

Barley plants overexpressing the NAC transcription factor gene *HvNAC005* show stunting and delay in development combined with early senescence.

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Table S1. Sequences of specific primers used for cloning, for check of transgenic barley plants, and generation of different constructs.

HvNAC005 cds PCR amplification – USER cloning	
HvNAC005-Fw	5'-ATGATCATGTCCGACCCGG-3'
HvNAC005-Rev	5'-GATCGGTACCTTTAGAAATGGAGCAGCGGCT-3'
Check of transgenic barley lines with qRT-PCR	
NAC5-prom_junc_Fw	5'-ACAACCAGATCTCCCCAAA-3'
NAC5-prom_junc_Rev	5'-TTGCGGAGGTAGTGCAGGAT-3'
NAC5-term_junc_Fw	5'-CCAAGTCGGCAACAACACCTTG-3'
NAC5-term_junc_Rev	5'-ATCATCGCAAGACCGGCAACAG-3'
Genome walking for promoter cloning	
Race-AP1:	5'-GTAATACGACTCACTATAGGGCACGCGTGG-3'
AP2:	5'-GGCACGCGTGGTCGACGGC-3'
HvNAC005-O:	5'-AACGCTCGAGGACGAAAACCCTAGCTG-3'
HvNAC005-I:	5'-AGCTGGCTAGCTAGAGCTTGGAGTGCT-3'
Constructs for transactivation analysis in yeast	
HvNAC005-FL-Fw	5'-ATATATCATATGATCATGTCCGACCCG-3'
HvNAC005-C-term-Fw	5'-GGAATTCCATATGAGCCAAGTGTCCCCGATGGCG-3'
HvNAC005-355-Rev	5'-CCGGAATTCCTTACACCGCGGCCTGCAGTGT-3'
HvNAC005-319-Rev	5'-CCGGAATTCCTTAGAGCGCGTGTCCGCTGTC-3'
HvNAC005-250-Rev	5'-CCGGAATTCCTTACGCTGGCTGGCTCGTCGA-3'
HvNAC005-218-Rev	5'-CCGGAATTCCTTAGAAATGGAGCAGCGGCTG-3'
Promoter cloning for EMSA analysis	
MLOC_61774 Fw	5'-GCTACGGGATCCCAACACCTCTCTTCCAATTTGA-3'
MLOC_61774 Rev	5'-CGTAGCTCTAGAGAAGCCGGTTGATGGCTTTGCT-3'
MLOC_61801 Fw	5'-GCTACGGGATCCACTCCAATAAAACAAATCTCACA-3'
MLOC_61801 Rev	5'-CGTAGCTCTAGAGGCAAGCATAGTGAGTGAACCT-3'

Table S2. Accession numbers of HvNAC005 related proteins/genes used for the phylogenetic tree in Fig. 2.

Name	Protein/gene acc. #	Database
ANAC018	AT1G52880.1	TAIR
ANAC025	AT1G61110.1	TAIR
ANAC047	AT3G04070.1	TAIR
ANAC056	AT3G15510.1	TAIR
ANAC060	AT3G44290.1	TAIR
AtNAP	AT1G69490.1	TAIR
BdNAC005	XP_003559782.1	GenBank
BdNAC006	XP_003562891.1	GenBank
BdNAC023	XP_003557998.1	GenBank
BdNAC090	XP_003577115.1	GenBank
BeNAC1	HM626401.1	GenBank
BnNAC2	AFI56994.1	GenBank
GmNAC26	NP_001238204.1	GenBank
HvNAC005	AK251058.1	GenBank
HvNAC023	CBZ41159.1	GenBank
HvNAC027	BAJ99416.1	GenBank
HvNAC030	ABI94358.1	GenBank
ONAC010	LOC_Os07g37920.1	MSU/RAP-DB
ONAC016	LOC_Os01g01430.1	MSU/RAP-DB
ONAC058	LOC_Os03g21060.1	MSU/RAP-DB
ONAC103	LOC_Os07g48450.1	MSU/RAP-DB
ONAC122	LOC_Os11g03300.1	MSU/RAP-DB
ONAC131	LOC_Os12g03040.1	MSU/RAP-DB
StNAC2	NP_001275015.1	GenBank
TaNAC005-like	W5EN00_WHEAT	uniprot_trembl
TaNAC25	ADG85701.1	GenBank
TaNAC69	AAU08785.1	GenBank
TtNAM-A1	A0SPJ3.1	GenBank
TtNAM-B1	A0SPJ4.1	GenBank
TtNAM-B2	A0SPJ6.1	GenBank
ZmNAC118	GRMZM2G109627_T01	MaizeGDB
ZmNAC122	GRMZM2G430849_T01	MaizeGDB
ZmNAC25	GRMZM2G127379_T01	MaizeGDB
ZmNAC44	GRMZM2G011598_T01	MaizeGDB
ZmNAC81	GRMZM2G042494	MaizeGDB

Table S3: Genes, accession numbers (GenBank), and primer sequences for qRT-PCR gene expression experiments performed in this work.

Gene	Accession	Fw-primer	Rv-primer
<i>18S ribosomal RNA</i>	AY552749.1	ACGGCTACCACATCCAAGGA	CAGGATTGGGTAATTTGCGC
<i>9-cis-epoxycarotenoid dioxygenase (NCED1)</i>	AK365103.1	CGCCCTCCATCCCTCCCATCTTCT	CCGCCGCTAACTGTTTCTCTTCC
<i>Amino acid permease</i>	AK361903.1	ACTTCGGCCCAGAAGATATGGC	AGTAGGCGAATGCGATGTTCCC
<i>APETALA2 and ethylene responsive element binding protein</i>	AK374826.1	TGATGGACGACGGCAACTTCTG	ATGTTGTGATGGGCGGTGATCC
<i>CYSPEP</i>	AM941122.1	AAAGGTGGCAAGGATTATTGG	TGCCACAGATACCTGACGAT
<i>HvNAC005</i>	AK251058.1	CCATGTGAACAGCAGCGGCAAC	CCGACGTTGAGGCTGGTGAATC
<i>HvNAC013</i>	AK376297.1	ATGCCGCCGCACATGATGTAC	ACAGGTCGCCGGAATTAGCG
<i>HvNAC027</i>	FR819765.1	ACGGCTACGTGAACCACGACAC	CAAGCTGCCGCTGGATCTCTTC
<i>Jasmonate ZIM domain-containing protein</i>	AK358513.1	CAGCAGAGCAACAAGCTCAACG	TGGGCTGGTGAGTAAGTATCTGC
<i>Nuclease I</i>	AB028448.1	ACTATTACTTCCGGGCGTTGCC	TCTGCAGCCTGCTATTCTTCCC
<i>Oxoglutarate/iron dependent dioxygenase</i>	AK355337.1	CAGCAGAGCAACAAGCTCAACG	TGGGCTGGTGAGTAAGTATCTGC
<i>Pheophorbide a oxygenase (PaO)</i>	AK358479.1	AGGCATCGAGAACTGGCAGAAG	ACCAGTTCCTGAATGCGACGAC
<i>Protein phosphatase 2C</i>	BM816007	TCGCAATGTCACGGTCAATCGG	ACGCTGACTTCAGGCTTTGGAATC
<i>Protein phosphatase 2C</i>	AK367469.1	TGCTCACCAAGATGGCTCTCAC	GAGATCGACGACGACAACAGAG
<i>Protein phosphatase 2C</i>	AK374059.1	TCTTCCTTCCGTTCCCTCGTTC	CAACAGCTGAGCTTCTCCGTTTC
<i>RUBISCO small subunit</i>	AB020943.1	TCTTCCGTGAGCACAACAGC	TCCAGTATCGGCCGTCGTAG
<i>Saccharopine dehydrogenase</i>	AJ508229.2	GTTTCAGAGGAAAGGCGTGATCC	ACTCTCTCGATCAGCTGAATGCC
<i>Stay green protein (SGR)</i>	AY850135.1	CGTGGTTGTTGTACGGGCAAAG	CAGTGGGATCTTGTTCAGCATCG
<i>Splicing factor 2 (SP2)</i>	AK249101.1	GAAGGATGAGTAGGCGCTGG	CTGGGAGGTTCCCAACGTAA
<i>Zeatin O-glucosyl-transferase (ZOG)</i>	AK364192.1	AGAAGAAGGCACACGAGTGGAG	TCGTCSAAGTTGCGGTGAGATG

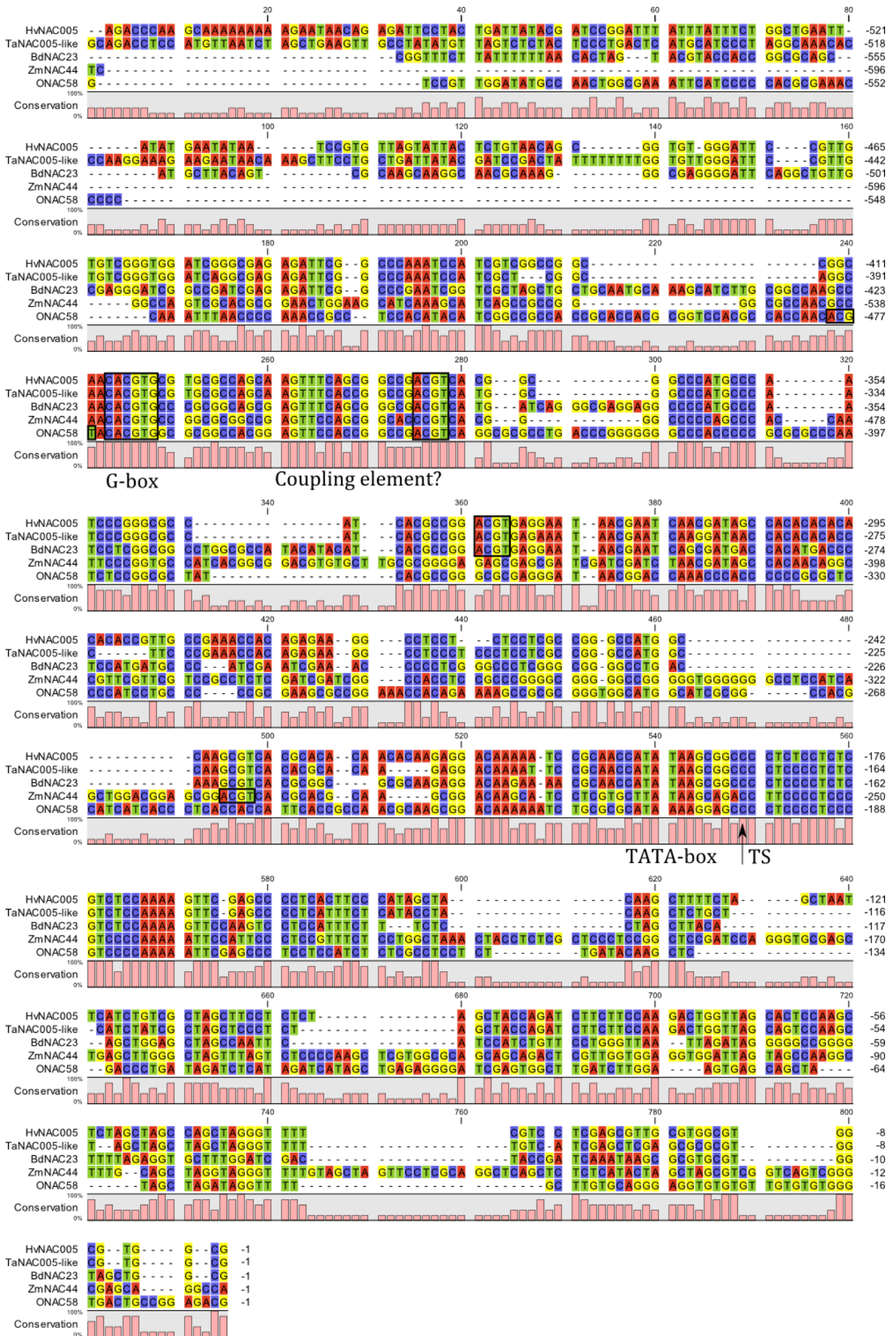


Fig S1. Alignment of the promoter (600 bp upstream of ATG) of HvNAC005 and the four most closely related genes from wheat, Brachypodium, maize and rice (see Fig. 2). G-box: an ABRE-like CACGTG motif. TS: transcription start. A potential ABRE coupling element is indicated, as well as the putative TATA box. ACGT containing motifs are delineated by black rectangles.

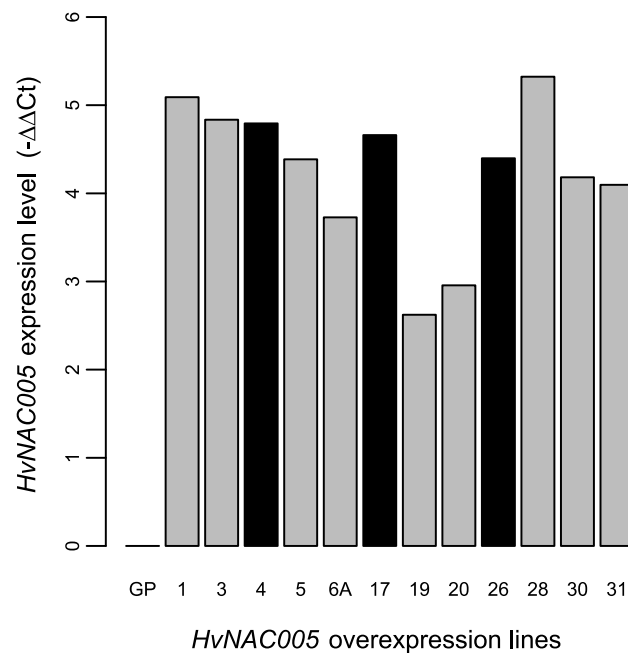


Fig. S2. *HvNAC005* expression level, as determined by qRT-PCR, for 12 *HvNAC005* overexpression T₀ lines. Gene expression levels (-ΔΔCt, i.e. log₂ scale) were normalized to the expression level of *18S rRNA* and with barley ‘Golden Promise’ (GP) control line as the calibrator sample. Since only one plant per line was available, the determinations are based on only one biological replicate with three technical replicates, except for GP and line 26 (T₁ plants), which are based on 4 biological replicates. Black bars: the three lines that were used for further characterization of *HvNAC005* overexpressing plants. Gray bars: lines that were not used for further characterization, due to bad seed setting or low expression of *HvNAC005*.

Table S4. Mean number of days after sowing when half of the final number of spikes with visible awns had appeared. Numbers of awns were recorded twice a week, when SPAD measurements were taken, placing the number of days into slots with 3-5 days intervals. A t-test was used to estimate statistical delays in this number in HvNAC005 over-expression lines.

Line	Number of plants	Days after sowing +/- SE	P-value, t-test
HvNAC005-OE-4	4	97.0 +/- 1.1	0.028*
HvNAC005-OE-4-null	6	93.0 +/- 1.0	
HvNAC005-OE-17	3	100.7 +/- 2.3	0.191 ^{NS}
HvNAC005-OE-17-null	3	96.3 +/- 0.75	
HvNAC005-OE-26	4	98.2 +/- 0.75	0.004**
HvNAC005-OE-26-null	5	94.0 +/- 0.0	
Golden Promise	4	95.5 +/- 0.87	
null vs. Golden Promise		94.4 vs. 95.5	0.335 ^{NS}
OE vs. Golden Promise		98.2 vs. 95.5	0.045*

Table S5. Mean number of tillers in plants of transgenic and control lines when the experiment was terminated 116 days after sowing. A t-test was used to estimate statistical differences among the lines.

Line	Number of plants	Final number of tillers +/- SE	P-value, t-test
HvNAC005-OE-4	5	96 +/- 13	0.19 ^{NS}
HvNAC005-OE-4- null	4	79 +/- 8	
HvNAC005-OE-17	3	130 +/- 27	0.57 ^{NS}
HvNAC005-OE-17- null	3	111 +/- 10	
HvNAC005-OE-26	3	100 +/- 3	0.049*
HvNAC005-OE-26- null	4	119 +/- 7	
Golden Promise	4	92 +/- 11	

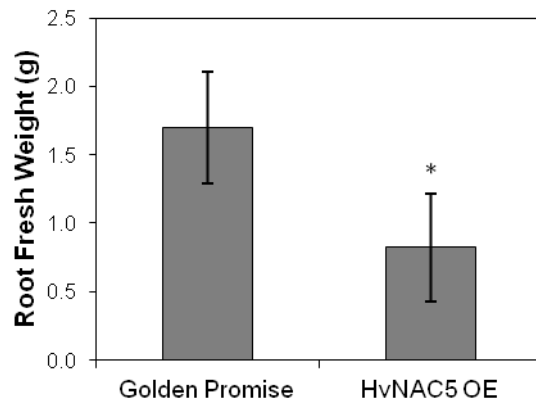


Fig. S3. Characterization of T₁ plants from *HvNAC005* overexpression line HvNAC005-OE-26. Fresh weight of roots from four week old seedlings. Error bars: standard deviation. Asterisk indicate reduced root mass in the HvNAC005-OE line, $p < 0.05$ (t-test).

A) MLOC_61774|MLOC_61774.1

CAACACCTCTCTTCCAATTTGAGCTTCAAGTACAACCACAAATGGAGTGGCATCTTCAA
CCGAGCACCATTCCCTTCACCTCTTAGCCTACACACTTCATCTCACTTATCTCCAATCAC
ATCAGTCAAAAACCTTCGGCAATGGATCATCATGCCATTCAATAAATCCACAACCACCATG
TTGTTCAATTTTAGTAATAGAAATCAATCAGTGGGGAGGAGAACAATGAAGAGAAACAA
ACAGAAATCCAACCTCACCATGGCATCGATGCATGCGTAGTACCTTCTGCCTGGATTATGA
ATGCTCGAGGAGATCCATCTTGACCTTCCGACAGGATTGCACCAGCAAAGCTTCTGC
GGCTCGAACCCATCGGGCTCTCCCTATAAAGCACCGATGACCTCGACTCCCCCTCTCC
TTCCTGCGGCTCAACGAAAGCCAGGAGCAGCAGAATGACCCGATGACCACGACATCCAT
TGGACAGATCCGCCGCCGCCCTCTCCCTAGTTCTCCCACTCGAAAATCCCCAATTA
ATTTGATGTTCCCTCATCTCTCCCCGCAACGCGTTATAATGCAACCTGGCCCATTAGTC
ATAAAGAATTCCGGTAGACTCTTGCCATGTCATCATGTTTAGCTCTAAATGACGTTAGGT
TTTAATTTAGACCGGAATTTATAAATTTAGAGCATAAACAATAAGGATTTTAACTTCAGA
GTTCCGGTTCGCTCAAGGACTAGAGTTCAATGTTTAAATGGACCTTTTCGTTTGTGAGCT
CTGTGTCTGCCGCCGAGTTAACAACCTGAACTGCGGCACGAGCGGAGTGCAGCCACCAC
GTGCGAAAATGTCTTGCTGTCCAGTAATCATTGTATAATCTCGTTCGACCAGCACGTGTT
CTTCGTCGAGAGACCTCCAGGAAGAAACACCCACATTAGACAGCAACAGCCCACAGGAC
ACGCTTCGATTCCTATAAAGCAAAGCCATCAACCGCTTC

B) MLOC_61801|MLOC_61801.1

ACTCCAATAAAACAAATCTCACATTCACCTAGCTCCACCTGTGCGCATACGTTGACAC
CCCTTTGCTAGAATCCTCATCCTCATCGCCGGCATCCTTAATGTCATCCACACTAGGGAG
AACATGAAGGCAACCAAGAAGACACACATGGAGGGAACCTGAGACAGGTGTCGCAACTAGG
AGTCCAAGCAAACATTTCAATACAATAGCCAAAGGAAATGGCCTTGAGAATAGCAGCTTG
TTTGCTGGCACGTTAGTGGTAGCAAACAAAACGTGATGATATTCTCAAGGTTGTTGTC
CTTGAAGACTACAACGCTTTCTTTCTTTGCACCAGTACGACACTTGTACAGGTCCAAT
CCTTGTGCTTCTTCTTGGTCGAGCATGGCCACATAAATTTGGATATTTATCATTGTC
GGCGCTCAAACCTCCGGATGACCATTCAACTCTTCTTGCATGTGGCGATGCAGATCTCCC
AATCTACGATAATGCTGTGTCTAGTTTGATCTTGATGATTAATTTGTGCTAACATAA
TTTTTCATGTTCTTTTTTTGGATTAATCTAATTTGAAAATTAATATTTTTATTTAACACA
GTGTGATTTGGACAGGACAAGGAGCATCGGATGATGGTTCCAGTACGGACTTAGGAGAG
AAAACCTACGACCGCCATTTTGGGGAAGACTCGAGCAAGGCTGCCATGTAAAACCTTCTA
TTCCTTCTGTATCTAAATAATTATATTTGAAAAAACTAAAGTAAAAGAGATTATATTTA
GAAACGGAACAAGTAGCAATTGAAGCGAGTAATAGATCCACCCGACATGTCCATGTGGAG
CTTAATTCCTGAAAGTTTTATCCTCGATCCAAGTTAATTATATGAACTACAATAAA
GAACCAATACCTCCCAATCGGTCGACCTAAATCACCTTTCGTTACCTCCATCATAACCAGACA
CTATTTGAGAGACGTCTTAGGTTCACTCACTATGCTTGCC

Fig. S4. Promoter sequences of two genes selected among the upregulated genes in the gene expression microarray for *HvNAC005* overexpressing plants. CGT/ACG (yellow/green) motifs potentially contained in NACBSs are highlighted. A) Gene encoding a ZIM domain protein (EnsemblPlants: MLOC_61774.1; GenBank: AK358513.1). B) Gene encoding a putative zeatin glucosyltransferase (ZOG) (EnsemblPlants: MLOC_61801.1; GenBank: AK364192.1).