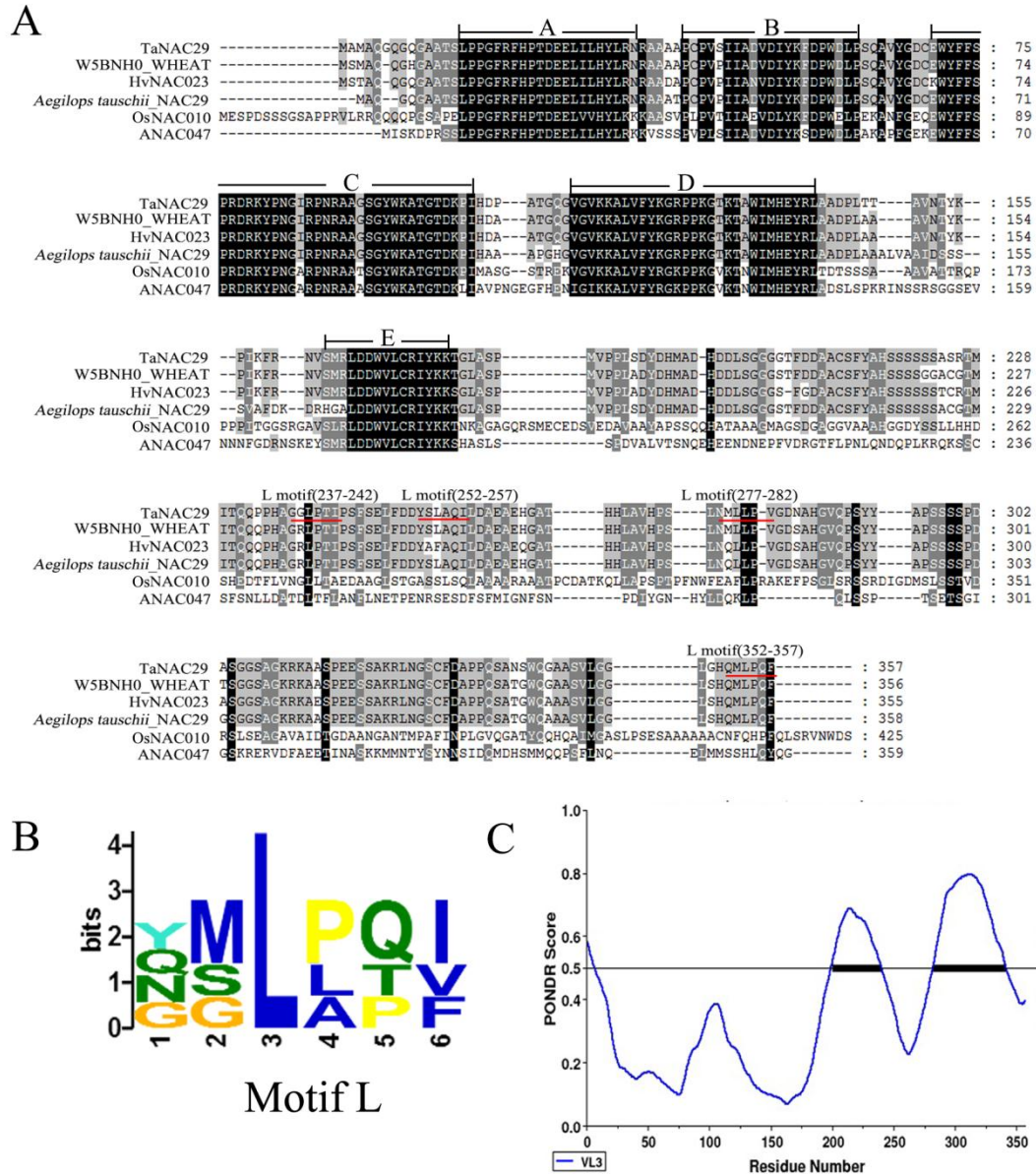


TaNAC29, a NAC transcription factor from wheat, enhances salt and drought tolerance in transgenic *Arabidopsis*. Quanjun Huang, Yan Wang, Bin Li, Junli Chang, Mingjie Chen, Kexiu Li, Guangxiao Yang and Guangyuan He.

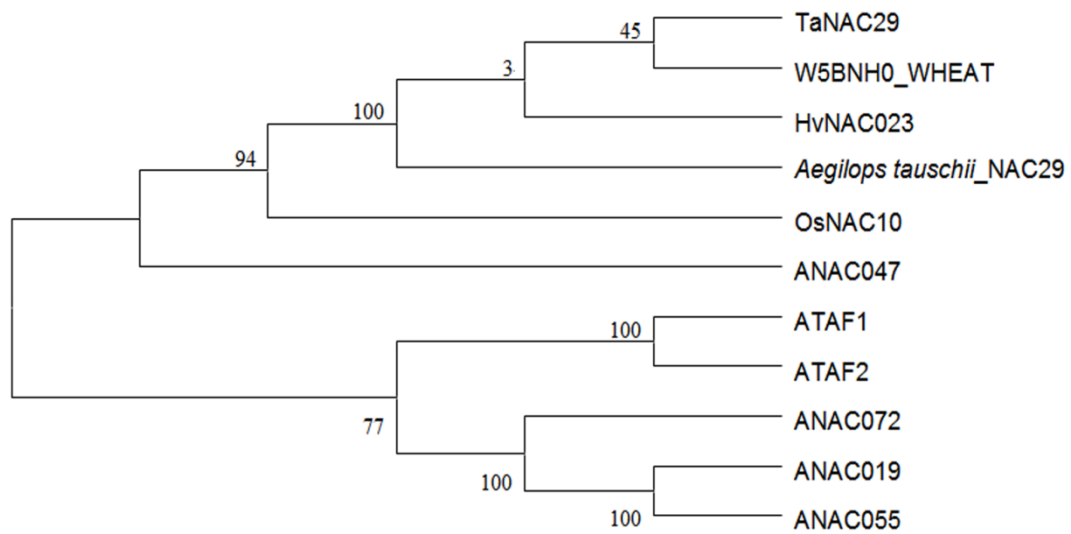


**Figure S1. Structural features of TaNAC29 protein**

(A) Multiple alignment of amino acid sequences of TaNAC29 and other related NAC proteins from selected plant species. Alignments were performed using MEGA5.1 program. L motif was indicated by the red bar after MEME searching. Five conserved NAC-subdomain were indicated.

(B) Weblogo of L motif.

(C) PONDR VL3 analysis for ID of TaNAC29.



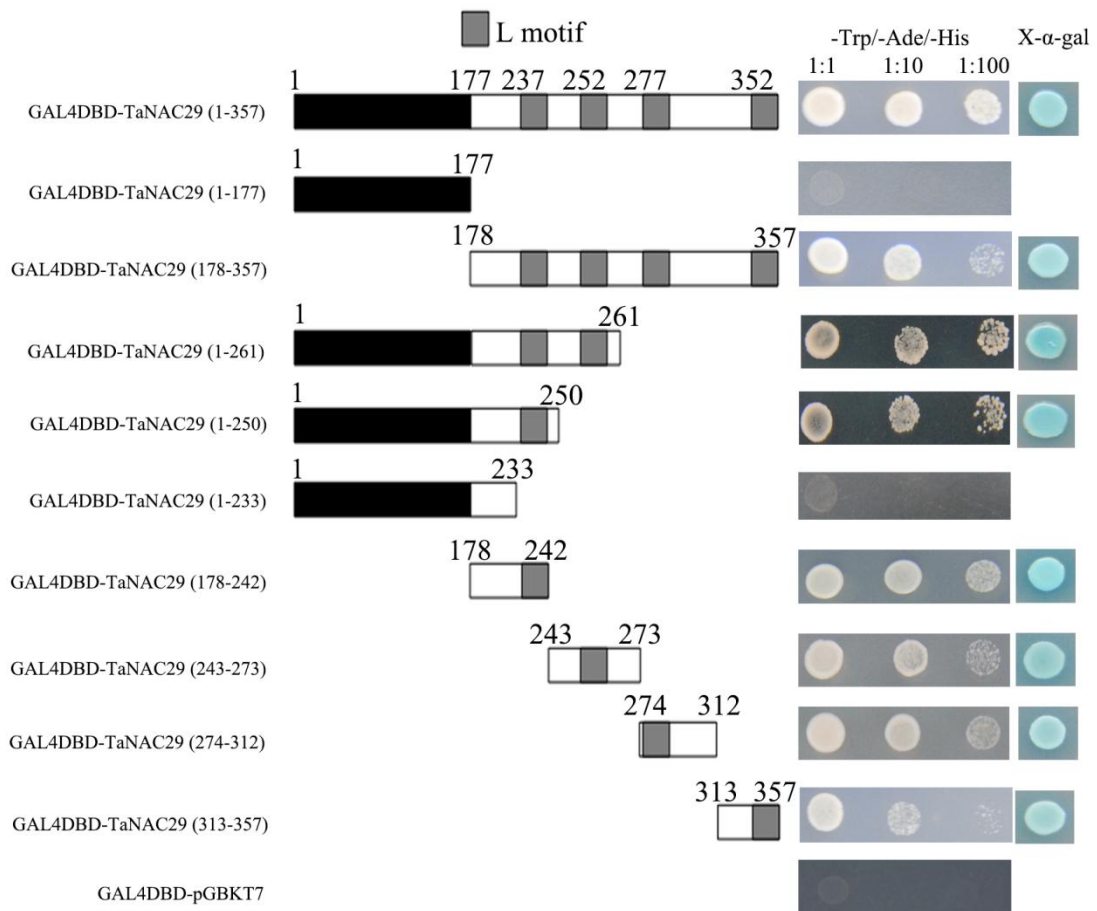
**Figure S2. Phylogenetic relationship of TaNAC29 with other NAC members.** Phylogenetic relationship of TaNAC29 with W5BNH0 (EMBL: C116E5668.1) from *Triticum aestivum*, HvNAC023 (GenBank: CBZ41159.1) from *Hordeum vulgare*, NAC29 (GenBank: EMT28859.1) from *Aegilops tauschii*, OsNAC10 (GenBank: EAZ40329.1) from *Oryza sativa*, ANAC047 (AT3G04070), ATAF1 (AT1G01720), ATAF2 (AT5G08790), ANAC072 (AT4G27410), ANAC019 (AT1G52890) and ANAC055 (AT3G15500).

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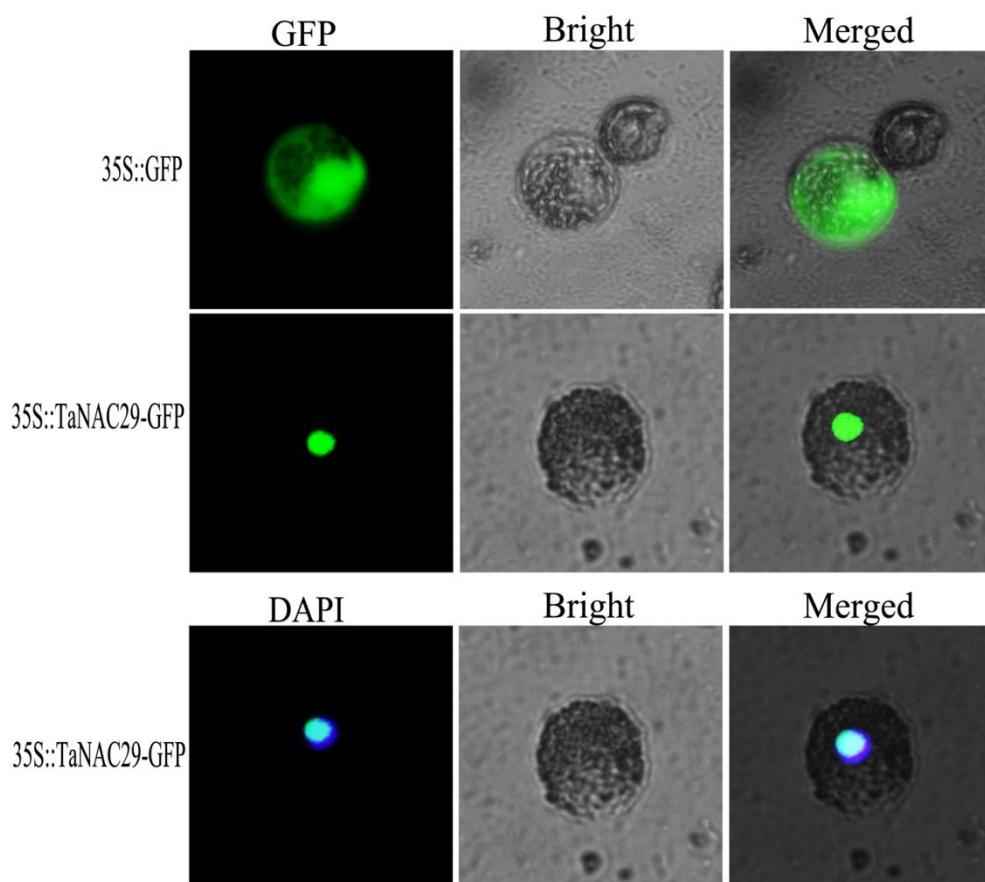
v60    v70    v80    v90    v100   v110   v120   v130   v140   v150   v160   v170   v180
TaNAC29  GTGGTGTGATCGGGTGGTGGATC----ATGGCGATGGCGCAGGGGCGAGGGGCGGGGCGGACGTCGCTGCGCGGGGTTCCGGTCCACCGACGGACGAGGAGCTCATCTCGACTACCTTCG
WSBNHO_WHEAT GTGGTGTGCGCGGGTGGTGGATCGATCATGTGCGATGGCGCAG---CAGGGGACAGGGGCGGGGCGGACGTCGCTGCGCGGGGTTCCGGTCCACCGACGGACGAGGAGCTCATCTCGACTACCTTCG
^10    ^20    ^30    ^40    ^50    ^60    ^70    ^80    ^90    ^100   ^110   ^120
v190   v200   v210   v220   v230   v240   v250   v260   v270   v280   v290   v300   v310
TaNAC29  CAACCGCGCCCGCGCCGCGCGTCCATCATGCGCGACGTAGACATCTACAAGTTCGATCCGTGGGACCTCCCTTCCAGGGCGGTGTACGGCGACTCGGAGTGGTACTTCTTCAGCCCGCGG
WSBNHO_WHEAT CAACCGCGCCCGCGCCGCGCGTCCATCATGCGCGACGTGACATCTACAAGTTCGATCCATGGGACCTCCCTTCCAGGGCGGTGTACGGCGACTCGGAGTGGTACTTCTTCAGCCCGCGG
^130   ^140   ^150   ^160   ^170   ^180   ^190   ^200   ^210   ^220   ^230   ^240   ^250
v320   v330   v340   v350   v360   v370   v380   v390   v400   v410   v420   v430   v440
TaNAC29  GATCGCAAGTACCCCAAGGCATCGGCCCAACCGCGCGCGGCTCCGCTACTGGAAGGCCACCGCACCAAGCCATCCACGACCCCGCCACCGCCAGGGCGTCGGGCTCAAGAAGGCGCTCG
WSBNHO_WHEAT GACCGCAAGTACCCCAAGGCATCGGCCCAACCGCGCGCGGCTCCGCTACTGGAAGGCCACCGCACCAAGCCATCCACGACCCCGCCACCGCCAGGGCGTCGGGCTCAAGAAGGCGCTCG
^260   ^270   ^280   ^290   ^300   ^310   ^320   ^330   ^340   ^350   ^360   ^370   ^380
v450   v460   v470   v480   v490   v500   v510   v520   v530   v540   v550   v560   v570
TaNAC29  TCTTCTACAAGGGCGCCGCCAAGGGCACCAAGACTGCTGGATCATGCACGAGTACCGGCTCGCGCGGACCCCTCACCAACCGCCCTCAACACTACAAGCCATCAAGTCCGCAACGCTCCAT
WSBNHO_WHEAT TCTTCTACAAGGGCGCCGCCAAGGGCACCAAGACTGCTGGATCATGCACGAGTACCGGCTCGCGCGGACCCCTCACCAACCGCCCTCAACACTACAAGCCATCAAGTCCGCAACGCTCCAT
^390   ^400   ^410   ^420   ^430   ^440   ^450   ^460   ^470   ^480   ^490   ^500   ^510
v580   v590   v600   v610   v620   v630   v640   v650   v660   v670   v680   v690   v700
TaNAC29  GAGGTGGATGACTGGGTGCTATGCGGATCTACAAGAAGACGGGCTGGGCTCGCGGATGGTGGCGCGCTGTCGGACTACGACCATGGCCGACCCAGCAGACTCTCCGCGCGCGGCGGACCTTC
WSBNHO_WHEAT GAGGTGGATGACTGGGTGCTATGCGGATCTACAAGAAGACGGGCTGGGCTCGCGGATGGTGGCGCGCTGCGGACTACGACCATGGCCGACCCAGCAGACTCTCCGCGCGCGGCGGACCTTC
^520   ^530   ^540   ^550   ^560   ^570   ^580   ^590   ^600   ^610   ^620   ^630   ^640
v710   v720   v730   v740   v750   v760   v770   v780   v790   v800   v810   v820   v830
TaNAC29  GACGACCGCGCTGCAGCTTCTACGCGCACTCCAGCAGCAGCAGCAGCGCGCAGCAAGACCATGATCAGCAGCAGCGCCGCGCACGCGGGGACTCCCCAGATCCCGTCTTCCGAGCTCTTCGAGC
WSBNHO_WHEAT GACGACCGCGCTGCAGCTTCTACGCGCACTCCAGCAGCAGCAGCGCGCGCGCTGCGGAACCATGATCAGCAGCAGCAGCGCCGCGCACGCGGGGACTCCCCAGATCCCGTCTTCCGAGCTCTTCGAGC
^650   ^660   ^670   ^680   ^690   ^700   ^710   ^720   ^730   ^740   ^750   ^760   ^770
v840   v850   v860   v870   v880   v890   v900   v910   v920   v930   v940   v950   v960
TaNAC29  ACTACTGCTCGCGAGATCCTCGACCGGAGGCGAGCAGCGGCGCCACCCACACCTCGCGTCCACCTTCCTCGAAGATGCTCTCCCGTGGCGACAAACCGCGCAGGAGTACAGCGTCTACTA
WSBNHO_WHEAT ACTACTGCTCGCGAGATCCTCGACCGGAGGCGAGCAGCGGCGCCACCCACACCTCGCGTCCACCTTCCTCGAAGATGCTCTCCCGTGGCGACAAACCGCGCAGGAGTACAGCGTCTACTA
^780   ^790   ^800   ^810   ^820   ^830   ^840   ^850   ^860   ^870   ^880   ^890   ^900
v970   v980   v990   v1000  v1010  v1020  v1030  v1040  v1050  v1060  v1070  v1080  v1090
TaNAC29  CGCGCGTCTGCTGATCGCCGGAACCGCGCGCGGGAGCGCGGGGAAACGCAAGGCGCGGAGCCCGGAAGATCATCGGCCAAGAGGCTTAACGGTCTGCTTCGACGCGCGCGCGAGTCCGGCAAC
WSBNHO_WHEAT CGCGCGTCTGCTGATCGCCGGAACCGCGCGCGGGAGCGCGGGGAAACGCAAGGCGCGGAGCCCGGAAGATCATCGGCCAAGAGGCTCAACGGTCTGCTTCGACGCGCGCGCGAGTCCGGCAAC
^910   ^920   ^930   ^940   ^950   ^960   ^970   ^980   ^990   ^1000  ^1010  ^1020  ^1030
v1100  v1110  v1120  v1130  v1140  v1150  v1160  v1170  v1180  v1190
TaNAC29  AGCTGGCAGGGGGCGGCGTCCGCTGCTGGGCGGCTCGGTCATCAGATGCTTCTCAGTCTAAGCTGTAATTAATTAACCTTAATAATCGGTGCTGTAAGCGG
WSBNHO_WHEAT GGCTGGCAGGGGGCGGCGTCCGCTGCTGGGCGGCTCAGCCATCAGATGCTTCTCAGTCTAAGCTGTAATTAATTAACCTTAATAATCGGTGCTGTAAGCGG
^1040  ^1050  ^1060  ^1070  ^1080  ^1090  ^1100  ^1110  ^1120  ^1130

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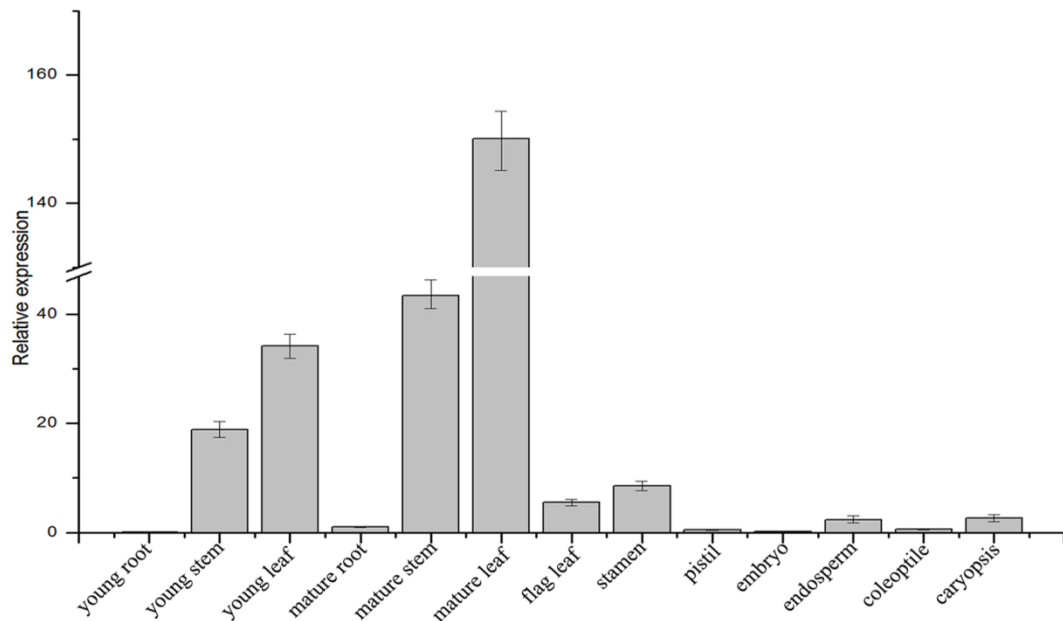
**Figure S3. Alignment of the nucleic acid sequences.** Alignment of the nucleic acid sequences of *TaNAC29* and *WSBNHO\_WHEAT*. Alignments were performed using MegAlign of DANStar program.



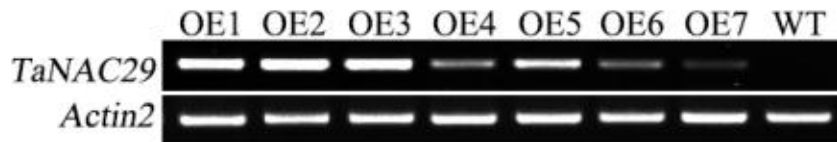
**Figure S4. Transactivation activity analysis of TaNAC29 protein in yeast.** Fusion proteins were expressed in yeast strain *AH109*, and then analysed for their transactivation activity of the *HIS3*, *ADE2* and *LacZ* reporter genes. Gal4-(DBD)-pGBKT7 served as negative control. Left, schematic diagram of full-length and truncated versions. A black bar indicates NAC domain, a gray bar indicates L motif. Right, transactivation activity assay.



**Figure S5. Subcellular localization of TaNAC29.** The fusion protein 35S::TaNAC29-GFP and 35S::GFP (control) constructs were transiently expressed in wheat mesophyll protoplasts and visualized with fluorescence microscope. Top, 35S::GFP (control); middle, 35S::TaNAC29-GFP; bottom, 35S::TaNAC29-GFP+DAPI.



**Figure S6. Organ-specific expression patterns of *TaNAC29* in wheat.** Different organs include young root (at four-leaf stage); young stem (at four-leaf stage); young leaf (at four-leaf stage); mature root (at anthesis stage); mature stem (at anthesis stage); mature leaf (at anthesis stage); flag leaf; stamen; pistil; embryo; endosperm; coleoptile; caryopsis. The  $2^{-\Delta\Delta CT}$  method was used in qRT-PCR analysis. Values are means  $\pm$ SE of three replicates.



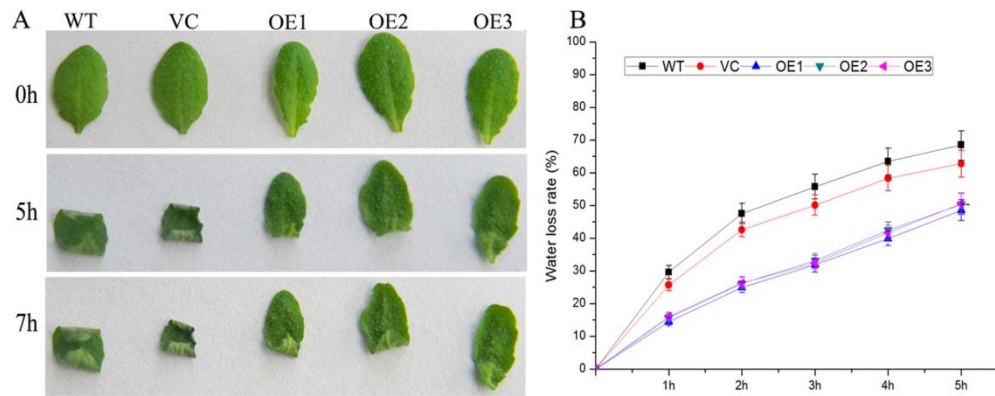
**Figure S7. Expression of *TaNAC29* in transgenic *Arabidopsis* lines.** The WT and transgenic *Arabidopsis* lines were cultured in 1/2 MS medium for two weeks and the whole seedlings were used to extract total RNA. *Actin2* as the internal control gene was used to examine the gene overexpression (OE) of *TaNAC29*. Three independent biological experiments were performed.



WT

OE1

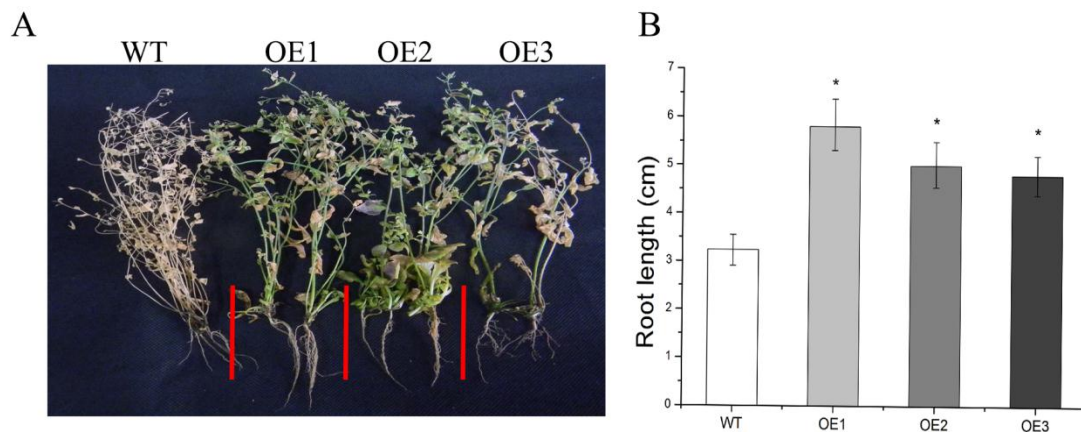
**Figure S8. Delaying in bolting and flowering of *TaNAC29*-overexpression line 1 (OE1) in comparison to the WT plants.**



**Figure S9. *TaNAC29* overexpression (OE) lines exhibit enhanced tolerance to water deficiency.**

(A) Phenotypes of detached leaves under water deficiency treatment for 0 h, 5 h, and 7 h.

(B) Comparison of water loss rate. Values are means  $\pm$  SE of three independent experiments.



**Figure S10. Root growth of WT and *TaNAC29*-overexpression (OE) lines in response to drought stress.**

(A) Comparison of root length of WT and *TaNAC29*-overexpressing lines (65-day-old seedling) after drought stress.

(B) Quantitative analysis of root length. Values are means  $\pm$  SE (n = 10 to 15 plants). Asterisks indicate statistically significant differences from WT (\* $P < 0.05$ ).