

Supplemental Table S1. Sequence similarity of B73 and teosinte *ZCN7* and *ZCN8* genes. The sequences were aligned with ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>; Larkin et al., 2007) followed by the calculation of the nucleotide identity and amino acid similarity using the SIAS software (<http://imed.med.ucm.es/Tools/sias.html>) with the BLOSSUM62 matrix. For sequence identity estimation the gaps obtained after the alignment were not considered and the percentage was referred to the shortest sequence. The cDNA includes the predicted coding region plus the 5'- and 3'-end untranslated regions amplified in RT-PCR; the gene region includes the cDNA plus the introns and the 5'- and 3'-end sequences that were not detected in RT-PCR with the selected primer combinations.

Nucleotide sequence identity in the cDNA

<i>ZCN7</i> B73	100%			
<i>ZCN7</i> teosinte	99.5%	100%		
<i>ZCN8</i> B73	92.2%	92.7%	100%	
<i>ZCN8</i> teosinte	91.2%	91.7%	99.5%	100%
	<i>ZCN7</i> B73	<i>ZCN7</i> teosinte	<i>ZCN8</i> B73	<i>ZCN8</i> teosinte

Nucleotide sequence identity in the gene

<i>ZCN7</i> B73	100%			
<i>ZCN7</i> teosinte	99.1%	100%		
<i>ZCN8</i> B73	88.9%	87.8%	100%	
<i>ZCN8</i> teosinte	87.0%	87.2%	99.1%	100%
	<i>ZCN7</i> B73	<i>ZCN7</i> teosinte	<i>ZCN8</i> B73	<i>ZCN8</i> teosinte

Amino acid sequence identity

<i>ZCN7</i> B73	100%			
<i>ZCN7</i> teosinte	98.9%	100%		
<i>ZCN8</i> B73	90.9	92.0	100%	
<i>ZCN8</i> teosinte	90.9	92.0	100%	100%
	<i>ZCN7</i> B73	<i>ZCN7</i> teosinte	<i>ZCN8</i> B73	<i>ZCN8</i> teosinte

Supplemental Table S3. Analysis of the mC profile of *ZCN7* and *ZCN8* by bisulfite sequencing. Percent mC for the upper strand is reported as an average (\pm SD) from 10 independent clones for each locus region and each treatment/developmental stage analyzed. The treatments are: wild-type (wt) and *idl* mutant for B73 inbred line and SD and NB condition for teosinte plants. The developmental stages are: immature leaf (IL) and mature leaf (ML). The mC is divided in subcategories CG, CHG, or CHH (H = A or T or C) and summed (total). The number of occurrences of each cytosine in a given context and in the sequenced region, for the total of the clones, is reported (n). No statistically significant differences (t test, $p < 0.01$) between wt vs *idl* and between SD vs NB for all locus regions analyzed was found.

Locus (region)	Treatment / Dev. Stage	% Methylation (n)							
		CG	CHG	CHH	Total				
ZCN7 B73 (5'-end)	wt IL	n=90	87.0 \pm 9.0	n=180	1.1 \pm 1.3	n=620	0.3 \pm 0.7	n=890	9.2 \pm 1.3
	<i>idl</i> IL		87.0 \pm 5.0		1.7 \pm 3.7		0.5 \pm 1.1		9.4 \pm 1.2
ZCN7 B73 (gene body)	wt IL	n=100	96.0 \pm 7.0	n=260	21.0 \pm 34.0	n=1010	2.0 \pm 0.8	n=1370	12.0 \pm 6.0
	<i>idl</i> IL		92.0 \pm 10.0		21.0 \pm 35.0		1.3 \pm 2.0		11.0 \pm 8.0
ZCN7 B73 (3'-end)	wt IL	n=60	92.0 \pm 9.0	n=220	14.0 \pm 21.0	n=720	2.1 \pm 2.6	n=1000	10.0 \pm 5.4
	<i>idl</i> IL		93.0 \pm 9.0		15.0 \pm 30.0		2.1 \pm 2.3		11.0 \pm 6.5
ZCN8 B73 (5'-end)	wt ML	n=60	88.0 \pm 16.0	n=220	0.5 \pm 1.4	n=760	0.3 \pm 0.6	n=1040	5.4 \pm 1.2
	<i>idl</i> ML		88.0 \pm 11.0		0.5 \pm 1.4		0.1 \pm 0.4		5.3 \pm 0.8
	wt IL		83.0 \pm 18.0		0.0 \pm 0.0		0.4 \pm 0.6		5.1 \pm 1.1
	<i>idl</i> IL		80.0 \pm 20.0	n=220	0.0 \pm 0.0		0.0 \pm 0.0		4.6 \pm 1.2
ZCN8 B73 (gene body)	wt IL	n=70	87.0 \pm 9.0	n=100	1.1 \pm 1.3	n=530	0.3 \pm 0.7	n=700	9.2 \pm 1.3
	<i>idl</i> IL		87.0 \pm 5.0		1.7 \pm 3.7		0.5 \pm 1.1		9.3 \pm 2.3
ZCN8 B73 (3'-end)	wt IL	n=70	91.0 \pm 14.0	n=270	9.0 \pm 14.0	n=720	2.1 \pm 1.9	n=1060	9.6 \pm 3.7
	<i>idl</i> IL		91.0 \pm 15.0		5.0 \pm 8.4		1.4 \pm 1.6		8.3 \pm 1.9
ZCN7 teosinte (5'-end)	SD IL	n=90	84.0 \pm 20.0	n=180	1.1 \pm 2.3	n=600	0.5 \pm 1.1	n=870	9.3 \pm 2.4
	NB IL		86.0 \pm 9.0		1.7 \pm 2.7		0.2 \pm 0.5		9.3 \pm 1.1
ZCN7 teosinte (gene body)	SD IL	n=100	97.0 \pm 5.0	n=270	14.0 \pm 28.0	n=1040	2.1 \pm 1.6	n=1410	11.0 \pm 6.0
	NB IL		96.0 \pm 7.0		15.0 \pm 29.0		2.5 \pm 1.8		12.0 \pm 7.0
ZCN7 teosinte (3'-end)	SD IL	n=60	93.0 \pm 9.0	n=210	12.0 \pm 19.0	n=750	2.2 \pm 2.2	n=1020	10.0 \pm 4.4
	NB IL		92.0 \pm 12.0		13.0 \pm 21.0		3.3 \pm 2.6		11.0 \pm 4.8
ZCN8 teosinte (5'-end)	SD ML	n=60	85.0 \pm 12.0	n=220	1.4 \pm 3.1	n=760	0.1 \pm 0.4	n=1040	5.3 \pm 0.9
	NB ML		83.0 \pm 19.0		0.9 \pm 1.9		0.3 \pm 0.6		5.2 \pm 1.3
	SD IL		78.0 \pm 11.0		0.5 \pm 1.4		0.4 \pm 0.6		4.9 \pm 0.8
	NB IL		82.0 \pm 12.0		0.9 \pm 1.9		0.3 \pm 0.6		5.1 \pm 0.9
ZCN8 teosinte (gene body)	SD IL	n=60	95.0 \pm 8.0	n=110	2.7 \pm 4.3	n=520	1.0 \pm 1.3	n=690	9.4 \pm 1.2
	NB IL		95.0 \pm 8.0		1.8 \pm 3.8		0.6 \pm 0.9		9.0 \pm 1.3
ZCN8 teosinte (3'-end)	SD IL	n=80	94.0 \pm 12.0	n=260	5.8 \pm 12.0	n=720	1.9 \pm 1.5	n=1060	9.8 \pm 3.9
	NB IL		94.0 \pm 9.0		8.8 \pm 14.0		2.0 \pm 1.3		10.7 \pm 4.3

Supplemental Table S4. List of primers used for B73 and teosinte cDNA synthesis in strand-specific reverse transcription. a: Orientation of the RNA strand used as template for cDNA synthesis. b: Primer sequences are indicated in 5'-3' orientation.

Primer name	RNA strand^a	Primer sequence^b
GAPC2rev	sense	TTCATGTGGCGGATCAGGTCGA
EF-1αrev	sense	CAGAGATTGGAACGAAGTGG
ZCN7for	antisense	GTTAAAAAATCATGCTTTGACAAAG
ZCN7rev	sense	ATTATTGATGATAATTATATAGTGTAG
ZCN8for	antisense	GCAACGGCCAATACCATTAG
ZCN8rev	sense	ATTATTGATGATATTCTATAGTGTGA

Supplemental Table S5. List of primers used in PCR. a: The amplified regions are indicated using the description employed in the text and Figures. b: Forward (F) and reverse (R) primers used in this study are indicated in 5'-3' orientation.

Sequence and amplified region ^a	Experiment	Primer combinations ^b
ZCN7 5'-end genomic region	cloning of teosinte ZCN7 genomic sequence by PCR	F AGTACCCAGCCACCCAGAA R TCCTGTAGGACACGAGCCA
ZCN7 internal genomic region and unspliced antisense transcript	i) cloning of teosinte ZCN7 genomic sequence by PCR ii) cloning of B73 and teosinte ZCN7 antisense transcript by strand-specific RT-PCR with ZCN7for-primed cDNA	F GACAAAGTACGTAAAAAATCATGC R AGAGGTGGTGCATTGACATTC
ZCN7 3'-end genomic region	cloning of teosinte ZCN7 genomic sequence by PCR	F TGTCAAAGGGAAGGTGGATCG R GACGATAGGACGAGGCGTG
ZCN8 5'-end genomic region	cloning of teosinte ZCN7 genomic sequence by PCR	F GTACATGTTCAAACAAACCAGGGA R CTTAGTGGAAATGGTTGGTGTAAAGG
ZCN8 internal genomic region, unspliced antisense transcript, and spliced and unspliced transcripts	i) cloning of teosinte ZCN7 genomic sequence by PCR ii) cloning of B73 and teosinte ZCN8 antisense transcript by strand-specific RT-PCR with ZCN8for-primed cDNA iii) RT-PCR with oligo(dT)-primed cDNA for detection of spliced and unspliced RNAs and cloning of B73 and teosinte ZCN8 sense spliced mRNA (ZCN8-1 and ZCN7-2 primers in Fig. 3A)	F AAGAGCAACGGCCAATACCATTAGCGA R CAGTGGCCTATGGTTTCTACTCTTCCCT
ZCN8 3'-end genomic region	cloning of teosinte ZCN8 genomic sequence by PCR	F CACGGCAATATCACCTCAGC R TGCAGCTAGTATATCAGAACGGTG
ZCN7 unspliced transcript	RT-PCR with oligo(dT)-primed cDNA for detection of unspliced B73 and teosinte RNAs (ZCN7-1a and ZCN7-2 primers in Fig. 3A)	F GACAAAGTACGTAAAAAATCATGC R AGAGGTGGTGCATTGACATTC

ZCN7 spliced and unspliced transcripts	RT-PCR with oligo(dT)-primed cDNA for detection of spliced and unspliced RNAs and cloning of B73 and teosinte ZCN7 sense spliced mRNA (ZCN7-1b and ZCN7-2 primers in Fig. 3A)	F CTTCCCTCAGTCCCATAGGGTTA R AGAGGTGGTGCATTGACATTC
ZCN7 and ZCN8 spliced and unspliced transcripts	RT-PCR with cDNA synthesized with locus and strand specific primers for detection of spliced and unspliced ZCN7 and ZCN8 RNAs from B73 and teosinte (ZCN7/8-1 and ZCN7/8-2 primers in Fig. 3B)	F TCATTGGTTATGGCTCGTGT R AGTGATGGATGGCTTGGACTT
ZCN7 and ZCN8 spliced transcripts	qRT-PCR with oligo(dT)-primed cDNA for detection of spliced transcripts of ZCN7 and ZCN8 (experiments of Supplemental Fig. S2)	F ACACCCTGGTACTGATTGACCC R CCAGTGCAAGTACTCCCTTAGTGA
ZCN7 spliced sense transcript (F primer spanning two exons and specific for spliced form)	strand-specific qRT-PCR with ZCN7rev-primed B73 and teosinte cDNA	F GGGAGTACTTGCACTGGATGG R CATATAGAGGTGGTGCATTGACA
ZCN7 unspliced sense and antisense transcript (F and R primers located in the intron and specific for unspliced form)	strand-specific qRT-PCR with ZCN7rev-primed and ZCN7for-primed B73 and teosinte cDNA	F TTTAATTGGTTGGCAACATCAG R TGTGGGGACATCGACATTTATT
ZCN8 spliced sense transcript (F primer spanning two exons and specific for spliced form)	strand-specific qRT-PCR with ZCN8rev-primed B73 and teosinte cDNA	F GGGAGTACTTGCACTGGATGG R TTGACAGTTGAAATATGTAGCGG
ZCN8 unspliced sense and antisense transcript (F and R primers located in the intron and specific for unspliced form)	strand-specific qRT-PCR with ZCN8rev-primed and ZCN8for-primed B73 and teosinte cDNA	F TGGTTGGTAACCTTGAGGTGTC R CAAGGAGCACTGAAGTATGTGG
ZCN7 A region	ChIP – MspJI B73 and teosinte	F ATGCAACAAAGAGAGCACAAAA R TACCTTAGTGTTTGTTCTTCCGC
ZCN7 B region	ChIP – MspJI B73 and teosinte	F GTTAAAAAATCATGCTTTGACAAAG R TGTAGGACACGAGCCATAACC
ZCN7 C region	ChIP – MspJI B73 and teosinte	F GCAATTTACAGATAATGCTACCCGA R CTGCACCAATTCAGTGGTCAAC
ZCN7 D region	ChIP – MspJI B73 and teosinte	F TTTAATTGGTTGGCAACATCAG R TGTGGGGACATCGACATTTATT
ZCN7 E region	ChIP – MspJI B73 and teosinte	F GAACAGGATTCAGCGAGCA R GCTCTTTGGCACATACGCT

ZCN8 A region	ChIP – MspJI B73 and teosinte	F CTCCAGCCATTCCTTCGGT R CATACTCAGTGCATGTTCTTCCAA
ZCN8 B region	ChIP – MspJI B73 and teosinte	F GCAACGGCCAATACCATTAG R TAGCTCAGCACTTGGCAGA
ZCN8 C region	ChIP – MspJI B73 and teosinte	F TGACATGAGGGCTTTCTACACC R CTACAACCTAAGCTCCACAGATAC
ZCN8 D region	ChIP – MspJI B73 and teosinte	F TGGTTGGTAACCTTGAGGTGTC R CAAGGAGCACTGAAGTATGTGG
ZCN8 E region	ChIP – MspJI B73 and teosinte	F CGCTCCCACCTCCTAACAAA R CGGCGTCCAACCTTACACA
ZCN7 5'-end region	Bisulfite sequencing B73 and teosinte	F GTTTTGTATTYYTAYTGTAATTTAT R TTCTTTRTCAAARCATRATTTTTTTAAC
ZCN7 internal region	Bisulfite sequencing B73 and teosinte	F GTTAAAAAATYATGYTTTGAYAAAGAA R CCAATATCATAATAARRCAAATTAATA
ZCN7 3'-end region	Bisulfite sequencing B73 and teosinte	F AAGAGYTAGTATTTTATGAGAGA R TTCCATRAAATATACTTTRTATTATCA
ZCN8 5'-end region	Bisulfite sequencing B73 and teosinte	F ATGTGGGTTYTYAAYAAGTGAAATA R ATATATTRCARAAAAATAACTCRCTAAT
ZCN8 internal region	Bisulfite sequencing B73 and teosinte	F TAAGAATAAYGTAYAAAYAATAGG R AACATATTRCRCTTTTTTTACCA
ZCN8 3'-end region	Bisulfite sequencing B73 and teosinte	F AAGAGYTAATATTTTATGAGAGG R TTCCATRAAATATACTTTRRATAATTCA