SUPPORTING ONLINE MATERIAL

"A NAC Gene Regulating Senescence Improves Grain Protein, Zinc, and Iron Content in Wheat"

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MATERIALS AND METHODS

Plant Materials

Tetraploid Recombinant Substitution Lines (RSLs) (121 and 300) and parental lines (LDN and RSL65 including a 30-cM DIC chromosome segment) used to map the *Gpc-B1* locus were previously described (*S1, S2*). RSLs 2-15 and 34-17 were generated by screening 3,900 additional F_2 plants from the cross RSL65 x LDN, followed by selection for plants with recombination events between PCR markers *Xuhw89* and *Xucw71* (Fig. 1B, manuscript). The selected F_2 RSLs were self-pollinated and F_3 homozygous recombinant plants were selected and seed increased for replicated field trials. Over 9000 gametes were screened with flanking molecular markers to select all the recombinant lines used in this study.

Sequencing and annotation of the *Gpc-B1* region

Overlapping BAC clones 916O17 and 409D13 (*S3*) from a LDN(DIC6B) tetraploid BAC library (*S4*), including flanking markers *Xuhw89* and *Xucw71*, were sequenced and annotated (Genbank DQ871219). Over 65 % of the sequence was identified as repetitive by searching the *Triticeae* Repeat Sequence Database (TREP http://wheat.pw.usda.gov/ITMI/Repeats/index.shtml). The non-repetitive sequence was further characterized by performing BLAST searches to GenBank and the wheat EST collection at GrainGenes (http://www.graingenes.org/) and using gene prediction programs (Genscan (http://genes.mit.edu/GENSCAN.html) and FGENESH (http://www.softberry.com/berry.phtml)).

Five genes were annotated and molecular markers were developed for these and other sequences within this region to further delimit the *Gpc-B1* locus (table S1). Sequences for all primers in the Supplemental Online Materials are indicated in the 5' to 3' direction. Markers *Xucw71* and *Xuwh89* have been published before (*S3*).

The structure of *TtNAM-B1* was confirmed by cDNA sequencing and no splice variants were detected. The *TtNAM-B1* transcription start was determined by 5' RACE and the transcription end by 3' RACE (FirstChoice® RLM-RACE Kit, Ambion, USA).

RNA extraction and cDNA synthesis

Tissue for RNA extraction was taken from the middle of the flag leaf and RNA was extracted using Aurum Total RNA Kit (Bio-Rad). After extraction, RNA concentration and quality were determined using the Agilent 2100 bioanalyzer. Only high quality RNA samples were used for further cDNA synthesis. Immediately after RNA quantification, 1 µg of RNA was used for cDNA synthesis with iScript cDNA Synthesis Kit (Bio-Rad) in

a 20 µl reaction volume. After inactivation of the reverse-transcriptase, the cDNA was diluted to a final volume of 100 µl by adding 80 µl of dH_20.

Quantitative real-time RT-PCR (Q-PCR)

Aliquots of the cDNA served as templates for quantitative real-time RT-PCR analysis. The Q-PCR reaction mixture contained the following components: 5 µl cDNA, corresponding to 50 ng of total RNA, 10 µl iTaq™ SYBR® Green supermix with ROX, 0.5 µl of each of the forward and reverse primers (10µM, final concentration 250 nM), and 4 µl of water in a 20 µl final reaction volume. Quantitative PCRs were carried out in an ABI Prism 7000 (Applied Biosystems) using the following condition: 50°C – 2 min, 95°C – 3 min, 40 cycles of 95°C – 15 sec, 60°C – 30 sec. The specificity of the PCR amplification was checked by dissociation curve analysis (from 60°C to 94°C). The gene specific primers used for Q-PCR are listed in table S9.

The specificity of all primer pairs was confirmed by cloning the PCR amplification products and sequencing (*TtNAM-A1*, *B1*, *B2* into pDrive plasmid (QIAgen) and *ACTIN* into pGEM T-Easy plasmid (Promega)) or by testing in nulli-tetrasomic lines (*TaNAM-D1, D2*). Recombinant plasmid DNA was purified using miniprep extraction kits (QIAgen), and plasmid DNA concentrations were measured by the GeneQuant spectrophotometer (Amersham Biosciences). Efficiency of each pair of primers was calculated using five 4-fold dilutions (1:1, 1:4, 1:16, 1:64, 1:256) in triplicate. Amplification efficiencies varied between 92% and 98% (table S9). Transcript levels are presented as normalized linearized values using the $2^{-\Delta\Delta C_T}$ method (*S5*), where C_T is the threshold cycle and *ACTIN* was used as the internal control. The same calibrator (10 days before anthesis) was used for all genes so their values are comparable. Values represent number of RNA copies per copy in the calibrator sample.

Production of the RNA interference (RNAi) transgenic plants

We used a 475-bp segment from *TtNAM-A1* (GenBank DQ869672, 1163-bp to 1637-bp, excluding the five domains characteristic of the NAC family of transcription factors) as our RNAi trigger. This 475-bp stretch includes multiple regions of 40 or more bp that are identical to the other target *TaNAM* genes (*TaNAM-D1*, *B2* and *D2*; fig. S4). This segment was cloned in forward and reverse orientations into vector pMCG161 provided by V. Chandler as described before (*S6*).

Embryogenic callus of hexaploid spring variety Bobwhite were bombarded with a 2:1 molar ratio of the RNAi and UBI::BAR selectable marker plasmids. Transformants were selected as described previously (*S7*), except that the callus recovery media after bombardment contained 5 µM CuSO4 and the regeneration media contained 5 µM CuSO4 and 0.1 mg/L 6-benzylamino purine.

Characterization of the RNAi transgenic plants

Transgene-positive plants were confirmed by PCR using primers designed from the vector sequence flanking the sense (primers Ri_S_F and Ri_S_R) and antisense insertions (primers Ri_AntiS_F and Ri_AntiS_R) (table S5). Transcription of the transgene in the T_1 plants and positive T_2 progeny was confirmed by RT-PCR using primers for the transcribed region of the Octopine Synthetase PolyA region of the pMCG161 vector (primers OCS_PolyA_F and Ri_AntiS_R; table S9). No RT-PCR product was detected in the negative plants.

Transcript levels of the *TaNAM* genes in the transgenic plants were determined in T_2 plants using Q-PCR. To avoid amplification of the construct, we designed new primers to quantify the transcript levels of endogenous *TaNAM-A1* (primers NAM_F2 and NAM_A1_R1; table S9). Since the transgenic plants were hexaploid, we also designed Q-PCR SYBR GREEN® systems specific for each of the two D genome copies of *TaNAM* (primers NAM_D1_F/R and NAM_D2 _F/R; table S9).

We confirmed the stay-green phenotype in the T_2 plants used in the expression study by using their flag leaf chlorophyll degradation profiles and the maximum quantum efficiency of photosystem II (*Fv/Fm*) at 40 days after anthesis (DAA) (fig. S6).

Determination of senescence parameters

RNAi Transgenic plants: For each transgenic event, the main spike was tagged at anthesis and the date was recorded. The corresponding flag leaf of each spike was later used to measure relative chlorophyll content in a non-destructive manner using a hand-held chlorophyll meter (SPAD-502, Minolta, UK). Leaf chlorophyll was measured every other day; each value corresponds to the average of 10 sampling positions per flag leaf. These chlorophyll content measurements are given in relative SPAD units.

The tagged main spike and its corresponding peduncle were evaluated for yellowing every other day in a subjective manner (by the same observer). DAA in which the peduncle turned completely yellow was recorded. In the same manner, the main spike was evaluated every other day by the same observer and the DAA upon complete dryness of the spike was recorded.

For the fluorescence measurements (*Fv/Fm*), we used a portable leaf chamber fluorometer (model LI-6400-40, LI-COR Biosciences, NE, USA) according to manufacturer's instructions. Flag leaves from nine transgenic and nine non-transgenic T_2 plants from each event were measured at 40 DAA.

Tetraploid RSL: Chlorophyll content of the critical tetraploid RSL was determined 20 DAA by extracting chlorophyll with *N,N*-dimethylformamide as described previously (*S8*).

Nitrogen and micronutrient determinations

Grain and leaf samples were dried to constant weight and ground to a fine powder with a mortar and pestle. The samples were analyzed for N concentration by a continuous-flow mass spectrometer (Europa Scientific, Cambridge, UK) and for *Zn* and *Fe* content by ICP-MS (Inductively Coupled Plasma- Mass Spectrometer) (Agilent Technologies, CA, USA) at the University of California, Davis.

Determination of grain size/ thousand kernel weight

For each transgenic event, T_1 plants were individually harvested and each spike threshed separately. Grains from the tagged main spike were dried to constant weight and counted. The average grain size is reported as thousand kernel weight ([total grain weight/ number of grains] * 1000).

Statistical analysis

Analyses of variance were performed using the SAS Version 9.1 program (*S9*). The general linear model (PROC GLM) was used to assess the effect of the DIC *TtNAM-B1* allele in the tetraploid RSLs and to evaluate the effect of the RNAi construct in the transgenic plants. The procedures for these analyses were previously described (*S1*).

SUPPORTING FIGURES

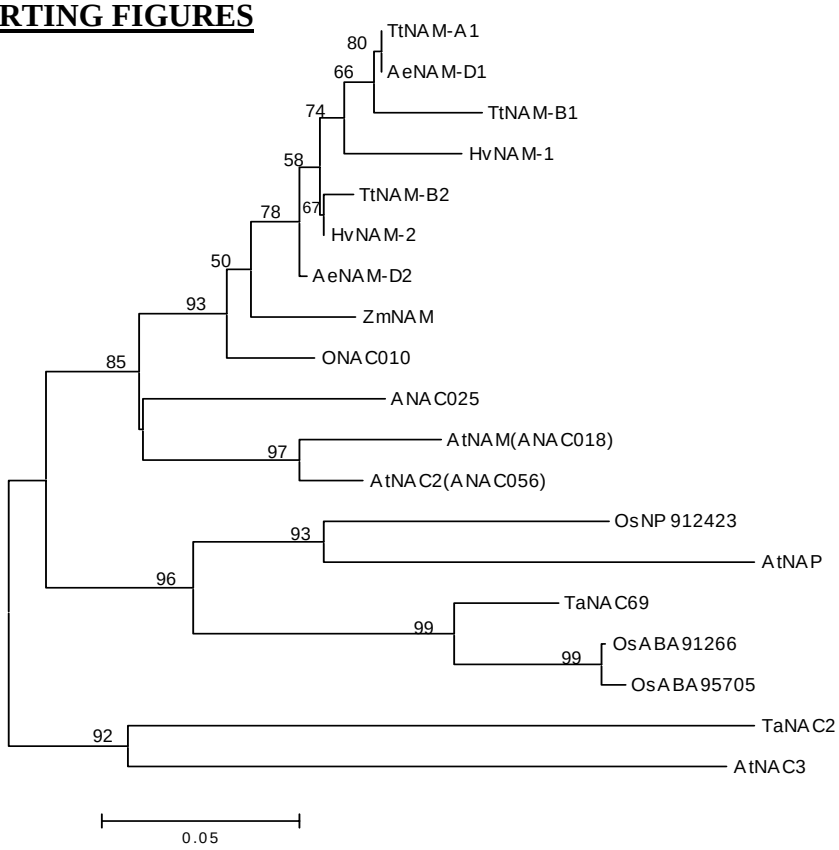

Figure S1. Best Neighbor Joining tree using the complete deletion option from MEGA3 (*S10*). The tree is based on the complete region including the NAC domain and includes *Arabidopsis* and grass species accessions with the most significant BLASTP values. Bootstrap values are based on 1000 replications and are indicated in their respective nodes (only values higher than 50 are reported). The tree shows that the closest rice homologue to the *NAM* genes is *ONAC010* (NP_911241) and that the two *NAM* copies in wheat cluster alongside their respective copies in barley. The *AtNAP* gene which was recently shown to affect senescence in *Arabidopsis* (*S11*) is not the closest homologue in this species. There are at least three other *Arabidopsis* genes (*ANAC025*, *AtNAM*, *AtNAC2*) with closer homology to the wheat *NAM* genes. The ONAC and ANAC designation are similar to those used by Ooka *et al.* (*S12*). Species abbreviations: Ae= *Aegilops tauschii*, Tt = *T. turgidum* , Hv= *Hordeum vulgare*, Os & ONAC= *Oryza sativa*, At & ANAC= *Arabidopsis thaliana*, Zm=*Zea mays* (To avoid confusion with the *Arabidopsis thaliana* species abbreviation (At), *Aegilops tauschii* was abbreviated as 'Ae'). GenBank accession numbers are: *TtNAM-A1* (DQ869672), *TtNAM-B1* (DQ869673), *AeNAM-D1* (DQ869675), *HvNAM-1* (DQ869678), *TtNAM-B2* (DQ869676), *AeNAM-D2* (DQ869677), *HvNAM-2* (DQ869679), *ONAC010* (NP_911241), *ANAC025* (NP_564771), *AtNAM* (NP_175696), *AtNAC2* (NP_188170), *OsNP_912423* (NP_912423), *AtNAP* (CAA10955), *TaNAC69* (AAY44098), *OsABA91266* (ABA91266), *OsABA95705* (ABA95705), *TaNAC2* (AAU08786), *AtNAC3* (BAB20599). The maize homologue *ZmNAM* was obtained from Plant Genome Database (http://www.plantgdb.org) accession ZmGSStuc11-12-04.2640.2.

Figure S2. Comparison of the 5 characteristic NAC domains from the closest *Arabidopsis* and grass proteins. Sequences were aligned using CLUSTALW and ordered according to the phylogenetic tree (fig. S1). Similar amino acids are marked with the same color across proteins. The GenBank accession numbers are the same as those presented in figure S1.

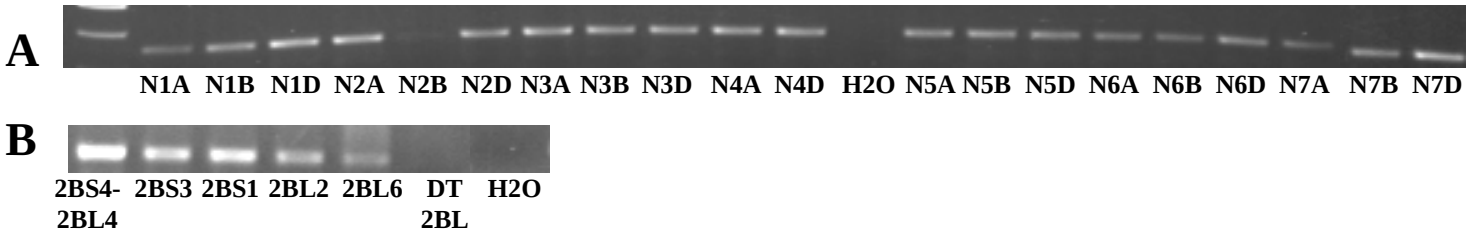

Figure S3. Mapping of the paralogous copy *TtNAM-B2* in centromeric BIN C-2BS1-0.53 (*S13*) using (**A**) nulli-tetrasomic lines and (**B**) ditelocentric and deletion lines.

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TtNAM-A1      1 ACCTCGCTGAGCCACCTGGCCGCGGCGGCGAGGGCGAGCCCGGCTCCGACCAAACAGTTT
TtNAM-B2      1 ACCTCGCTGAGCCACCTAGCCGCGGCGGCGAGGGCGAGCCCGGCTCCGACCAAACAGTTT
AeNAM-D2      1 ACCTCGCTGAGCCACCTAGCCGCGGCGGCGAGGGCGAGCCCGGCTCCGACCAAACAGTTC
AeNAM-D1      1 ACCTCGCTGAGCCACCTGGCCCCGGCGGCGAGGGCGAGCCCGGCTCCGACCAAACAGTTT

TtNAM-A1     61 CTCGCCCCGTCGTCTTCAACCCCGTTCAACTGGCTCGATGCGTCACCCGCCGGCATCCTG
TtNAM-B2     61 CTGGCCCCGTCGTCTTCAACCCCGTTCAACTGGCTCGATGCGTCAACCGTTGGCATCCTC
AeNAM-D2     61 CTGGCCCCGTCGTCCTCAACCCCGTTCAACTGGCTCGATGCGTCAACCGTTGGCATCCTC
AeNAM-D1     61 CTCGCCCCGTCGTCTTCAACCCCGTTCAACTGGCTCGAGGCGTCAGCCGCTGGCATCCTG
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Figure S4. ClustalW alignment of RNAi target sequences of *NAM* genes (*A1*, *B2*, *D2* and *D1*). In bold text is part of the sequence used in the RNAi construct as trigger for the silencing response. All copies have identical stretches longer that 40-bp, indicating the potential of the RNAi response to interfere with all of them. Additional conserved regions were detected in the rest of the sequence alignments (not shown).

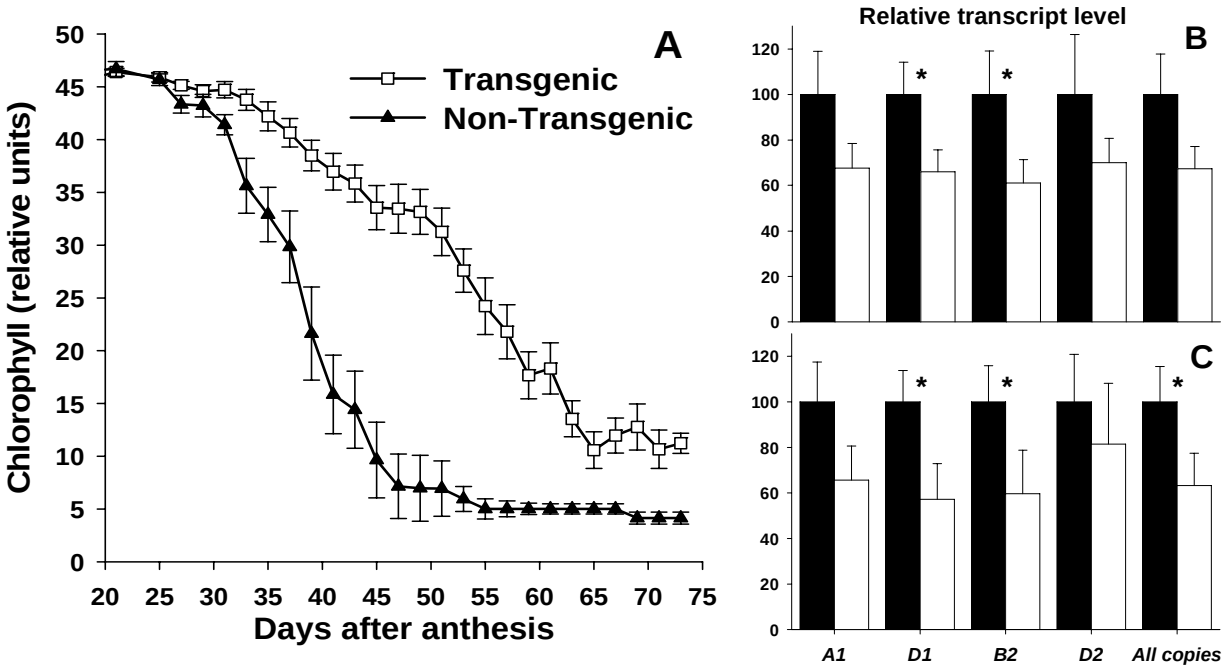

Figure S5. Characterization of plants from the second transgenic event (L23-119). (**A**) Flag leaf chlorophyll content profile of transgenic Bobwhite T_1 plants segregating for the presence (transgenic, n = 17) and absence (non-transgenic, n = 9) of the RNAi construct for *TaNAM*. (**B**) Relative transcript level of endogenous *TaNAM* genes in T_2 plants segregating for the presence (white bars, n = 12) and absence (black bars, n = 12) of the RNAi construct at 4 and (**C**) 9 days after anthesis. Asterisks indicate significant differences (P<0.05). Error bars represent standard error of the means.

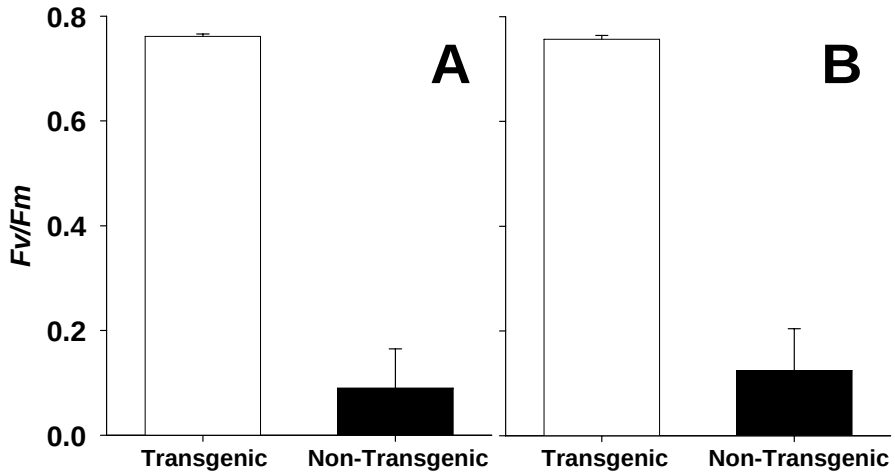

Figure S6. Maximum quantum efficiency of photosystem II (*Fv/Fm*) at 40 DAA in T_2 plants segregating for the presence (transgenic, n = 9) and absence (non-transgenic, n = 9) of the RNAi construct from event (**A**) L19-54 and (**B**) L23-119.

SUPPORTING TABLES

Table S1. Primer sequence and amplification conditions for PCR-based markers employed in the mapping of *Gpc-B1*.

Locus	Marker type	Primer name	Primer sequence	Rest. Enzyme /indel	Ann. (°C) /Ext. (s)	Expected size LDN (bp)
Xucw97	CAPS	Xucw97_F Xucw97_R	GGAGATTGTGGTGGGTTCAG GCTGCATCTATGCTCCAAAA	*Hinc*II	58 / 120	1300 + 380 + 53
Xucw87	CAPS	Xucw87_F Xucw87_R	ACCCGCTGTTCTAGTCCAAA CTGCACGACAGATCAACGTC	*Msp*I	57 / 30	279 + 195
Xuhw106	CAPS	Xuhw106_F Xuhw106_R	AGAGCAGGGGAGAAATACGG TAAGGTAGGAAGCGGAATGG	*Hpy*CH4	59 / 30	159
Xucw101	dCAPS*	Xucw101_F1 Xucw101_R1	AGGGACTACCACAAACTGCAA CTCCGTTCCTTCCTTCACAC		59 / 120	
		Xucw101_F2 Xucw101_dR2	CCCTAATTAAGCTGGAGAGCAA ATGAGGTCCATGGG**G**AGCT†	*Sac*I	54 / 60	620 + 135 + 111
Xucw107	dCAPS*	Xucw107_dF2 Xucw101_R1	ATTCGATTATTTAATTGCA**T**C† CTCCGTTCCTTCCTTCACAC	α*Taq*I	54 / 60	357 + 96 + 5
Xucw108	SSR	Xucw108_F1 Xucw108_R1	AGCCAGGGATAGAGGAGGAA AGCTGTGAGCTGGTGTCCTT	30-bp indel	58 / 40	217
Xucw109	SSR	Xucw109_F1 Xucw109_R1	ATCTGCAATTCCAGGCACAC CCAGCAGATCAAGGAGAATTG	2-bp indel	58 / 40	212

CAPS: Cleavage Amplified Polymorphic Sequences; dCAPS: degenerate CAPS; LDN: durum wheat cultivar Langdon

* For marker *Xucw101* and *Xucw107*, an initial PCR was done with primers Xucw101_F1 /R1. The PCR product was then re-amplified with primers Xucw101_F2/dR2 (for *Xucw101*) or with primers Xucw107_dF2 and Xucw101_R1 (for *Xucw107*) and subsequently digested with the indicated restriction enzyme.

† The bold underlined letters indicate a degenerate base pair introduced to generate a polymorphic restriction site

Table S2. Wild and domesticated emmer germplasm used in the allelic diversity study. All germplasm listed below contains the ancestral functional *TtNAM-B1* allele, except for the two accessions in bold text which carry the +1-bp frameshift allele.

Wheat	N	Germplasm numbers *
T. turgidum ssp. *dicoccoides* (wild emmer)	42	PI428020, PI428028, PI428036, PI428041, PI428047, PI428055, PI428058, PI428061, PI428065, PI428066, PI428070, PI428072, PI428079, PI428082, PI503310, PI538626, PI554580, PI554581, PI554582, PI428098, 1-22, 5-61, 7-4, 8-12, 9-36, 10/85-21, 11-15, 17-41, 19-14, 23-17, 24-34, 27-37, 28-50, 29-9, 30-22, 32-70, 33-22, 40-600, 41-8541, 42-8736, 43-8811, 44-8821
T. turgidum ssp. *dicoccum* (domesticated emmer)	19	PI355498, PI254180, PI254158, PI347230, PI94640, PI319868, PI319869, PI352329, PI470737, PI470738, PI94626, PI352347, PI470739, PI352367, PI182743, **PI352352**, **PI355454**, CItr 17675, CItr 17676

* PI and CItr germplasm correspond to Germplasm Resources Information Network (GRIN) numbers. Other numbers correspond to 'Location-Genotype' identification numbers from the University of Haifa wheat germplasm collection (*S14*).

Table S3. Cultivated durum varieties carrying the +1-bp frameshift mutation at *TtNAM-B1*.

Country of Origin	Variety Names *
France	Durfort, Exeldur, Nefer, Neodur
Greece	Minos
Italy	Adamello, Appio, Appulo, Capelli, Ciccio, Cirillo, Colosseo, Duilio, Karel, Lira B 45, L35, Latino, Messpia, Ofanto, Rusello SG7, San Carlo, Saragolla, Trinakria, Valbelice, Valforte, Valnova, Varano, Vitron, Zenit
Mexico	Aconchi 89, Altar 84, Carcomun 'S', Mexicali 75
Tunisia	Inrat 69, Karim, Khiar
USA	Aldura, Aruba, Colorado, Duraking, Durex, Imperial, Kofa, Kronos, Langdon, Leeds, Maier, Ocotillo, Produra, Reva, Ria, Rugby, UC1112, UC1113, WB 881

* Two tetraploid wheat varieties were of unknown origin (Dunfati and Edolic).

Table S4. Hexaploid wheat accessions and varieties used in the allelic diversity study.

***TaNAM-B1* allele**	**N**	**Description**	**Varieties and germplasm numbers***
Deletion	29	*T. aestivum* ssp. *aestivum*	PI166698, PI166305, PI166792, PI119325, IWA10993, Anza, Bobwhite, Cayuga, Caledonia, Express, GRN*5/ND614, Harry, ID0444, Jagger, Jaypee, Louise, McNeal, P91193, P92201, Platte, Rio Blanco, Tan105, Thatcher, UC1041, UC1037, USG3209, Wesley, Yangxian Yangqiannai, Yecora Rojo
	1	*T. aestivum* ssp. *spelta*	405a(DV1132)
	1	*T. aestivum* ssp. *compactum*	PI410595 (Pakistan)
Mutant allele with +1 frameshift mutation†	5	*T. aestivum* ssp. *aestivum*	IWA10940, Chinese Spring, CO940610, NY18/CC401, Panawawa
Wild type allele	1	*T. aestivum* ssp. *compactum*	PI350731 (Austria)‡

* PI corresponds to Germplasm Resources Information Network (GRIN) numbers. More details on varieties can be found at http://maswheat.ucdavis.edu/Mapping/index.htm.

† Determined with *NAM-B1* specific primer pairs 1-4 from Table S5.

‡ The presence of the wild type allele in this accession agrees with recent work by Dvorak *et al.* (*S15*) showing that segments of tetraploid wild emmer were introgressed in some European *T. aestivum* ssp. *compactum* accessions.

Table S5. Markers used to study hexaploid allelic diversity, to map the paralogous *NAM-B2* locus and to identify the transgenic plants.

Description	Primer name	Primer sequence
NAM-B1 specific pair 1	NAM_B1_F1	TTGTCCACTGCGCCAGC
	NAM_B1_R1	AAAAAGCAACGCGGTATCAC
NAM-B1 specific pair 2	NAM_B1_F2	GGAAGAATATAAAAATACTACTTGTGC
	Xucw101_R1	CTCCGTTCCTTCCTTCACAC
NAM-B1 specific pair 3	NAM_B1_F3	TGGAGAGCAATTAAATACTGCTAGAC
	NAM_B1_R2	GCCGGCCGTGCTTACT
NAM-B1 specific pair 4	NAM_B1_F1	TTGTCCACTGCGCCAGC
	NAM_R2	GGCTCCGACCAAACAGTTTC
Mapping of *NAM-B2* copy	NAM_B2_F2	TGAATGGAGGCATGGTGTTT
	NAM_B2_R2	TCTCTCAGGGTACGTGTCGAT
DNA integrity control	Xucw101_F1	AGGGACTACCACAAACTGCAA
	NAM_R1	TCGCTGAGCCACCTAGCA
RNAi sense	Ri_S_F	GTTGAGTGGCCCTGTTTCTC
	Ri_S_R	CATTGATCAGCCTAACCAAACA
RNAi antisense	Ri_AntiS_F	CAAATTCTAATCCCCAATCCAA
	Ri_AntiS_R	GGCGGTAAGGATCTGAGCTA

Table S6. Functional *NAM* copies in tetraploid and hexaploid wheat used in this study.

Wheat	Variety	Functional *NAM* copies*
Tetraploid	DIC	*A1*, *B1*, *B2*
	LDN	*A1*, *B2*
	RSL300†	*A1*, *B1*, *B2*
Hexaploid	Bobwhite	*A1*, *D1*, *B2*, *D2*

* Functional copies of *NAM* are defined as those whose predicted protein includes a NAC domain.

† RSL = Recombinant substitution line

Table S7. Characterization of grain and senescence related traits of transgenic Bobwhite T_1 plants (second event, L23-119) segregating for the presence (transgenic, n = 17 plants) or absence (non-transgenic, n = 9 plants) of the *TaNAM* RNAi construct.

	GPC (%)	***Zn* (ppm)**	***Fe* (ppm)**	**TKW* (g)**	**Dry Peduncle (DAA)***	**Dry Spike (DAA)**
Transgenic	12.81	53.87	37.29	34.63	67.9	52.0
Non-transgenic	17.31	70.45	57.08	32.23	37.2	36.7
Difference	-4.49	-16.58	-19.79	+ 2.04	+ 30.7	+ 15.3
P value	<0.001	<0.001	<0.001	0.24	<0.001	<0.001

* TKW = Thousand kernel weight, DAA = Days after anthesis.

Table S8. Residual nitrogen, *Zn* and *Fe* in flag leaves of transgenic Bobwhite T_1 plants segregating for the presence (transgenic, n = 20 plants) or absence (non-transgenic, n = 16 plants) of the *TaNAM* RNAi construct. Events L19-54 and L23-119 were analyzed together.

	***N* (%)**	***Zn* (ppm)**	***Fe* (ppm)**
Transgenic	2.74	168.73	189.85
Non-transgenic	2.20	93.59	150.11
Difference (absolute)	+ 0.54	+ 75.14	+ 39.74
Difference (%)	+ 24.4	+ 80.29	+ 26.47
P value	0.01	<0.01	<0.01

Table S9. Primers used for Q-PCR and their amplification efficiencies.

Locus or marker	Primer name	Primer sequence	Amplification efficiency (%)
NAM-A1 (RSL)	NAM_A1_F1 NAM_R2	GGAGAGGATTCATAAATGCGGA GGCTCCGACCAAACAGTTTC	93.0
NAM-A1 (transgenic plants)	NAM_F2 NAM_A1_R1	GGGCGAGAAACTGTTTGGTC ACAGCCATTTCCTGGAGGG	92.4
NAM-B1	NAM_B1_F1 NAM_R2	TTGTCCACTGCGCCAGC GGCTCCGACCAAACAGTTTC	93.4
NAM-D1	NAM_D1_F NAM_D1_R	GCCTGCGGTGGCCTC CATGTCTGCATTTATGAATCCTCTTC	93.6
NAM-B2	NAM_B2_F1 NAM_B2_R1	AAAGCGGGTATGCGGTGAC CCTCTCTCAGGTTGGACGAC	96.6
NAM-D2	NAM_D2_F NAM_D2_R	GGACAGCCACGAGGACAACT GGGCCAGGAACTGTTTGGT	98.0
All copies	NAM_F3 NAM_R3	TCCACGGAGTCCTCGCAC AAGAAGATCAACAAGGCCGC	97.8
RNAi construct	OCS_PolyA_F Ri_AntiS_R	AGTGGGTCTAGAGTCCTGCTT GGCGGTAAGGATCTGAGCTA	--
Actin	Actin_F Actin_R	ACCTTCAGTTGCCCAGCAAT CAGAGTCGAGCACAATACCAGTTG	97.7

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