

Supplemental Table 1

Segregation ratios of opaque vs. vitreous kernels from T1 ears of selected α -zein RNAi events

Event	Observed opaque/vitreous	Expected opaque/vitreous	Chi² (1 degree of freedom)	Two-tailed P value
609-2-1	26/8	25.5/8.5	0.039	0.84
615-1-1	54/18	54/18	0.000	1.00
615-1-1	36/11	35.25/11.75	0.064	0.80
653-3-1	70/24	70.5/23.5	0.014	0.91

Supplementary table 2. Primers for qRT-PCR of zein expression

z1A

F: 5'-GCTCCTTGGTCTTTCTGCAA-3'

R: 5'-GGTAACTGCTGTAATAGGGCTGATG-3'

z1B

F: 5'-CCAGCCCTATCTTTGGTGCA-3'

R: 5'-TCAGTGCGGCCAATTGGTTA-3'

z1C

F: 5'-TTCCACAATGCTCACTTGCT-3'

R: 5'-GTTGTTGTAAGACGCTCGCC-3'

z1D

F: 5'-GCACAACAACACTACAACAACA-3'

R: 5'-AATGGTAGTAGCTGTTGTGC-3'

27-kD γ -zein

F: 5'-GTGTCCCCCACTGAAGAACTAT-3'

R: 5'-CAATTGTTCAATTCAGGTCATTGCT-3'

50-kD γ -zein

F: 5'-TGTGCAACAACACGAACAGA-3'

R: 5'-ATTACCAAAGGGCTGCACTG-3'

16-kD γ -zein

F: 5'-TGGAGAACTCGACACCATGA-3'

R: 5'-GGTGGTTGAGTGGGGTATTG-3'

15-kD β -zein

F: 5'-CAGCAGATGAGGATGATGGA-3'

R: 5'-TCAGTAGTAGGGCGGAATGG-3'

Tubulin

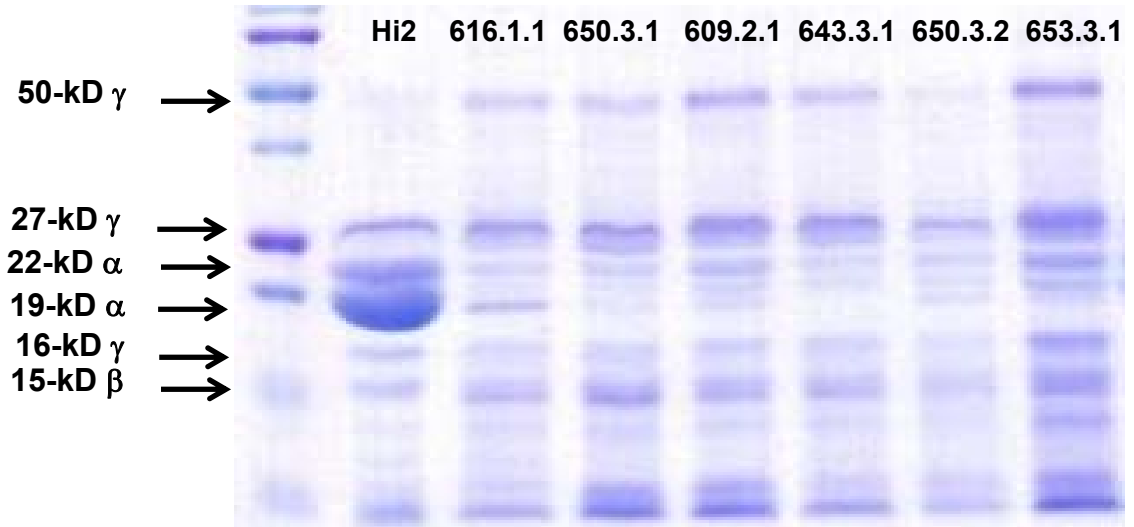
F: 5'-GTGTCCTGTCCACCCACTCTCT

R: 5'-GGAACGTTTCACATCAACGTTT

Supplemental Figure 1

Purified zeins from transgenic mature kernels showing increased 50-kD γ -zein

α -zein RNAi, zein accumulation in mature endosperm (6 events)



Supplemental Figure 2 Design of synthetic zein RNAi transgenes

Total alpha zein RNAi synthetic gene (EU116446 for Z1A, EU116472 for Z1B, EU116497 for Z1D, and EU116477 for Z1C)

>RNAi_Z1A^BDC

Tccaacaggcaatcgcagcaggcatcttacctttatcaccccttggtcctcca
acaaccgtcagccctattacagcagttacctttggtgcatttggtggcacia
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**Gamma zein RNAi synthetic gene AF371263 (50-kD),
DQ400403 (16-kD) and NM_001112534 (15-kD)**

>RNAi_50_gamma_16_gamma_15_beta

Ttcctaaggcagcagtgccagccctttggtaatgccttttctccaatcacggt
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