Overexpression of *BoNAC019*, a NAC transcription factor from *Brassica oleracea*, negatively regulates the dehydration response and anthocyanin biosynthesis in *Arabidopsis*

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Table S1. List of primers used in this study.

Purpose	Gene	Primer
ORF of NAC gene	*BoNAC019*	F: ATGGGTATCCAAGAAACCGA
	Bol039157	R: TCATATATAACCAAACCCACCA
QPCR of CsNAC gene	*BoNAC019*	F: GGTTCAACGGTTTAGCGGACA
		R: CCCACCAACTTGCCCTGAATA
	BoActin	F: TAACAGGGAGAAGATGACTCAGATCA
		R: AAGATCAAGACGAAGGATAGCATGAG
OE construct	*BoNAC019*	F: ggactctagaggatccccggg ATGGGTATCCAAGAAACCGA
		R: ataagggactgaccacccggg TCATATATAACCAAACCCACCA
Subcellular location construct	*BoNAC019*	F: accatggtagatctgactagt ATGGGTATCCAAGAAACCGA
		R: aagttcttctcctttactagtTATATAACCAAACCCACCA
Transactivation assay	*BoNAC019*	F: GGAATTCC ATGGGTATCCAAGAAACCGA
		R: GAAGATCTTC TCATATATAACCAAACCCACCA
	BoNAC019-N	F: GGAATTCC ATGGGTATCCAAGAAAC
		R: GAAGATCTTC CCGTTTGCACGAGAGGG
	BoNAC019-C	F: GGAATTCC AAGCTCTAAGTTAGATG
		R: GAAGATCTTC TCATATATAACCAAACCC
QPCR of stress-responsive genes	*AtRD29A*	F: TCTTGATGGTCAACGGAAGGTC
	AT5G52310	R: CCGGTACTCCTCCAGTTTCTTC
	AtRAB18	F: GGGAGGAGGAAGAAGGGAATA
	AT5G66400	R: CGTAGCCACCAGCATCATATC
	AtDREB2A	F: ATTGTCTGGAGAATGGTGCGG
	AT5G05410.	R: GATAGCGAATCCTGCTGTTGT
	AtDREB2B	F: AACCGAAGCAGGAAGAAGAGG
	AT3G11020	R: TTTTGGTCTTGGCTCTGATGG
	AtP5CS1	F: TGTGTGTTTGTGTATTTGGTTGAGAC
	AT2G39800	R: TGAGTACTAAGCAGAGAGGAAACAAAA
	AtABI3	F: TGGAGACGCTGATGATACTGT
	AT3G24650	R: AGGAGACGATTGCGTTGACAG
	AtABI4	F: GGCGTTAGGGCAGGAACAAGG
	AT2G40220	R: AATCCCAAATACTCCCCCAAT
	AtABI5	F: AAGAGAGGGATAGCGAACGAG
	AT2G36270	R: CTAACCACACCAGCCTTCACC
	AtNCED3	F: CAATCATCAAACTCTCCCGCC
	AT3G14440	R: TCTCGTGGCTGACAAGGAAAC
	AtSOD	F: ATCTCCTCCCTGCTTTCAACAAC
	AT1G08830	R: CCCAGACATCAATGGTAAGCAAT
	AtPOD	F: CGGCAAGAGGATTTGAAGTTA
	AT1G14540	R: TGTCTGCGATAGCCCTAAACG
	AtCAT	F: TCACTTGTGCTGACTTTCTCCGA
	AT1G20630	R: TCTTGGATGTGAGATTTTGGGTT

	AtPAL1	F: GAGGAGTGGACGCTATGTTATGC
	AT2G37040	R: CACCATAACTATCAGTGCCTTTG
	AtCHS	F: CTTCCCTCAAATGTCCGTCTATG
	AT5G13930	R: CGTGTCCTCGTTGTCTGCTCT
	AtCHI	F: CCTTTTCGTCCTTGTTCTTCATCAT
	AT3G55120	R: GAGGCGGTTCTGGAATCTATCA
	AtC4H	F: ATCTCAACCACCGTAATCTCGTC
	AT2G30490	R: TTCCTCCAATGCTCGCCGTAAAC
	AtF3H	F: TGGGTGAAAGTGACGGAGGAGTA
	AT3G51240	R: GTAATGGTTCCAGGGTCAGTGTG
	AtF3'H	F: GACACCGATGGAGACTGTTGAGA
	AT5G07990	R: AGCGACGCCTTGTAAATCTAACC
	AtDFR	F: AGGCGGCATAGACGTTGTGA
	AT5G42800	R: GGAGAAAGCAGCGTGGGATT
	AtANS	F: CTTTTCTACGAGGGCAAATGGGT
	AT4G22880	R: CACAGCCCAAGAAATCCTAACCT
	AtUF3GT	F: TCCACACCATCTCTATCCCTCAG
	AT5G54060	R: AGAAGGGACAAGAGACAGTGCGA
	AtTT2	F: GATGGATCAAGGTGGATCTTCG
	AT5G35550	R: AAGTGAAGTCTCGGAGCCAATC
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	AT1G66370	R: TCGTTCATCGTGCTTCTTACTCA
	AtMYB114	F: AAGTATGGAGAAGGCAAATGGCA
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	AtPAP1	F: CCATCTCAATGCCCCACCA
	AT1G56650	R: TTCTGTTGTCGTCGCTTCAGG
	AtPAP2	F: ATAGGTGGTCCTTGATTGCTGG
	AT1G66390	R: AGGATCGAGGTCGAGGCTTAA
	AtTTG1	F: TTGGAACGATGTAGAGCCGAAAC
	AT5G24520	R: CAGGCTGAGGACTCTCGTAAATG
	AtTT8	F: GCAACAGCATAAGCGGACG
	AT4G09820	R: CCTCTTACTTTCGCCCTTATCTCC
	AtEGL3	F: TTCAAGCGATTGCGTTTCCC
	AT1G63650	R: GATGATCGCTTCCACCTTGTGA
	AtGL3	F: TTTGCTTCCCGTTCCTTGG
	AT5G41315	R: AGGCTCCGTACTGAACATAGGC
	AtACTIN	F: CTGGAATGGTGAAGGCTGGTT
	AT1G13320	R: CGATTGGATACTTCAGAGTGAGGAT
	CYP707A1	F: AGAGTCTCTAACTTGGGGAGAT
	AT4G19230	R: TGGATCAAATTTCCCCGGATTA
	CYP707A2	F: ATTTAAATGGTGGTTGCACTGG
	AT2G29090	R: GCGAAGAAGGAATTGGGATTTT
	CYP707A3	F: TGATATATTTTCGGATCCGGGG
	AT5G45340	R: TGATATATTTTCGGATCCGGGG

	CYP707A4	F: TTTAGCTAACTCATTGCCTGGA
	AT3G19270	R: TGCCACTGACACATAAGGTTAT
	AtPP2CA	F: GTTAATGGTGCTACTCGGAGTA
	AT3G11410	R: GTGATCTACGGAGAGAGGAATG
	PYL1	F: GTGAGTGAAGATTTCGAGATGC
	AT5G46790	R: CGATCATCGTCCAACAGATCTA
	PYL6	F: ACAAACACTTCGTGAAAAGCTG
	AT2G40330	R: TGTAGTTCATGAGTCTGTGGTC
	SnRK2.2	F: TTAAAGAGGTGATTTTGACGCC
	AT3G50500	R: AATCCGCTCATAAAGTTCTCCA
	SnRK2.4	F: AAGGGAACAAGAGAAAACATGC
	AT1G10940	R: GAGCTTCAGATCTCTATGGCAT
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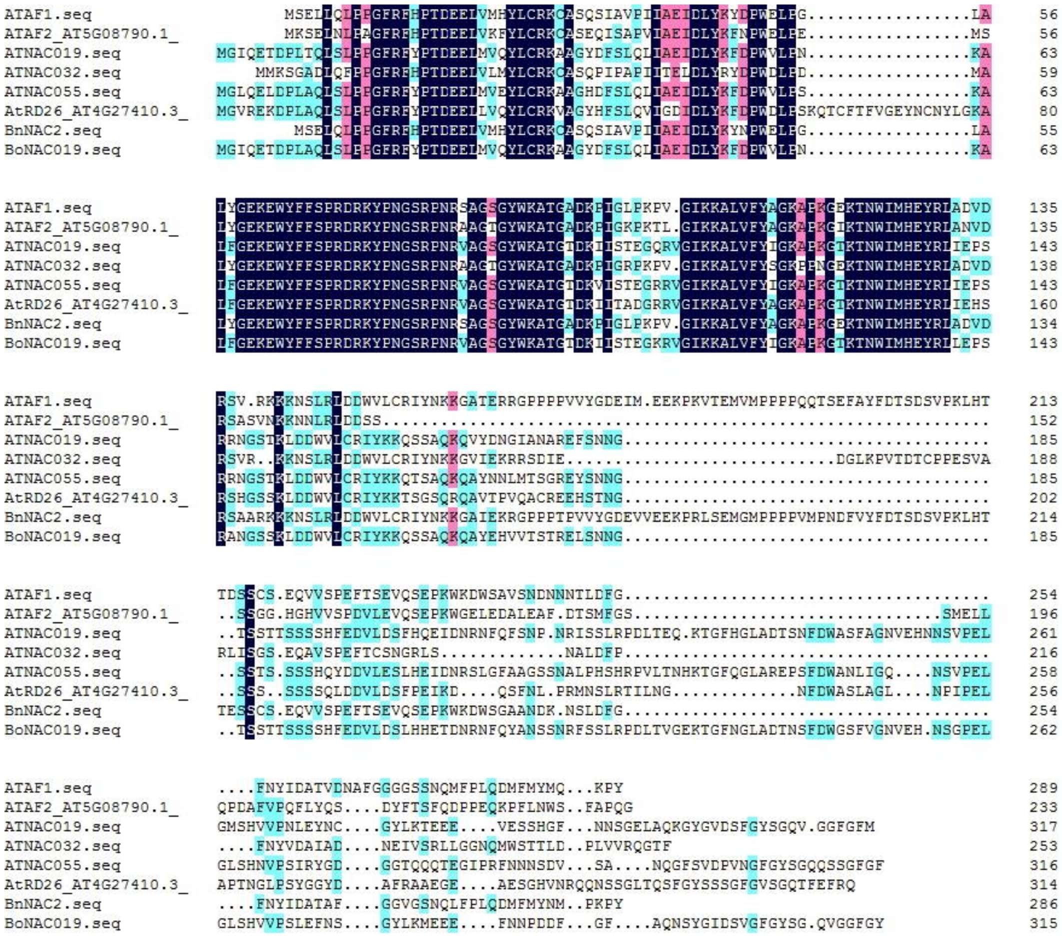

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ATNAC019.seq         LFGEKEWYFFSPRDRKYPNGSRPNRVAGSGYWKATGTDKIISTEGQRVGIKKALVFYIGKAPKGTKTNWIMHEYRLIEPS   143
ATNAC032.seq         LYGEKEWYFFSPRDRKYPNGSRPNRAAGTGYWKATGADKPIGRPKPV.GIKKALVFYSGKPPNGEKTNWIMHEYRLADVD   138
ATNAC055.seq         LFGEKEWYFFSPRDRKYPNGSRPNRVAGSGYWKATGTDKVISTEGRRVGIKKALVFYIGKAPKGTKTNWIMHEYRLIEPS   143
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BnNAC2.seq           LYGEKEWYFFSPRDRKYPNGSRPNRSAGSGYWKATGADKPIGLPKPV.GIKKALVFYAGKAPKGEKTNWIMHEYRLADVD   134
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Fig. S1. Sequence analysis of BoNAC019. Amino acid alignment of BoNAC019 and other NAC family members from selected plant species, including AtNAC032 (GenBank accession no. NP_177869.1), ATAF1 (GenBank accession NP_171677.1), ATAF2 (GenBank accession: X74756.1), AtNA019 (GenBank accession: NM_104167.6), AtNAC055 (GenBank accession: NM_112418.4), AtRD26 (GenBank accession: NM_118875.4) from *Arabidopsis*, BnNAC2 (GenBank accession no. NP_001302866.1) from *Brassica napus*. The amino acid residues are exactly the same as the black shadow, the 75% is the same as the pink shadow, and the 50% is the same as the blue shadow Alignments were performed using DNAman.

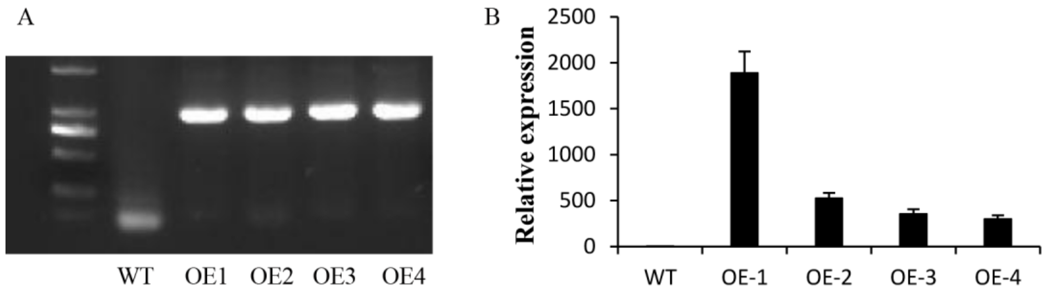

Fig. S2. PCR and qPCR analysis of transgenic Arabidopsis plants. A. PCR analysis of overexpressing transgenic Arabidopsis plants. B. qPCR analysis of overexpressing transgenic Arabidopsis plants. Error bars show the standard deviations for three independent replicates.

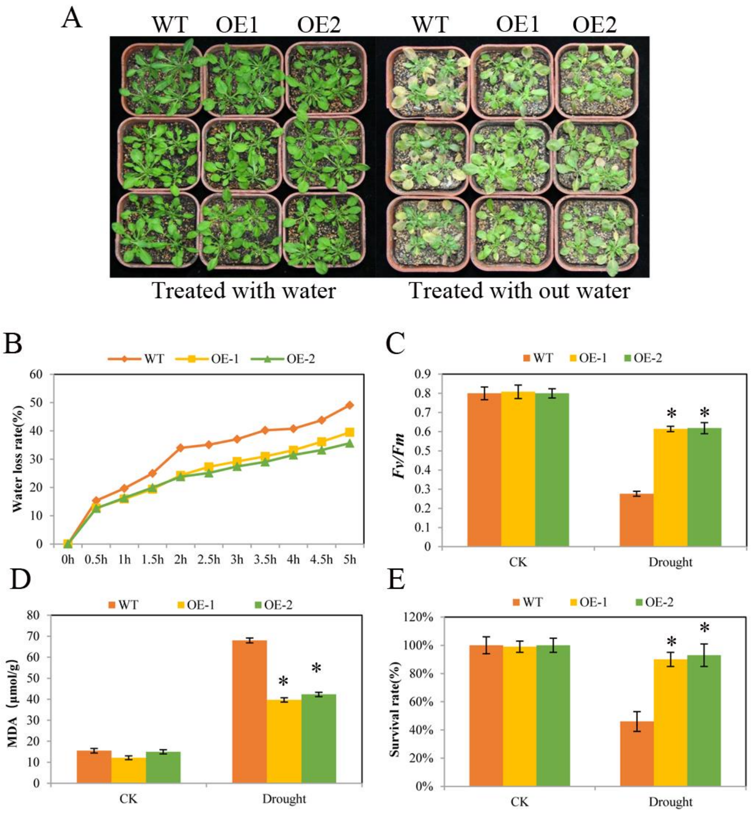

Fig. S3. Overexpression of AtNAC019 enhanced tolerance to drought stress. A. Phenotypes of WT and OE plants after water withholding for three weeks. B. Water loss rate. C. *Fv/Fm* ratios. D. MDA content. E. Survival rate of WT and OE plants after drought treatment. Seedlings treated with water were used as a mock control (CK). Error bars show the standard deviations for three independent replicates. Asterisks indicate statistically significant differences compared to WT at the same time point (*P < 0.05).

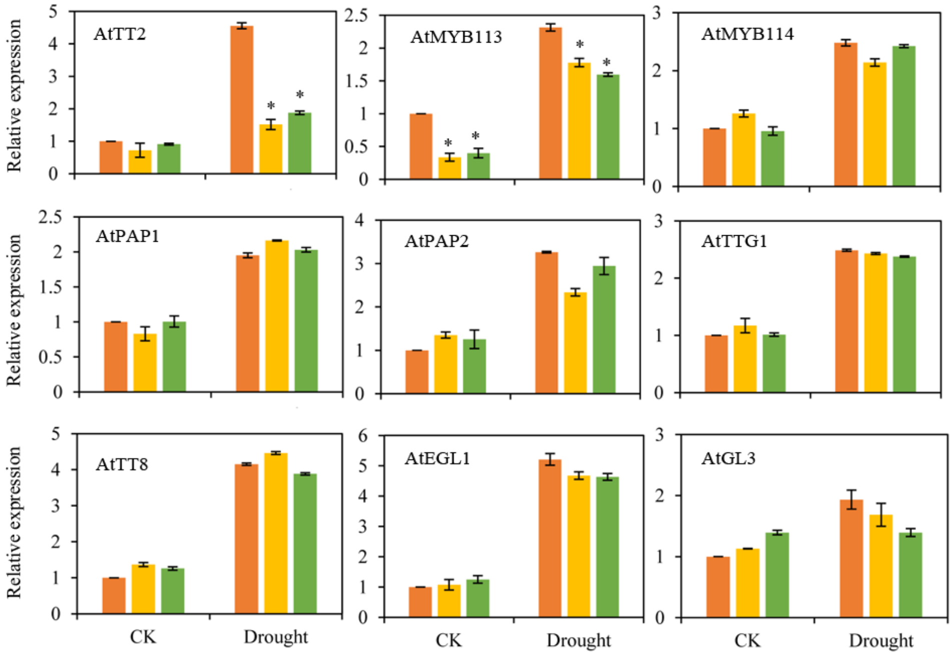

Fig. S4. Expression levels of transcription factors involved in anthocyanin biosynthesis of WT and OE plants. Cabbage seedlings on MS medium containing 0 mannitol were used as a mock control (CK). Error bars show the standard deviations for three independent replicates. Asterisks indicate statistically significant differences compared to WT at the same time point (*P < 0.05).

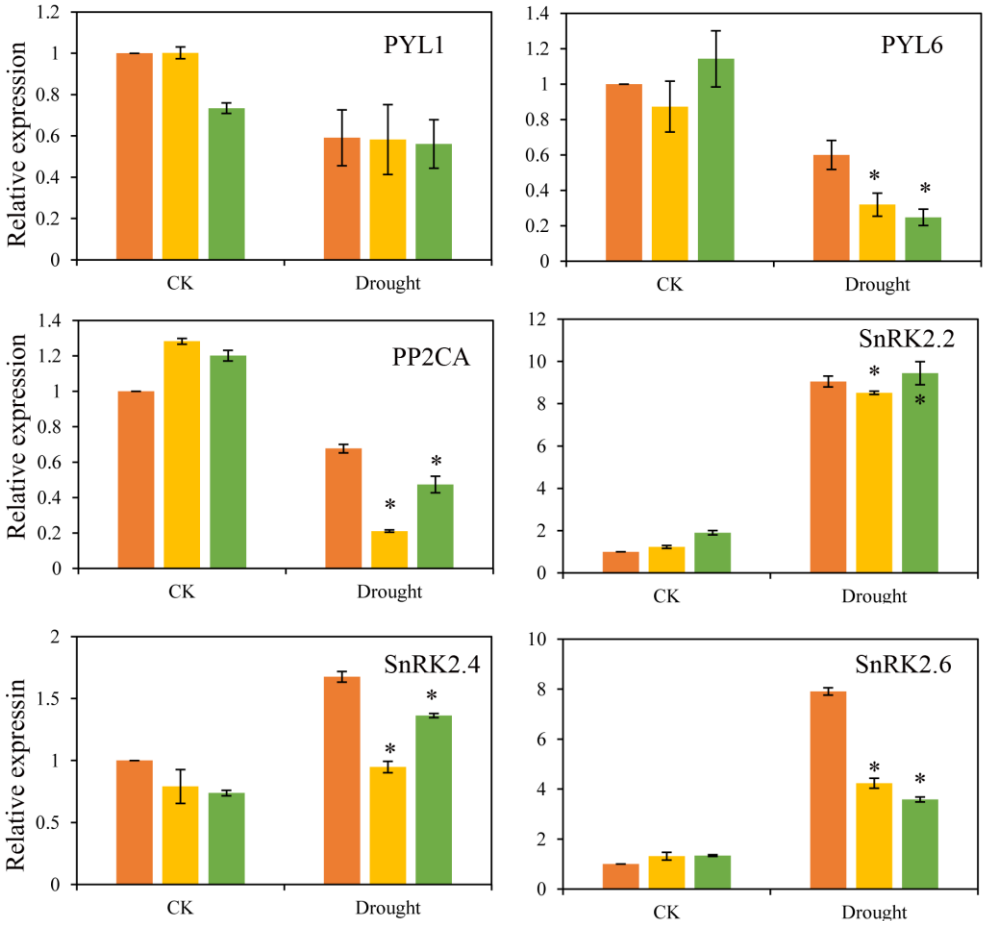

Fig. S5. Expression levels of ABA signaling genes of WT and OE plants. Cabbage seedlings treated without water were used as a mock control (CK). Error bars show the standard deviations for three independent replicates. Asterisks indicate statistically significant differences compared to WT at the same time point (*P < 0.05).

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AtNAC019.seq   MGIQETDPLTQLSLPPGFRFYPTDEELMVQYLCRKAAGYDFSLQLIAEIDLYKFDPWVLPNKALFGEKEWYFFSPRDRKY    80
BoNAC019.seq   MGIQETDPLAQLSLPPGFRFYPTDEELMVQYLCRKAAGYDFSLQLIAEIDLYKFDPWVLPNKALFGEKEWYFFSPRDRKY    80
Consensus      mgiqetdpl qlslppgfrfyptdeelmvqylcrkaagydfslqliaeidlykfdpwvlpnkalfgekewyffsprdrky

AtNAC019.seq   PNGSRPNRVAGSGYWKATGTDKIISTEGQRVGIKKALVFYIGKAPKGTKTNWIMHEYRLIEPSRRNGSTKLDDWVLCRIY   160
BoNAC019.seq   PNGSRPNRVAGSGYWKATGTDKIISTEGKRVGIKKALVFYIGKAPKGTKTNWIMHEYRLLEPSRANGSSKLDDWVLCRIY   160
Consensus      pngsrpnrvagsgywkatgtdkiisteg rvgikkalvfyigkapkgtktnwimheyrl epsr ngs klddwvlcriy

AtNAC019.seq   KKQSSAQKQVYDNGIANAREFSNNGTSSTTSSSSHFEDVLDSFHQEIDNRNFQFSNP.NRISSLRPDLTE.QKTGFHGLA   238
BoNAC019.seq   KKQSSAQKQAYEHVVTSTRELSNNGTSSTTSSSSHFEDVLDSLHHETDNRNFQYANSSNRFSSLRPDLTVGEKTGFNGLA   240
Consensus      kkqssaqkq y       re snngtssttsssshfedvlds h e dnrnfq  n  nr sslrpdlt   ktgf gla

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Consensus      dt  fdw sf gnvehn s pelg shvvp le n gylk eee      f      aq  yg d   fgysgqvggfg
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Fig. S6. Sequence analysis of BoNAC019 and AtNAC019. The amino acid residues are exactly the same as the black shadow and the 50% is the same as the blue shadow Alignments were performed using DNAman.