500), *TaNPP*, purple acid phosphatase gene (TraesCS4B02G348600), *TaF3H*, flavanone 3-hydroxylase gene (TraesCS2A02G493500). Sen 2, a transgenic line overexpressing *TaPYL5*. Anti 1, a transgenic line with *TaPYL5* knockdown expression. The constitutive gene *Tatubulin* was used as internal standard to normalize target gene transcripts, whose expression level was set as 1. *: significant difference from the WT ($P<0.05$).



Supplemental Figure S5. qRT-PCR results in roots of *TaPYL5* transgenic wheat lines for the differentially expressed genes with downregulated expression pattern identified based on RNA-seq analysis. **(A)** Expression levels in Sen 2. **(B)** Expression levels in Anti 1. In **(A,B)**: *TaWAT1*, WAT1-related protein gene (TraesCS3B02G006900), *TaADH2D*, alcohol dehydrogenase ADH2D gene (TraesCS4A02G202300), *TaSADP*, S-adenosylmethionine decarboxylase proenzyme gene (TraesCS6D02G202500), *TaCBF*, AP2 domain CBF protein gene (TraesCS6D02G238200), *TaH2B*, histone H2B gene (TraesCS5D02G063200). Sen 2, a transgenic line overexpressing *TaPYL5*. Anti 1, a transgenic line with *TaPYL5* knockdown expression. The constitutive gene *Tatubulin* was used as internal standard to normalize target gene transcripts, whose expression level was set as 1. *: significant difference from the WT ($P<0.05$).



Supplemental Figure S6. Characterization on *TaPYL5* and its homeologs. **(A)** Alignment results among *TaPYL5* and its homeologs at nucleic acid level. **(B)** Alignment results among *TaPYL5* and its homeologous proteins situated on 1A and 1B. **(C)** Expression levels of *TaPYL5* and its homologs in roots under normal growth and drought treatment. In **(A,B)**, the sequence alignment analyses were conducted based on MEGA8. In **(C)**, root tissues of wheat seedlings (cv. Shimai 26) at the third-leaf stage grown in standard MS solution (control) and modified MS solution provided by PEG-6000 (10%, w/v) were collected and subjected to qRT-PCR analysis using specific primers. Constitutive gene *Tatubulin* was used as an internal standard to normalize the target transcripts, whose expression level was set as 1. *: significant difference compared with Control ($P < 0.05$).

Supplementary Table S1. PCR primers used in this study

Purpose	Accession number	Forward primer (5'-)	Reverse primer (5'-)
<i>TaPYL5</i> expression	TraesCS1A02G1 26800	ATGGTGGGCCCTCGTAGGTGGCGG	CTATTGTTCAAGCGGCATGTAGGT
<i>Tatubulin</i> expression	U76558	CATGCTATCCCTCGTCGACCT	CGCACTTCATGATGGAGTTGTAT
<i>TaPYL5</i> location	XM_037633867	AAAGGTACCATGGTGGCCCTCGTAGGTGGC GG	AAAAAGCTTCTATTGTTCAAGCGGCAT GTAGGT
<i>TaPYL5</i> overexpression	XM_037633867	TTTCCATGGTGGCCCTCGTAGGTGG	TTTGGTAACCTATTGTTCAAGCGGCAG
<i>TaPYL5</i> knockdown expression	XM_037633867	AAACCATGGCAAAGAGTATCACAGCA	AAAGGTAACCAAGTGCACCTCCGCA
<i>TaPYL5</i> bait in two-hybrid	XM_037633867	AAAGAATTCATGGTGGCCCTCGTAGGTGGC GG	AAAGGATTCCATTGTTCAAGCGGCAT GTAGGT
<i>TaPP2C6</i> prey in two-hybrid	TraesCS1A02G3 58600	TTTGAATTCATGGCGCGCGCGCGCG	TTTCTGCAGGTACGTTGCTCATCCG
<i>TaPP2C8</i> prey in two-Hybrid	TraesCS1A02G4 41200	TTTGAATTCATGGACGCCCTAGGCGCC	TTTCTGCAGGCTATCTGTTGTTGT
<i>TaPP2C9</i> prey in two-hybrid	TraesCS1B02G3 75100	TTTGAATTCATGGCGCGCGCGCGCG	TTTCTGCAGGTTAGGAATTATTCTTG
<i>TaPP2C50</i> prey in two-Hybrid	TraesCS1D02G3 63500	TTTGAATTCATGGCGCGCGCGCG	TTTCTGCAGGTTAGGAATTATTCTTG
<i>TaPP2C53</i> prey in two-hybrid	TraesCS1A02G4 41200	TTTGAATTCATGGACGCCCTAGGCGCC	TTTCTGCAGGCTATCTGTTGTTATTGT
<i>TaSnRK2.1</i> prey in two-hybrid	TraesCS2A02G4 93800	TTTGAATTCCATGGATCGGTACGAGGTG	TTTGGATCCTCACACGGCACACGAAA
<i>TaSnRK2.2</i> prey in two-hybrid	TraesCS2A02G1 63800	TTTGAATTCATGGAGCGGTACGAGGTG	TTTGGATCCTCACACCGCACACGAA
<i>TaSnRK2.3</i> prey in two-hybrid	TraesCS1A02G2 15900	TTTGAATTCATGGAGGAGAGGTACGAG	TTTGGATCCTCAGTAGGTCTCCCCCTC
<i>TaSnRK2.4</i> prey in two-hybrid	TraesCS3A02G3 81100	TTTGAATTCATGGAGAGTACGAGGCC	TTTGGATCCTCATGATATGCGTAGCGA
<i>TaSnRK2.5</i> prey in two-hybrid	TraesCS2A02G5 66700	TTTGAATTCATGGAGAGTACGAGCCGG	TTTGGATCCTCAGATTGGAGCTTGCT
<i>TaSnRK2.6</i> prey in two-hybrid	TraesCS2A02G3 03900	TTTGAATTCATGGAGAGGTACGAGCTG	TTTGGATCCTAGCTGATGTGAAACTC
<i>TaSnRK2.7</i> prey in two-hybrid	TraesCS1A02G2 70800	TTTGAATTCATGGACAAGTACGAGGAGG	TTTGGATCCTAGATGTGCAACACGCT
<i>TaPP2C53</i> expression	TraesCS1A02G4 41200	ATGGACGCCCTAGGCGCC	CTATCTGTTGTTGTCAG
<i>TaPP2C53</i> overexpression	TraesCS1A02G4 41200	AAACCATGGATGGACGCCCTAGGCGCC	AAAGGTAACCCATCTGTTGTTGT
<i>TaPP2C53</i> knockdown expression	TraesCS1A02G4 41200	AAACCATGGATGGACGCCCTAGGCGCC	AAAGGTAACCCTAGGCGCCGCTGCC
<i>TaSnRK2.1</i> expression	TraesCS2A02G4 93800	GGGACATAGGAAGAAAGAGGG	GGAAGCATGACAAAACAGGGAT
<i>TaSnRK2.1</i> overexpression	TraesCS2A02G4 93800	AAAAGATCTATGGATCGGTACGAGGTG	AAAGGTAACCAACTCATGAGCAAT
<i>TaSnRK2.1</i> knockdown expression	TraesCS2A02G4 93800	AAAAGATCTATGACAAAACAGGGATCC	AAAGGTAACCAAGGAGCACTT
<i>TaABII</i> expression	AB238930	ATGGAGGACGTGGCCGTG	GGAAGTTCAAGAGCAAAACTTAA

<i>TaABII</i> overexpression	AB238930	AAACCATGGATGGAGGACGTGGCCGTG	AAAGGTAACCTTAAGTTTGCTCTGAA CTTCC
<i>TaABII</i> knockdown expression	AB238930	AAACCATGGATGTTGCCTTGCTCCCC	AAAGGTAACCTAGCCCCGCCACGGCC
<i>TaPYL5</i> -BiFC	XM_037633867	AAAGGATCCATGGTGGCCTCGTAGG	AAAGGTACCGTATTGTTCAAGCGGG
<i>TaPP2C53</i> - BiFC	TraesCS1A02G4 41200	AAAGGATCCATGGACGCCCTAGGCGC	AAAGTCGACGTATCTGTTATTGT
<i>TaSnRK2.1</i> - BiFC	TraesCS2A02G4 93800	AAAGGATCCATGGATCGGTACGAGGTG	AAAGTCGACCACTCATGAGCAAT
<i>TaPYL5</i> Pro-D1	XM_037633867	TTGGATCCGGTCATGTGTCACATGACC	TTGTCGACGCCAGTAACTCAGATAACC
<i>TaPYL5</i> Pro-D2	XM_037633867	TTGGATCCGGCGAAGGGATAGAAC	TTGTCGACGCCAGTAACTCAGATAACC
<i>TaPYL5</i> Pro-D3	XM_037633867	TTGGATCCGAAGATGGTCATGGTTGG	TTGTCGACGCCAGTAACTCAGATAACC
<i>TaPYL5</i> Pro-D4	XM_037633867	TTGGATCCGCCCGCGTAGTTTCCC	TTGTCGACGCCAGTAACTCAGATAACC
<i>TaPYL5</i> Pro-D5	XM_037633867	TTGGATCCCCTCCCGCAGAGATGGTCC	TTGTCGACGCCAGTAACTCAGATAACC
<i>TaPT5</i> expression	TraesCS3D02G4 39300	ATGGCGGTGCCTCTGAAAGC	CTAGATCTCATATGTCATACCC
<i>TaLD2</i> expression	TraesCS6D02G0 04300	ATGGCGCGCGTGGAGGC	TTAATTAACCTCACTAGGTTGGCT
<i>TaWRKY9</i> expression	TraesCS6A02G3 26500	ATGTCATCATCAAAGAGG	TCAACCACCCCCCCTTGT
<i>TaNPP</i> expression	TraesCS4B02G3 48600	ATGGGGATGGCGCATGTCG	CCACCACTCGCTAGCTGA
<i>TaF3H</i> expression	TraesCS2A02G4 93500	ATGGCGCCGGTGAGCAACGAG	TCAGGCAAGAATTCTGTTGAGAGA
<i>TaWAT1</i> expression	TraesCS3B02G0 06900	ATGGCGGACGCGGTGGATG	TCAGACATTGTCGGAGGTGGT
<i>TaADH2D</i> expression	TraesCS4A02G2 02300	ATGGCGGACGCGGTGGATG	TCAGACATTGTCGGAGGTGGT
<i>TaSADP</i> expression	TraesCS6D02G2 02500	ATGGCCGTCTCTGCGATTGG	TAAGAAGATGAAGAGCTCTGA
<i>TaCBF</i> expression	TraesCS6D02G2 38200	ATGGACCTCAGTGCCTC	TCAGTAGCTCCACAAGCTGACC
<i>TaH2B</i> expression	TraesCS5D02G0 63200	ATGGCCCCAAGCGGAGAAG	CAAGTTCACCTCCTCTAG

Supplementary Table S2. Drought-related *cis*-acting elements in the *TaPYL5* promoter

Site Name	Organism	Position	Matrix score.	Species	Function
MBS	CAACTG	1394	-		
MYB recognition site	CCGTTG	577	-	<i>Arabidopsis thaliana</i>	MYB binding site involved in drought-inducibility
MYB-binding site	CAACAG	997	-	<i>Nicotiana tabacum</i>	
MYB	CAACTG	1394	-		
MYB	CAACCA	974	-	<i>Arabidopsis thaliana</i>	<i>cis</i> -acting regulatory element
	CAACAG	997	-		
	CAACCA	1367	-		
	CAACCA	1949	+		
DRE core	GCCGAC	257	-		
		1415	-		
		1680	+		
ABRE	ACGTG	437	+	<i>Arabidopsis thaliana</i>	<i>cis</i> -acting element involved in the abscisic acid responsiveness
		1293	-		