

Figure S1 Cis-elements in zein-gene promoters.

ZM27	--AAAAATAA-AATAGGCCGGACAG--GACAAAAATCCTTGACGT TGTAAAG TAAATTAC	-267
SB27	GTAAAAAACAA-AGTAGGCCGGAGAGAGGACAATAATCCTTGACGT TGTAAAG TGAATTAC	-250
ZM16	--AAAAGCAAGAGTAGGACGGAGAG--GAAAATAATCCTTGACG-----ACGTGC	-183
	***** * * * ***** *	
ZM27	AACAAAAAAAAGCCATATGTCAAGCTAACATTCTAATTCTGT TTTACGTAGA T--CAACAACC	-209
SB27	AA-----AGCCATATATCAATTATCTAATTCTGT TTCATGTAGA TATCAACAACC	-198
ZM16	A-----CATGAGCCA-----	-173
	* *** *	
ZM27	TGTAGAAGGCAACAAAATGAGCCACGCAGAAGTACAGAATGATTCCAGATGAACCATCG	-149
SB27	TGTAAAAGGCAACAAA-TTGAGCCACGCAAATTACA-AGTGAGTCCAAATAAACCTC-	-141
ZM16	-----CACGCAAGAGTACTGAATAATCCAGATGAACCCTCC	-136
	***** *	
ZM27	ACGTGCTACGTAAAGA-GAGTGACGAGTCATATACATTGGCAAGAAACCATGAAGCTGC	-90
SB27	ACATGCTACATAAAAGTGAATGATGAGTCATGTATATCTGGCAAGAAACTGTAGA---AG	-84
ZM16	AAAAG-TGAATGAGAT-GAGTCATG----TATACATTGGCAAGAAACCGTAGA---AG	-86
	* *	
ZM27	CTACAGCCGTCTGGTGGCATAGAACACAAGAAATTGTGTTAATTAAATCAAAG CTATAA	-30
SB27	CTACAGTCATC--GGTAGCAAAGAACACAAGAAATGTGCTAATAA---AAG CTATAA	-30
ZM16	CTACCGCCATC--GATTTCATCATAGAAGAAGAAATTGTGGTAATCGG--AAAG CTATAA	-30
	***** +1 Transcription Start	
ZM27	ATAACGCTCGCATGCCTGTGCACTTCTCCATCACCACACTGGTCT---TCAGACCATT	28
SB27	ATAACCCTCGTACGCCATGCACATCTCCATCACCACACTGGTCTTCATT CAGCCTATT	31
ZM16	ATAACCGTCGTATGCCTATGCACTTCTCCACCACCACACTGGACGTAGCATT	31

Figure S2 Alignment of the 27- and 16-kDa γ -zein and 27-kDa γ -kafirin promoters.

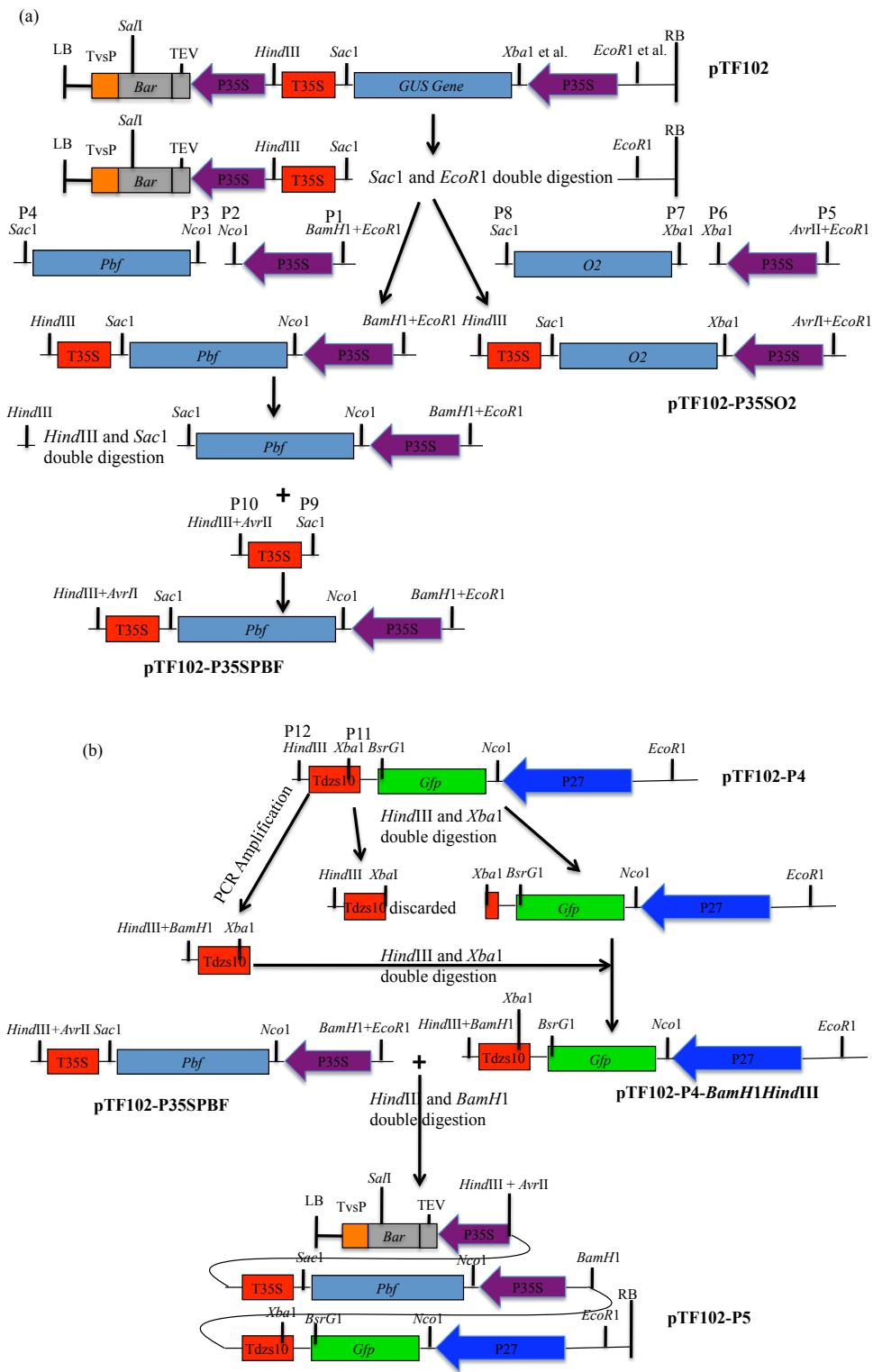


Figure S3 Cytosines in the 27-kDa γ -zein promoter. The 421-bp fragment immediately upstream of the transcription start site contains 90 cytosines, covering the P-box, O₂-like box and TATA box. P-box, "ACGT" core motif and TATA box are shaded. The cytosine positions indicated in Figs. 4a and S6d are numbered in the sequence.

		1	2	3	4							
zP22/6		TGTGAAAGGTGAAGAGATCTGCATGTCATTC										
azs22.11		TGTGAAAGGTGAAGAGATGATGCATGTCAT-C										
azs22.12		TGTGAAAGGTGAAGAGATGATGCATGTCAT-C										
azs22.13		TGTGAAAGGTGAAGAGATCATGCATGTCATTC										
azs22.4		TGTGAAAGGTGAAGAGATCATGCATGTCATTC										
azs22.1		TGTGAAAGGTGAAGCGATCATGCATGTCATTC										
azs22.3		TGTGAAAGGTGAAGAGATCATGCATGTCATTC										
azs22.5		TGTGAAAGGTGAAGAGATCATGCATGTCATTC										
azs22.19		TGTGAAAGGTGAAGAGATCATGCATGTCATTC										
azs22.7		TGTGAAAGGTGAAGAGATCATGCATGTCATTC										
azs22.8		TGTGAAAGGTGAAGAGATCATGCATGTCATTC										
azs22.2		TGTGAAAGGTGAAGAGATCATGCATGTCATTC										
azs22.20		TGTGAAAGGTGAAGAGATCATGCATTCATTC										
azs22.6		TGTTAAAGGTGAAGAGATCATGCATGTCATTC										
azs22.9		TGTTAAAGGTGAAGAGATCATGCATGTCATTC										
azs22.16		TGTTAAAGGTGAAGAGATCATGCATGTCATTC										
			5	6	7	8	9	10				
zP22/6		CACGTAGAT-AAAAGAATGCATATATAAAAATGGCACATTCTGTAGGTAGTGGAAA										
azs22.11		CAAGTATATGAAAAT-AATTCTATA-GAAAATGACACTTTCTTATAGTAGTGGAAA										
azs22.12		CAAGTATATGAAAAG-AATTCTATA-GAAAATGACAATTTCTGTAGGTAGTGGAAA										
azs22.13		CTCATGTATGGAAAG-AATTCCAATA-GAAAATGACGAATTTCTGTAGGTAGTGGAAA										
azs22.4		CACGTAGATGAAAAA--AATTCTATATAAAAATGACACCTTTCTGTAGGTAGTGGAAA										
azs22.1		CACGTAGATGAAAAAAATTCTATATAAAAATGACAATTCTGTAGGTAGTGGAAA										
azs22.3		CACGTAGATGAAAAAG-AATTCTATATAAAA-TGACACCTTTCTGTAGGTAGTGGAAA										
azs22.5		CACGTAGATGAAAAAG-AATTCTATATAAAA-TGACACCTTTCTGTAGGTAGTGGAAA										
azs22.19		CACGTAGATGAAAAAG-AATTCTATATAAAA-TGACACCTTTCTGTAGGTAGTGGAAA										
azs22.7		CACGTAGATGAAAAAG-AATTCTATATAAAA-TGACACCTTTCTGTAGGTAGTGGAAA										
azs22.8		CACATAGATAAAAAA-AAATTCTATATAAAAATGACAATTCTGTAGGTAGTGGAAA										
azs22.2		CACGTAGATAAAAAAAG-AGTCTCTATATAAGAATGGCACATTCTGTAGGTAGTGGAAA										
azs22.20		CACGTAGATGAAAAAG-AATTCTATATAAAA-TGACACATTCTGTAGGTAGTGGAAA										
azs22.6		CACGTAGATAAAAAAAG-AATGCCATATATAAAAATGGCACATTCTGTAGGTAGTGGAAA										
azs22.9		CACATAATGAAAAAG-AATTCTATATAAAAATGACATGTTCTGTAGGTAGTGGAAA										
azs22.16												
			11	12	13	14	15	16	17	18	19	20
zP22/6		GTATCTTCCAGCTAACGACC---ATATAATCGATAAAGGTGATAACTAAATGTCGAAA										
azs22.11		CTATCTTCTA-----TATAATCGATAAACCGATAACTAAATGTCGAAA										
azs22.12		CTAGCTTCCAGCAAAGACCA---TATAATCTGTGAAACTGATAACCAAATGTCGAAA										
azs22.13		CTATCTTATAGCAAAGTCCA---TATAATCATATGAAACTGATGACTAAATGTCAAA										
azs22.4		GTACCTTCCAGCAAAGATCATATATAATCGATAAAGCTGAAACTAAATGTCGAAA										
azs22.1		CTATCTTCTAGCAAAGACCATATATAATCGATAAACGCCATAACTAAATGTCGAAA										
azs22.3		GTATCTTCCAGCAAAGACCATATA---ATCGATAAAGTTGATAACTAAATGTCAAA										
azs22.5		GTATCTTCCAGCAAAGACCATATA---ATCCAATAAAGTTGATAACTAAATGTCGAAA										
azs22.19		GTATCTTCCAGCAAAGACCATATA---ATCGATAAAGTTGATAACTAAATGTCAAA										
azs22.7		GTATCTTCCAGCAAAGACCATATA---ATCGATAAAGTTGATAACTAAATGTCGAAA										
azs22.8		GTATCTTCCAGCAAATACCATATA---ATCGGATAAAGCGGATAACTAAATGTCGAAA										
azs22.2		GTATCTTCCAGCAAAGACTATATATAATCGGATAAAGCCGATAACTAAATGTCGAAA										
azs22.20		GTATCTTCCAGCAAATACCATATA---ATCGGATAAAGATGATAACTAAATGTCGAAA										
azs22.6		GTATCTTCCAGCAAAGACTATA----ATCGGATAAAGCTGATAACTAAATGTCGAAA										
azs22.9		GTATCTTCCAGCAAAGACCATATA---ATCGGATAAAGCTGATAACTAAATGTCGAAA										
azs22.16		TTATCTTCCAGCAAAGACCATATA---ATCGGATAAAGCTGATAACTAAATGTCGAAA										
			21	22	23	24	25	26	27	28	29	
zP22/6		TCGAGTAGATGCCATATCATCTACCTATCTGTGTTGG--AAAAAAAGACAAAT										
azs22.11		TTGAGTAGGTGCCATATCATCGATAGCTTATCTATTGTTGGC--AAAAA--GACAAAT										
azs22.12		TTGAGTAGGTGCCATATCATGTAGCTTATCTATTGTTGGC--AAAAA--GATAAAAT										
azs22.13		TTGAGTAGGTGCCATATCATCTACCTATCTATTGTTGGC--AAAAA--TACAAAT										
azs22.4		TCGAGTAGATGCCATATCATCTACCTATCTATTGTTGGC--AAAAA--GACAAAC										
azs22.1		TCGAGTAGATGCCATATCATCTACCTATCTATTGTTGGC--AAAAA--GACAAAT										
azs22.3		TAGAGTAGATGCCATATCATCTACCTATCTATTGTTGGC--AAAAAAAGACTAAAT										
azs22.5		TAGAGTAGATGCCATATCATCTACCTATCTATTGTTGGC--AAAAAA-GACTAAAT										

azs22.19	TAGAGTAGATGCCATATCATCTACCTTATCTGTTGGAA--AAAA---GACTAAAT	
azs22.7	TAGAGTAGATGCCATATCATCTACCTTATCTGTTGGAA--AAAA---GACTAAAT	
azs22.8	TCGAGTAGATGCCATATCATCTACCTTATCTGTTGGAA--AAAAA-GACAAAAT	
azs22.2	TCGAGTAGATGCCATATCATCTACCTTATCTGTTGGAA--AAAAA-GACAAAAT	
azs22.20	TCGAGTAGATGCCATATCATCTACCTTATCTGTTGGAA--AAAAAA-GACAAAAT	
azs22.6	AAGAGTAGATGCCATATCATCTACCTTATCTGTTGGAA--AAAAAA-GACTAAT	
azs22.9	TCGAGTAGATGCCATATCATCTACCTTATCTGTTGGAAAAAAAGACAAAAT	
azs22.16	TCGAGTAAGTGCCATATCATCTACCTTATCTGTTGGAA--AAAA---GACAAAAT	
3031	32333435	36373839 40
Zp22/6	CAAAAAAAA-TTTATAT----GAGATCTCACCTATATAAATAGCGCCAAATCAGTAGTTA	
azs22.11	CCAAATATATATATATAT----GAGATCTCACATGTATGAATATCTCCAAATCAGTAGTTA	
azs22.12	CCAAATATATATATATATATGAGATCTCACCTATATAAATATCTCCAAATCAGTAGTTA	
azs22.13	CCAAAAAA-ATATATAT----GAGACCTCACCTGTATATATAACCCCCAAATCAGTAGTTA	
azs22.4	CAAAAAAA-TATTAT----GAGGTCTCACTTGTATAAATAGCTCCAAATCAGTAGTTA	
azs22.1	CCAAAAAA-ATATATAT----GAGATCTCACTTGTATGAATAGCTCCAAATCAGTAATTA	
azs22.3	CCAAAAAA-ATATATAT----GAGATCTCACTTGTATAAATAGCTCCAAATTAGTAGTTA	
azs22.5	CC--AAA-ATATATAT----GACATCTCACTTGTATAAATAGCTCCAAATCAGCAGTTA	
azs22.19	CCAAAAAA-ATATATAT----GAGATCTCACTTGTATAAATAGCTCCAAATCAGTAGTTA	
azs22.7	CCAAAAAATATATATAT----AAGATCTCACTTGTATAAATAGCTCCAAATTAGTAGTTA	
azs22.8	CCAAAAAA-ATATATTT----GAGACCTCACCTGTATAAATAGCTCCAAATCAGTAGTTA	
azs22.2	CCAAAAAAATATATAT----GAGATCTCACTTGTATAAATAGCTCCAAATCAGTAGTTA	
azs22.20	CCAACAA-AAATATTC----GAGACCTCACCTATATAAATAGCTCCCATATCAGTAGTTA	
azs22.6	ACAAAAAA-ATATATAT----GAGATCTCACTTGTATAAATAGCTCCAAATCAGTAGTTA	
azs22.9	CCAAAAAAAATATAA---GAGATCTCACCTATATAAATAGCTCTGAAATCAGTAGTTA	
azs22.16	CCAAAAAAAATATAT---GAGATCTCACATGTATAAATAGCTCCAAATCAGTAGTTA	
4142		
Zp22/6	ATCCA	
azs22.11	ATTCA	
azs22.12	ATCCA	
azs22.13	ATTCA	
azs22.4	ATCCA	
azs22.1	ATCCG	
azs22.3	A-CCA	
azs22.5	A-CCA	
azs22.19	A-CCA	
azs22.7	A-CCA	
azs22.8	ATCCA	
azs22.2	ATCCA	
azs22.20	ATCCA	
azs22.6	A-CCA	
azs22.9	ATCCA	
azs22.16	ATACA	

Figure S4 The alignment of the 22-kDa α -zein promoters from B73 and the copy Zp22/6 from BSSS53. The cytosine positions indicated in Figs. 4b, 7c and 9 are marked in the corresponding places; P-box and “ACGT” core motif are shaded.



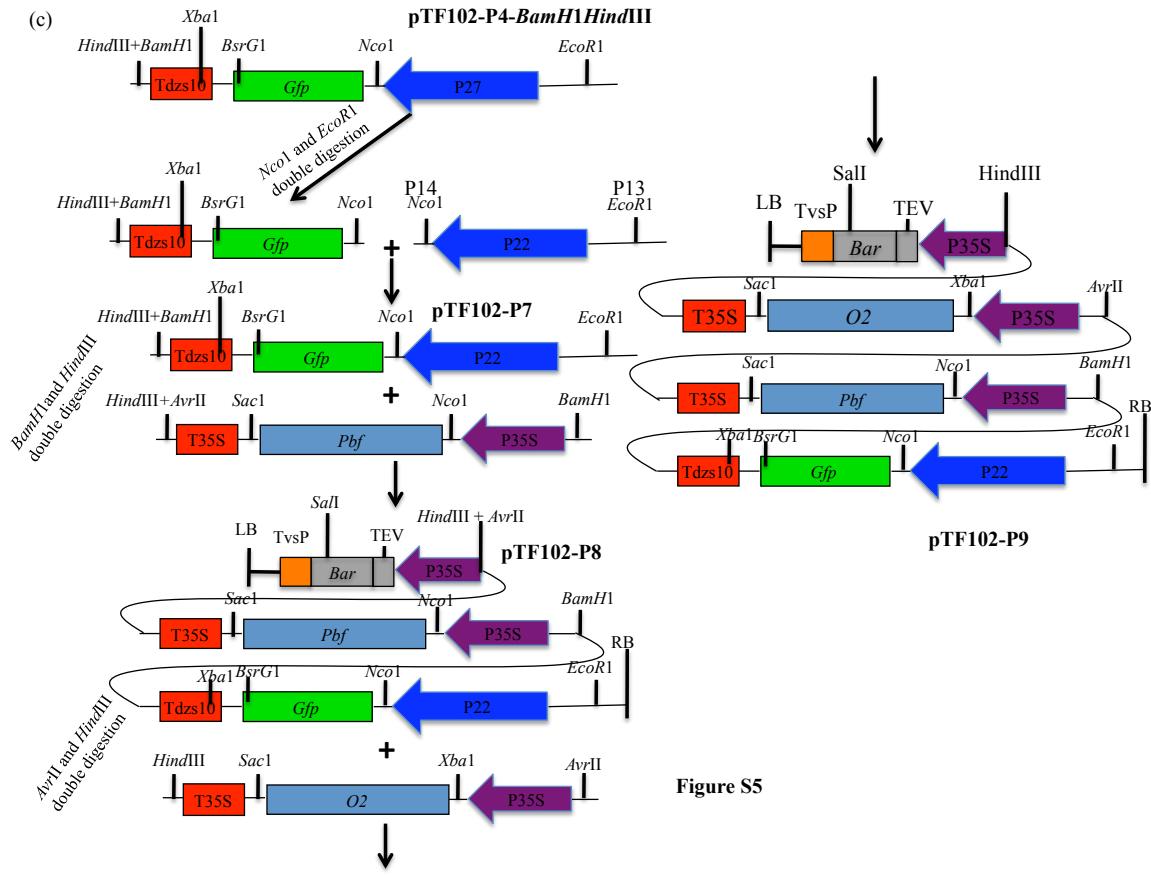


Figure S5

Figure S5 Strategies of plasmid construction. (a) P35SPBF and P35SO2 cassettes. (b) pTF102-P5. (c) pTF102-P9. Primers P1-P14 used for plasmid construction are indicated in the corresponding step.

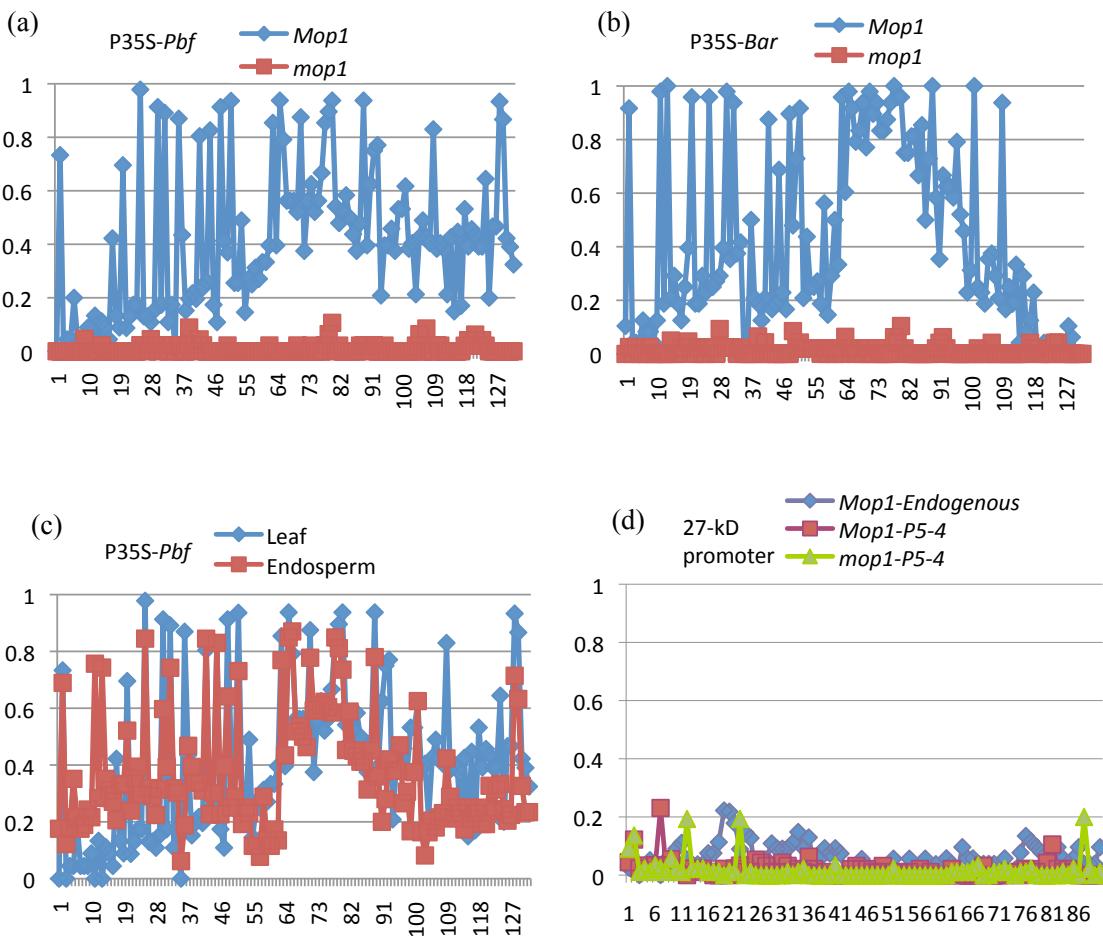


Figure S6 DNA methylation of 35S and transgenic 27-kDa γ -zein promoters in *Mop1-P5-4* and *mop1-P5-4*. (a) The 35S promoter driving the *Pbf* gene in *Mop1-P5-4* and *mop1-P5-4*. (b) The 35S promoter driving the selective marker *Bar* gene in *Mop1-P5-4* and *mop1-P5-4*. (c) The 35S promoter driving the *Pbf* gene in *Mop1-P5-4* leaf and endosperm. (d) The transgenic 27-kDa γ -zein promoter driving the *Gfp* gene.

G A G C A A T G T A T T G G T T G T G A G T T T
 1 2 3 4 5
 T T G A C G G C G C T C A C A G T G A T A G A T
 6 7 8 9 10 11 12
 T T G T T A T C T A T A T G C C C A G C C C C A G
 13 14 15 16 17 18
 C A T A T T C A T C C T T G T G C T G T G G G C
 19 20 21 22
 G T C T A G A G G A C C G A C A A T A T A T A T
 23 24
 A T T T T A A A A C A A A T T C G T G A A G A
 25 26 27 28 29 30
 A C A T C A C A A G T T A T G C A T G C A A A C
 31 32 33 34 35
 T G C T C A A G T C A T G T G G A T C C A A G G
 36 37 38 39 40 41 42 43
 C A T C C T A A C A A C T A G C A C A G C A T T
 44 45
 A C A A C A A A A T A T T G G T G T A T A T G T
 46 47 48
 G C C T A C A A T G A A G T G A A A G G T G A T
 49
 G A G T C A T G G T G A T G T G T A A A G A G G
 50 51 52 53 54 55
 C A T T A C A A A G T T A G C T T C A C A A G C
 56 57 58 59 60
 G T A T G A A A T T C A T T G A C A A C C C T T G
 61 62
 A C A T G T A A A G T T G A T T C A T A T G T A
 63 64 65
 T A A G A A A G C T T A A T G A T C T A T C T G
 66 67 68 69 70
 T A A A T C C A A A T C C A T G T A C T A T G T
 71 72 73 74 75 76 77 78
 T T C C A C G T C A T G C A A C G C A A C A T T
 79 80 81 82 83 84
 C C A A A A C C A T G G G T T G C A A G A T G C
 85 86 87 88 89
 T G C A G A A A T G C A A G C C A T G G A T C A T
 90 91 92 93 94 95 96
 C T A T A A A T G G C T A G C T C C C A C A T A
 97 98 99 100 101 102 103
 T G A A C T A G T C T C T A T C A T C A T C C A
 104 105 106 107 108 109
 A T C C A G A T C A G C A A A G C G G C A G T G
 110
 C G T A G A G A G G A T C G T C G A A C A G A A
 C A G C A T G

Figure S7 Cytosines in β -zein promoter. The 559-bp fragment upstream of the start codon contains 110 cytosines, covering the P-box, O2 box and TATA box. P-box, "ACGT" core motif and TATA box are shaded. The cytosine positions indicated in Fig. 11b are numbered in the sequence.

Table S1 List of primers

Primer	Sequence	Note
pbfBamH1	5'-AATT GGATCC ACTAACCTTATTGTCCCTTG-3'	
pbfBspE1	5'-TATA TCCGGA ATGGACATGATCTCCGGCAG-3'	
pbfBbIII	5'-AATA AGATCT ATGGACATGATCTCCGGCAG-3'	<i>Pbf</i> RNAi construct
pbfSac1	5'-AATT GAGCTC ACTAACCTTATTGTCCCTTG-3'	
Umc1066F	5'-ATGGAGCACGTCATCTCAATGG-3'	
Umc1066R	5'-AGCAGCAGCAACGTCTATGACACT-3'	<i>O2</i> polymorphic markers
P1	5'- TATAT GAATTCAAGGATCC GCATGCCGGTCAACATGGTG -3'	P35S forward, <i>EcoR1</i> and <i>BamH1</i> introduced
P2	5'- AAAT CCATGGTAGATCCCCGTTCGTAAATG -3'	P35S reverse, <i>Nco1</i> introduced
P3	5'- ATTAC CATGGACATGATCTCCGGCAG -3'	<i>Pbf</i> CDS forward, <i>Nco1</i> introduced
P4	5'- AATT GAGCTC ACTAACCTTATTGTCCCTTG -3'	<i>Pbf</i> CDS reverse, <i>Sac1</i> introduced
P5	5'- TATAT GAATTCAACCTAGG GCATGCCGGTCAACATGGTG -3'	P35S forward, <i>EcoR1</i> and <i>AvrII</i> introduced
P6	5'- AAAT TCTAGAGGTAGATCCCCGTTCGTAAATG -3'	P35S reverse, <i>Xba1</i> introduced
P7	5'- ATTAT TCTAGACCATGGAGCACGT CATCTCAATG -3'	<i>O2</i> CDS forward, <i>Xba1</i> introduced
P8	5'- TAAT GAGCTC CTTATTCAAGCGACGCCCTG -3'	<i>O2</i> CDS reverse, <i>Sac1</i> introduced
P9	5'-TATA GAGCTCGGCGCAAAATCACCAGTC -3'	T35S forward, <i>Sac1</i> introduced
P10	5'- ATT AAGCTTAATTCTAGG TGCAGGTCACTGGATTTGG -3'	T35S reverse, <i>HindIII</i> and <i>AvrII</i> introduced
P11	5'- AAAGCTGTACAAGTAAATAGAAATATTGTGTTGTATCG -3'	Terminator of 10-kDa δ -zein gene, forward
P12	5'- ATC AAGCTTACTAGTGGATCC TACCAAGCTGAGAATTAGGAG -3'	Terminator of 10-kDa δ -zein gene, reverse, <i>HindIII</i> and <i>BamH1</i> introduced
P13	5'- ATTT GAATTCGTCCACCGCGCAAATAGACC -3'	22-kDa zein promoter forward, <i>EcoR1</i> introduced
P14	5'- TAAT CCATGGTTGTTGGTCGTTGCTAGTG -3'	22-kDa zein promoter

		reverse, <i>Nco</i> 1
		introduced
γ 50F	5'-ATGAAGCTGGTGCTTGTGGTTC-3'	
γ 50R	5'-TAATGTCATTGCTGCTGCATGG-3'	For γ 50 RT-PCR
γ 27F	5'-ATGAGGGTGGTGCCTCGTTGC-3'	
γ 27R	5'-ACTCAACTAGCTAGCTAGCC-3'	For γ 27 RT-PCR
α 22F1	5'-ACACCATATGTTCATTATTCCACAATGCTCA-3'	
α 22R1	5'-TTAAGGATCCTATATAATCTAAAAGATGGCA-3'	For α 22 RT-PCR
α 19AF	CTCTTAa/gATTAGTAGCTAATAt/cATC	
α 19AR	CTGGGAAGGCCACAAACATCA	For α 19A RT-PCR
α 19BDF	ATTAGTCGGTAATCCATCAACC	
α 19BDR	CTAGAACATGGCACCCACCAATG	For α 19B and D RT-PCR
δ 18F	5'-CGCCATGGCAGCCAAGATG-3'	
δ 18R	5'-ATGCCGACTTCATTATTGGG-3'	For δ 18 RT-PCR
γ 16F	5'-TCGACACCATGAAGGTGCTG-3'	
γ 16R	5'-TGGTGATGGGTGACACTACG-3'	For γ 16 RT-PCR
β 15F	5'-AGGATCGTCGAACAGAACAGC-3'	
β 15R	5'-AGATGGATAGAGGGAGATTCCC-3'	For β 15 RT-PCR
δ 10F	5'-ATACTCTAGGAAGCAAGGAC-3'	
δ 10R	5'-TAAGAACATGGGTGGAATCG-3'	For δ 10 RT-PCR
δ 18-10F	5'-GCCATGGCAGCCAAGATG-3'	Common primer pair for
δ 18-10R	5'-TATCTAGAACATGCAGCACCAAC-3'	δ 18 and δ 10 RT-PCR
PbfF	5'-ATGGACATGATCTCCGGCAG-3'	
PbfR	5'-ACTAACCTTATTGTCCCTTG-3'	For <i>Pbf</i> RT-PCR
O2F	5'-ATGGAGCACGTATCTCAATG-3'	
O2R	5'-CCTTATTCAAGCGACGCCCTG-3'	For <i>O2</i> RT-PCR
GFPF1	5'-ATGGTGAGCAAGGGCGAGG-3'	
GFPRI1	5'-TTACTTGACAGCTCGTCC-3'	For <i>Gfp</i> RT-PCR
P22F1	5'-ACATGTGTAAAGGTGAAGAG-3'	
GFPRI2	5'-GTGAGCAAGGGCGAGGAGCT-3'	
P22bisulF	5'-ATATGTGTAAAGGTGAAGAG-3'	
α 22bisulR	5'-CTAATATCTTAATAACCATT -3'	
GFPbisulR	5'-AACTCCTCACCCCTACTCAC-3'	
P27bisulF	5'-GAAATATGGTGAGTTATGTTGAG-3'	
γ 27bisulR	5'-AAAACAACAAACACACCCCTC-3'	
P35SbisulF	5'-ATGTTGGTTAATATGGTGGAG-3'	
BarbisulR	5'-ACCACCAACATATCCACCTC-3'	
PfbisulR	5'-CTTAAATCCCTAACTTCCTCC-3'	

β 15bisuF 5'- GAGTAATGTATTGGTTGTGAG -3'

β 15bisuR 5'- CTATTCAACAATCCTCTAC -3'

Restriction enzyme cutting sites are colored in red.