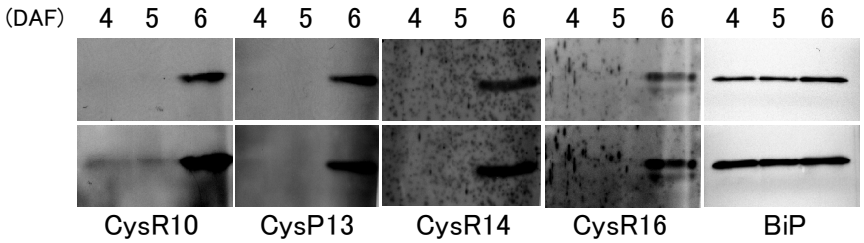
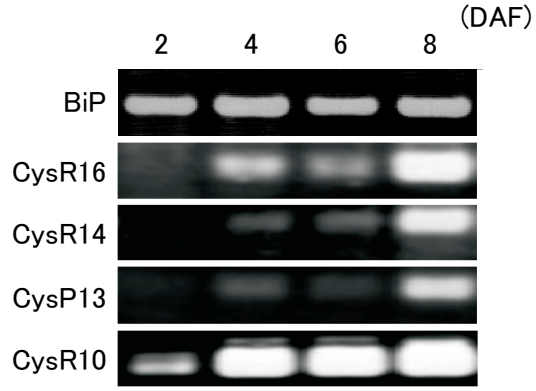


Supplemental Fig. 1. Immunoblot analysis of CysR10, CysP13, CysR14 and CysR16 between 4 to 6 DAF. The sample volume loaded on the SDS-PAGE gel was about 3-fold greater than that analyzed in Fig. 3. Bottom images are longer exposures than the upper images. BiP, whose levels remain constant per mg of seed, was used as an internal standard.

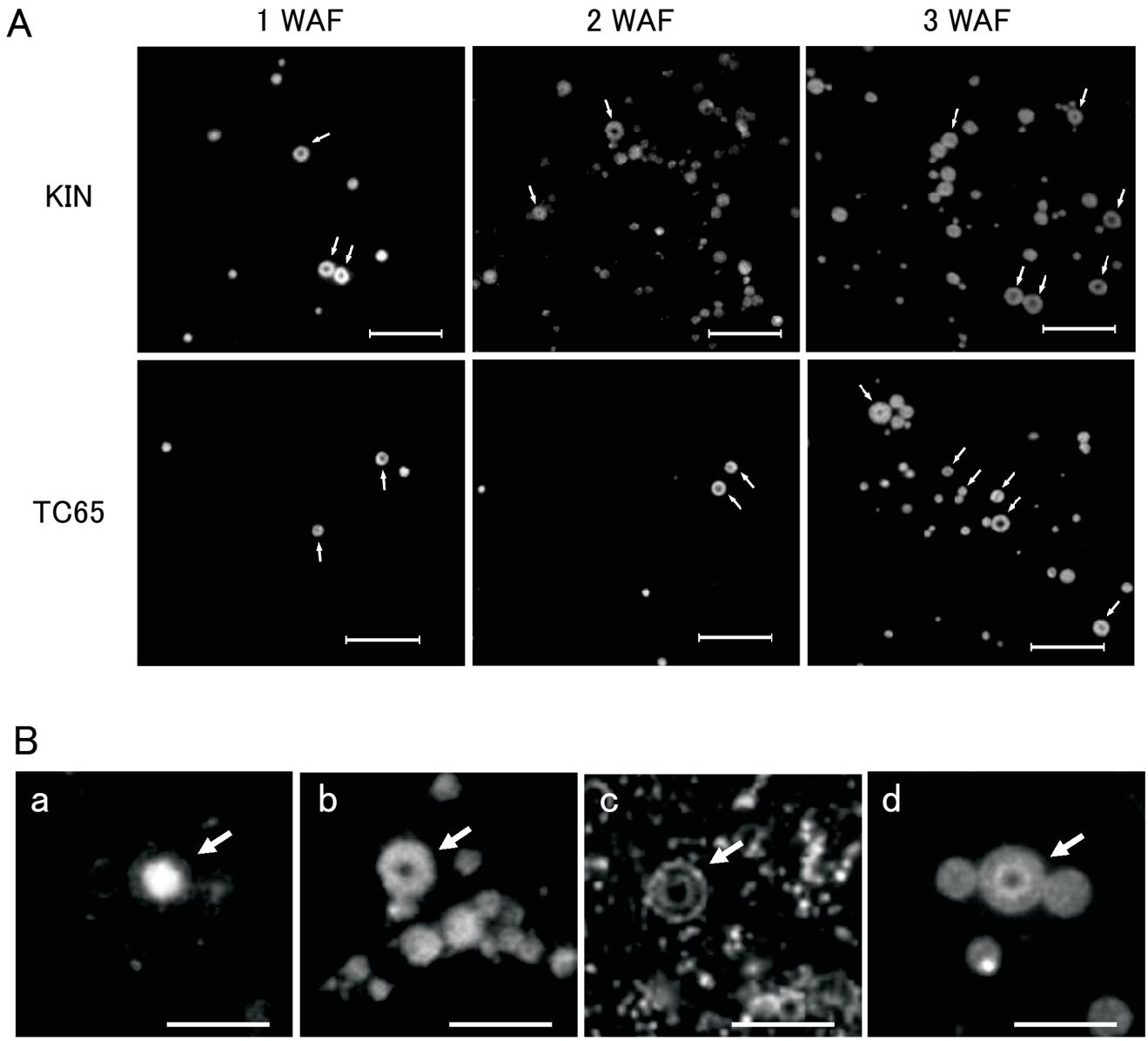
A



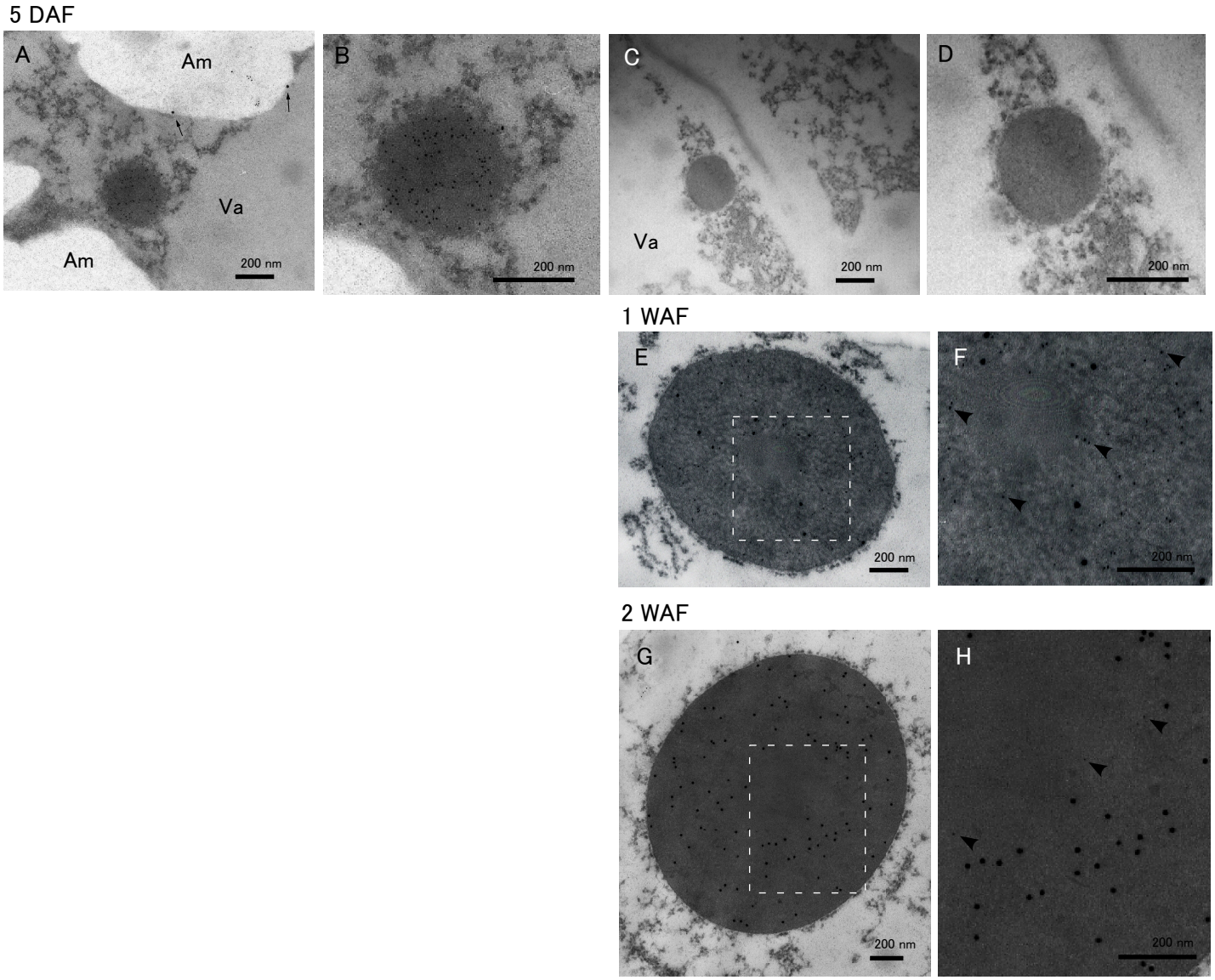
B

Clone name	Prolamin class	Accession number	Forward primer	Reverse primer	Amplicon length (bp)	Cycle number
λ RP10	10 kD prolamin	E09782	atggcagcatacaccagcaa	acaacaaccacaggaagaga	402	20
λ RP16	16 kD prolamin	AK107785	atgaagatctttgtcatcct	ccaagaaccgcaatgaccag	447	20
λ RM1	14 kD prolamin	AB016503	atgaagatcattttcgtatt	gtaccagacaccaccaacgg	471	20
λ RM4	13 kD prolamin	AB016504	atgaagatcattttcgtctt	caagacaccgccaagggtgg	450	20
BiP2		AF006825	cagctgtgaacccagagagg	tccttgctgatgtccttgct	592	25

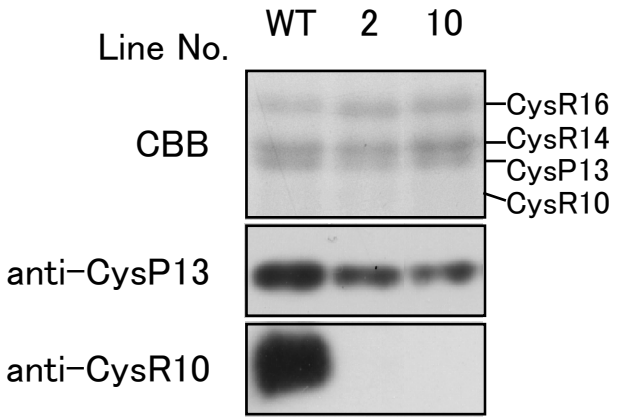
Supplemental Fig 2. **RT-PCR analyses of the steady state RNA levels for the various prolamin specie during early seed development.** Total RNA was extracted from developing seeds at 2, 4, 6 and 8 DAF using the RNeasy Plant Mini Kit (QIAGEN). Reverse transcription was performed using Ready-To-Go You-Prime First-Strand Beads (GE Healthcare Bioscience). The resulting cDNA samples were then analyzed by PCR using prolamin-specific primers shown in (B) using 20 cycles at 94 ℃ for 30 sec, 60 ℃ for 30 sec and 72 ℃ for 2 min. For an internal control, the same cDNA samples were analyzed by PCR using BiP-specific primers for 25 cycles at 94 ℃ for 30 sec, 60 ℃ for 30 sec, 72 ℃ for 2 min. PCR products were electrophoresed on agarose gels and visualized by staining with ethidium bromide. (B) The list of primers used for RT-PCR gene expression analysis. The PCR products were confirmed by sequencing.



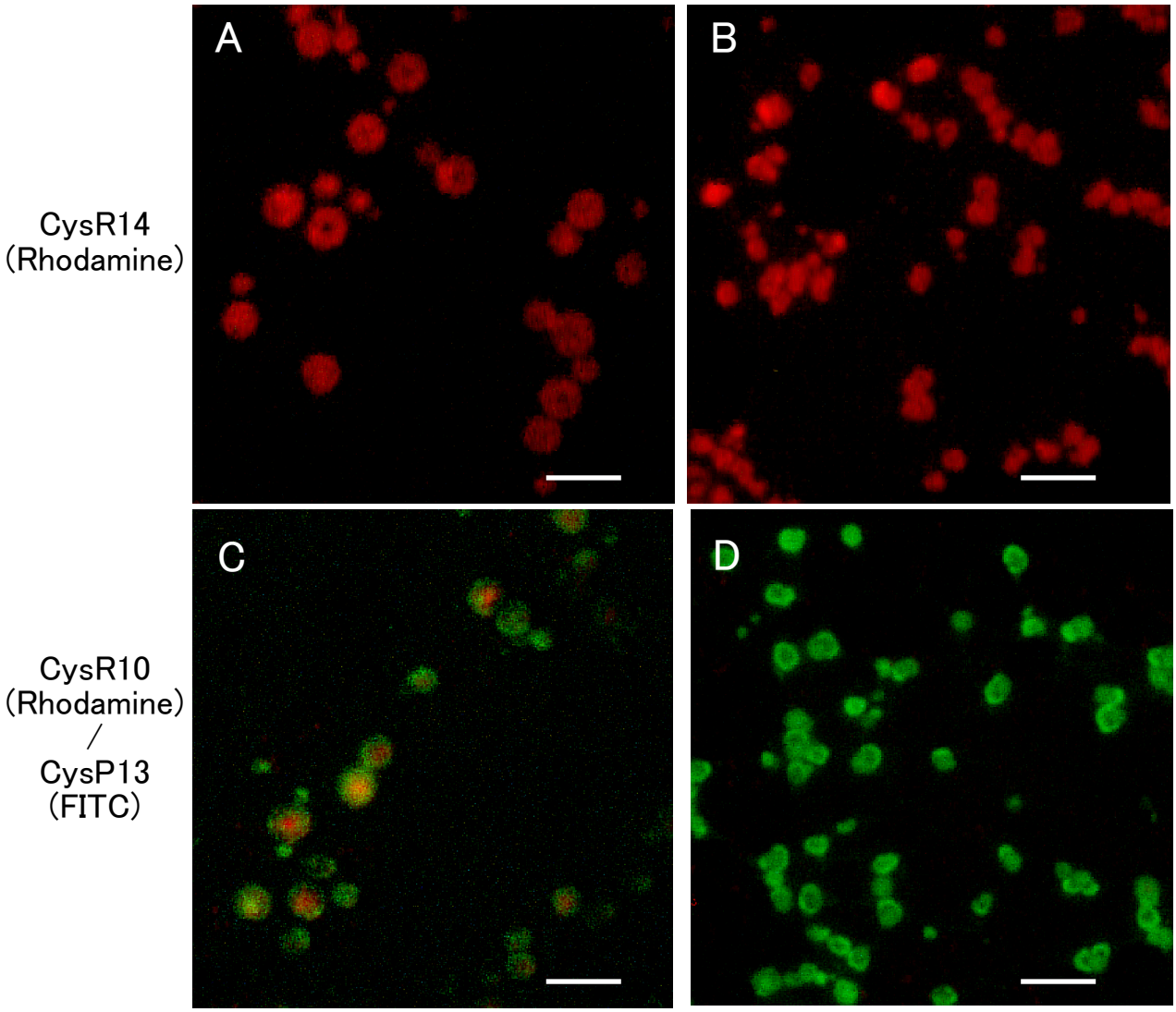
Supplemental Fig. 3. **The distribution of CysR14 in PB-I during seed development.**
(A) CysR14-containing PBs were visualized with anti-CysR14 and rhodamine-labeled secondary antibodies. The top images depict PBs from the endosperm of the rice japonica variety Kinmaze (KIN) while the bottom images show PBs from the endosperm of the rice japonica variety Taichung 65 (TC65). The CysR14 prolamins are distributed as a doughnut structure in many PBs (arrows) indicating that they surround the center core containing CysR10. Bars: 10 μ m.
(B) The distribution of CysR10 (a), CysR14 (b), CysR16 (c) and CysP13 (d) in PB-I from the endosperm of Kinmaze at 3 WAF. Sections were incubated with antibodies against the four prolamin types and then visualized with rhodamine-labeled secondary antibodies. Bars: 5 μ m.



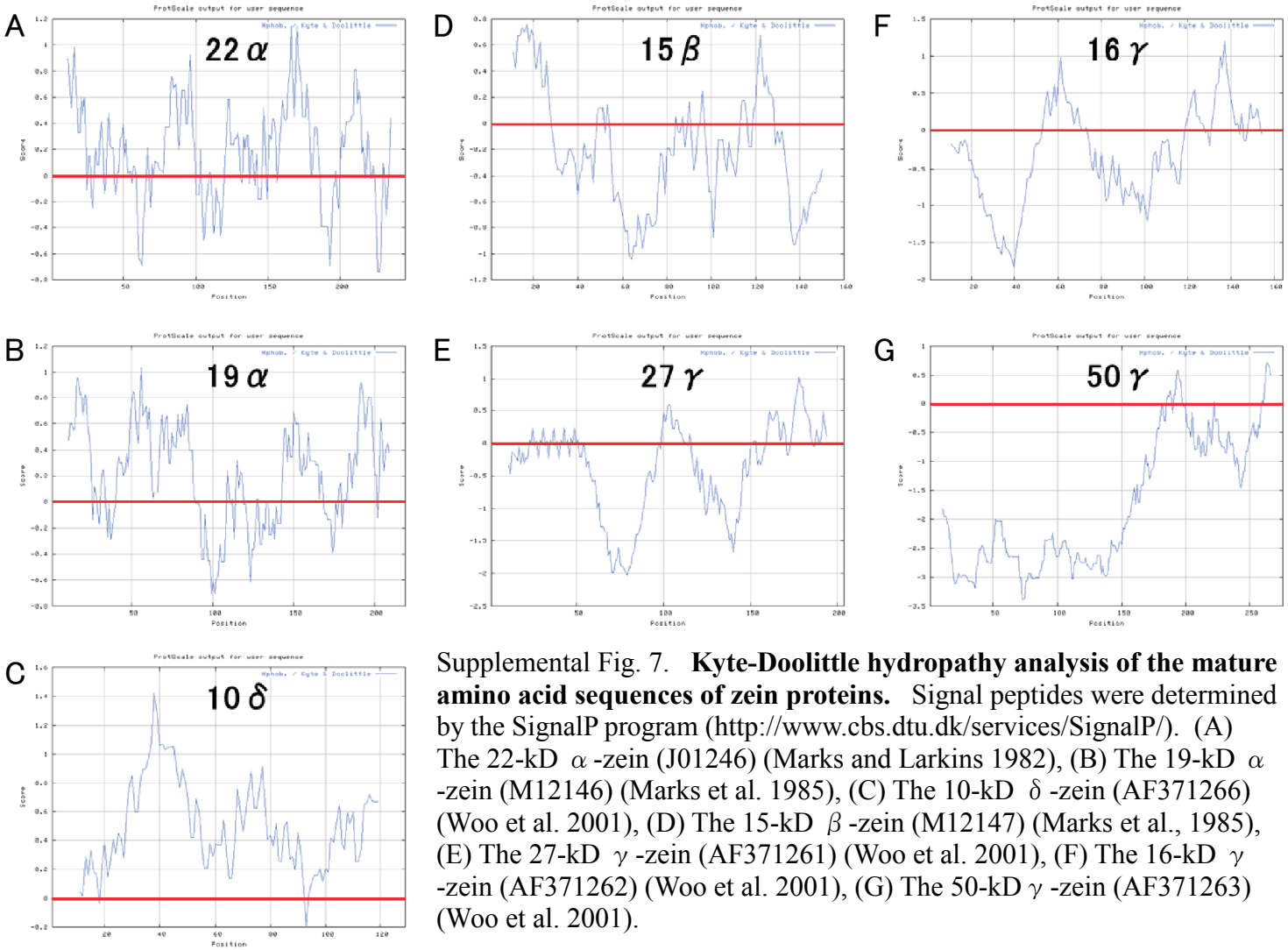
Supplemental Fig. 4. **The distribution of CysR14 in PB-I as viewed by immunoelectron microscopy.**
In panels (A, B), endosperm sections at 5 DAF were incubated with anti-CysR10 and anti-CysP13 antibodies followed by treatment with secondary antibodies which were conjugated with 5 nm and 15 nm gold particle, respectively. In panels (C-F), 1 WAF endosperm sections were incubated with anti-CysR14 (5 nm gold particles) and anti-CysP13 antibodies (15 nm gold particles). In panels (G, H), 2 WAF endosperm sections were incubated with anti-CysR14 (15 nm gold particle) and anti-CysP13 (5 nm gold particles) antibodies. (B, D, F and H) were enlarged images of the left panels. The high magnifying images of (F, H) are enlarged images of the areas denoted by the broken squares in (E, G), respectively. For particle size comparison, arrows in A show the 15 nm gold particle which were non-specifically attached. Arrow heads in the high magnifying images (F, H) show the 5 nm gold particles. Am: amyloplast, Va: vacuole. Bars: 200 nm.



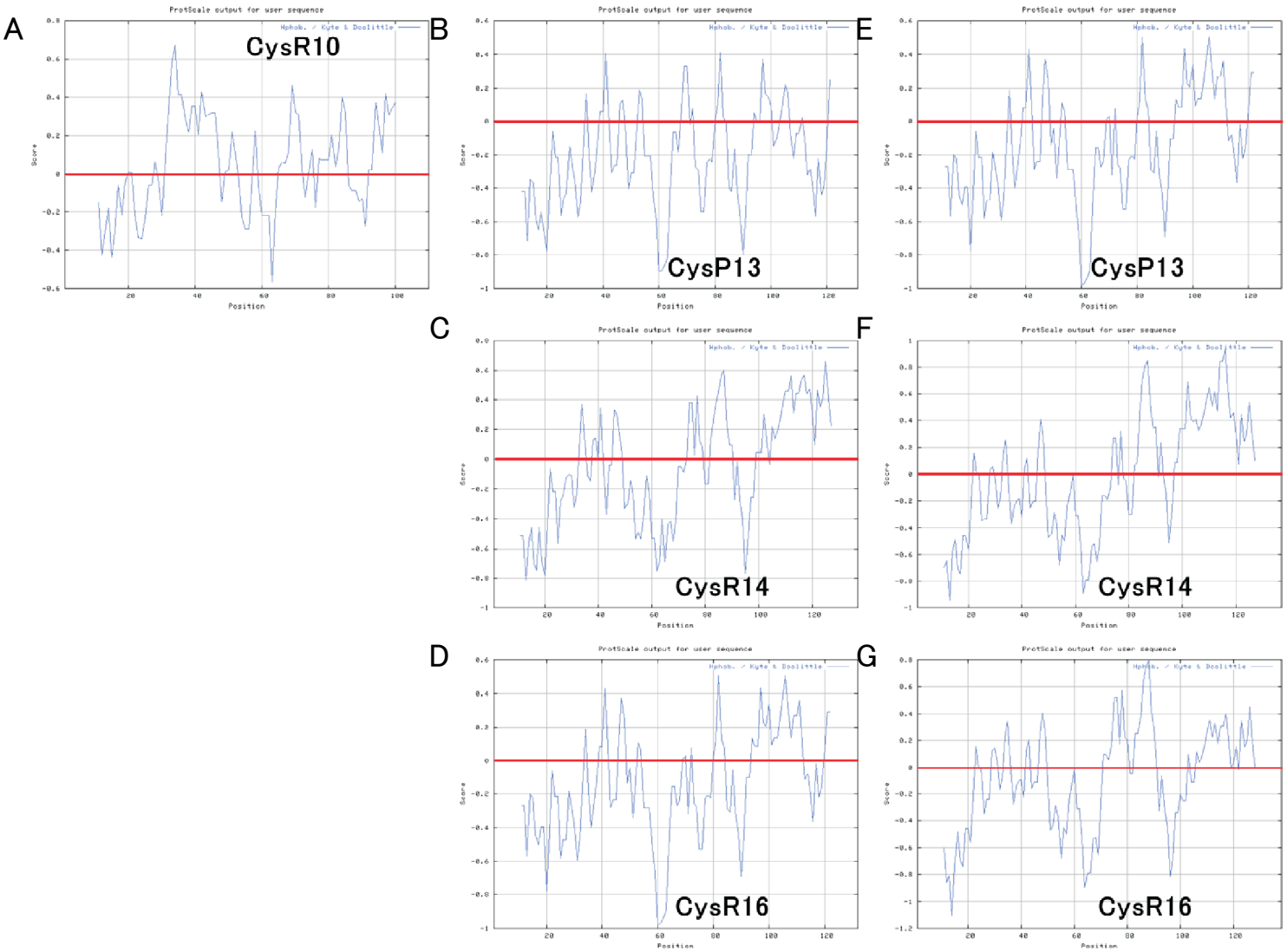
Supplemental Fig. 5. **Immunoblot analysis of CysR10 and CysP13 in seeds from wildtype (WT) and CysR10-repressed lines.**
The upper panel is a Coomassie blue stain polyacrylamide gel of WT (Yukihikari cultivar) and CysR10-repressed lines 2 and 10.
Note the absence of CysR10 and the reduction in CysP13 in the two CysR10-repressed lines.



Supplemental Figure 6. **Immunofluorescence microscopy of CysR10-repressed endosperm at 3 WAF.** Endosperm sections from wildtype (Yukihikari) (A) and from the CysR10-RNAi line (B) were incubated with anti-CysR14 antibody and reactive antigens visualized with rhodamine-conjugated secondary antibodies. The localization of CysR14 is similar to other wildtype cultivars, Kinmaze and Taichung 65 (See Supplemental Figure 3).
The PBs from Yukihikari endosperm (C) or CysR10-repressed line (D) were incubated with anti-CysR10 and anti-CysP13antibodies, and the reactive antigens then visualized with rhodamine-conjugated secondary antibodies and FITC-conjugated secondary antibodies, respectively. Bars: 10 μ m.



Supplemental Fig. 7. **Kyte-Doolittle hydropathy analysis of the mature amino acid sequences of zein proteins.** Signal peptides were determined by the SignalP program (http://www.cbs.dtu.dk/services/SignalP/). (A) The 22-kD α -zein (J01246) (Marks and Larkins 1982), (B) The 19-kD α -zein (M12146) (Marks et al. 1985), (C) The 10-kD δ -zein (AF371266) (Woo et al. 2001), (D) The 15-kD β -zein (M12147) (Marks et al., 1985), (E) The 27-kD γ -zein (AF371261) (Woo et al. 2001), (F) The 16-kD γ -zein (AF371262) (Woo et al. 2001), (G) The 50-kD γ -zein (AF371263) (Woo et al. 2001).



Supplemental Fig. 8. **Kyte-Doolittle hydropathy analysis of the mature amino acid sequences of rice prolamin proteins.** Signal peptides were determined by the SignalP program (http://www.cbs.dtu.dk/services/SignalP/). (A) λ RP10 (X15231) (Masumura et al. 1989), (B) λ RM4 (AB016504) (Mitsukawa et al. 1999a), (C) λ RM9 (AB016505) (Mitsukawa et al. 1999a), (D) λ RP16 (D88210) (Mitsukawa et al. 1999b), (E) λ RM2 (D11385) (Yamagata et al. 1992), (F) λ RM1 (AB016503) (Mitsukawa et al. 1999a), (G) λ RM7 (X14392) (Masumura et al. 1990).