The 13 kDa Prolamin Fused to Green Fluorescent Protein Accumulates Not Only in the Seeds But Also in the Vegetative Tissues of Transgenic Rice

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Introduction

- Plant seeds contain a large amount of storage proteins which serves as a reserve of nitrogen and sulfur for the developing seedlings.
- Prolamins, one group of the major storage proteins in rice endosperm, are synthesized on endoplasmic reticulum (ER) membrane, and stored in spherical intracisternal inclusion granules, referred to as protein body type I (PB-I), within ER lumen (Fig. 1).
- The formation of PB-Is is a complex process involving a number of regulatory events; (1) specific targeting of mRNAs encoding prolamin to the PB-Is, (2) interactions of prolamin molecules among themselves, (3) interaction of prolamins with ER-resident molecular chaperones.

To investigate whether accumulation of prolamin in ER and formation of the PB-I would be a seed-specific event, we examined the subcellular localization of the ectopically expressed 13 kDa prolamin fused to green fluorescent protein (GFP) in seeds, leaves and roots of transgenic rice plants.

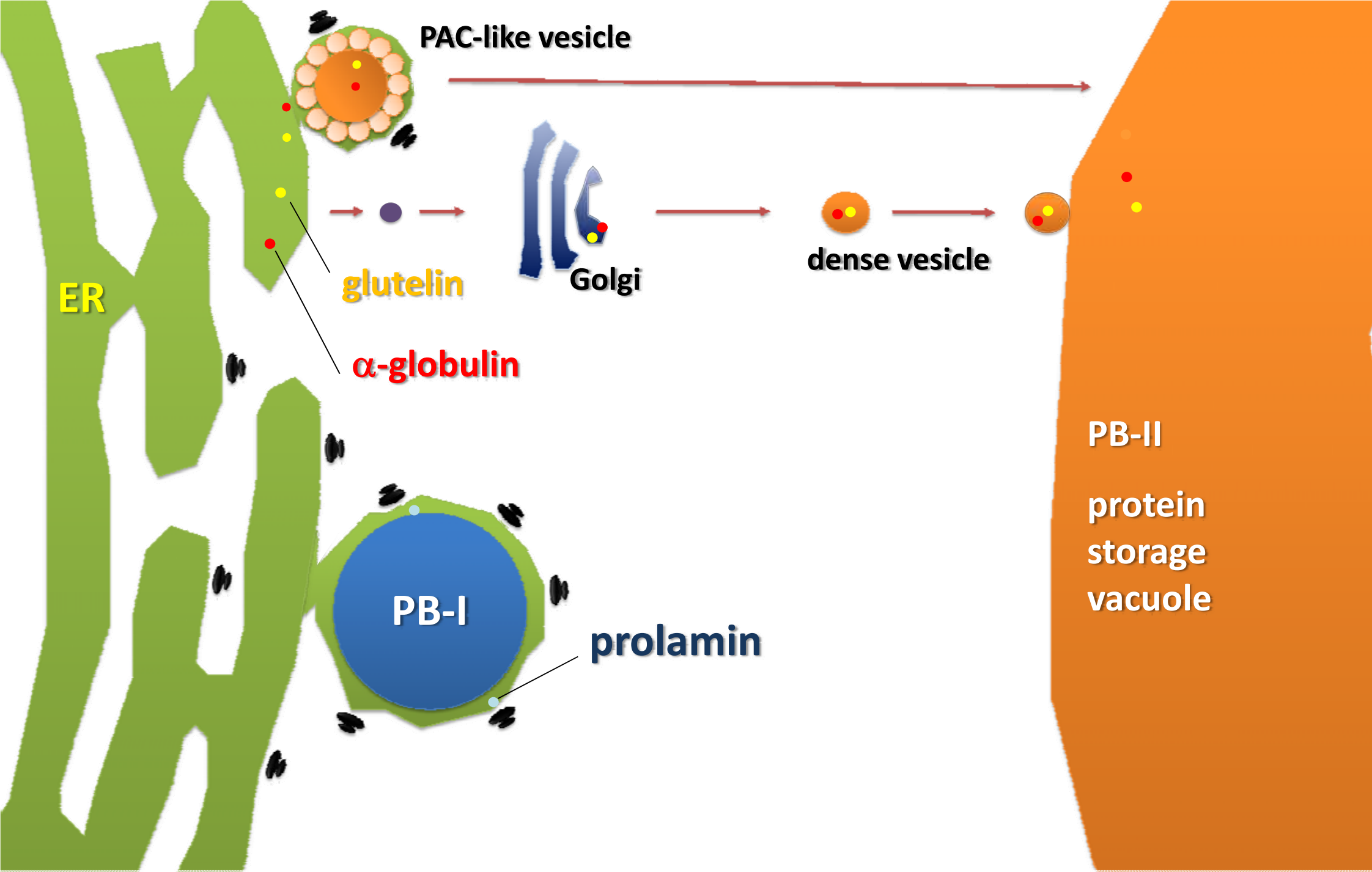

Figure 1. Accumulation of seed storage proteins in rice endosperm cell.

Materials & Methods

- The binary vectors expressing a RM1-GFP fusion protein (Fig. 2) were introduced into rice plants (*Oryza sativa* L. cv Nipponbare), using an *Agrobacterium*-mediated method described previously (Masumura *et al.*, 2006).

- We studied the expression of transgene by RT-PCR, western blot and fluorescence microscopy and immunoelectron microscopy in the trangenic rice plants.

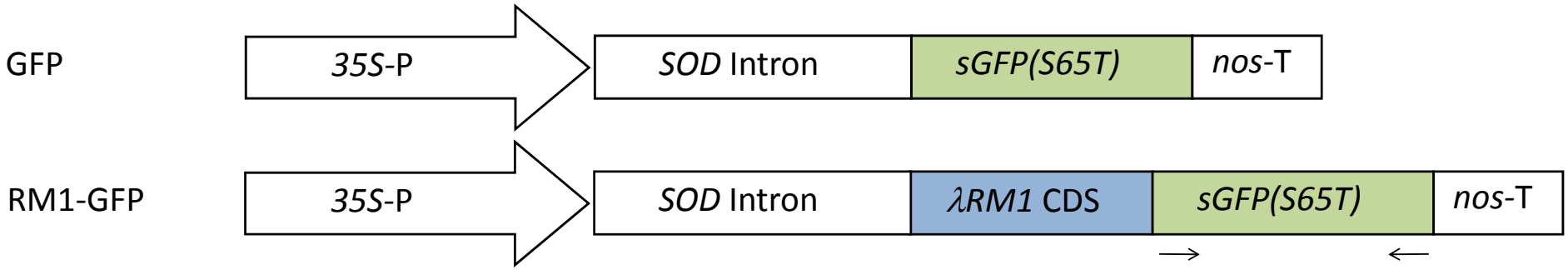

Figure 2. RM1-GFP and GFP constructs expressed in transgenic rice plants.
35S-P, 35S promoter of cauliflower mosaic virus; *SOD* Intron, the first intron of rice *sodCc2*; *λRM1* CDS, the coding sequence for 13 kDa prolamin gene; s*GFP(S65T)*, modified green fluorescent protein gene; *nos*-T, terminator of nopaline synthase gene. The horizontal arrows indicate RT-PCR primers.

Masumura T, Morita S, Miki Y, kurita A, Morita S, Shirono H, Koga J and Tanaka K. (2006) Production of biologically active human interferon-α in transgenic rice. *Plant Biotech*. 23, 91-97.

Results

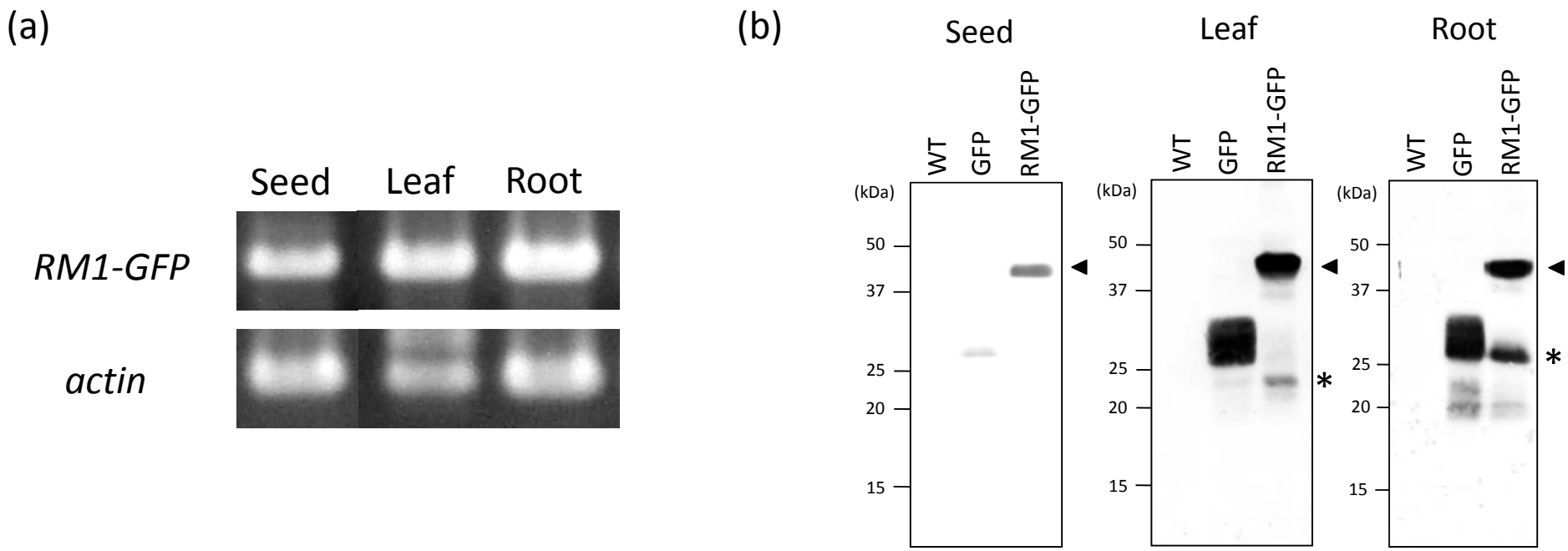

Figure 3. The mRNA expression and protein accumulation of RM1-GFP in transgenic rice plants.
(a) RT-PCR analysis of transcripts in seeds, leaves and roots of RM1-GFP plants. (b) Immunoblot analysis of mature seeds, leaves and roots from WT, GFP and RM1-GFP plants with anti-GFP antibodies. The upper bands (arrowheads) show RM1-GFP fusion proteins. The lower bands (asterisks) are putative processed products of RM1-GFP proteins.

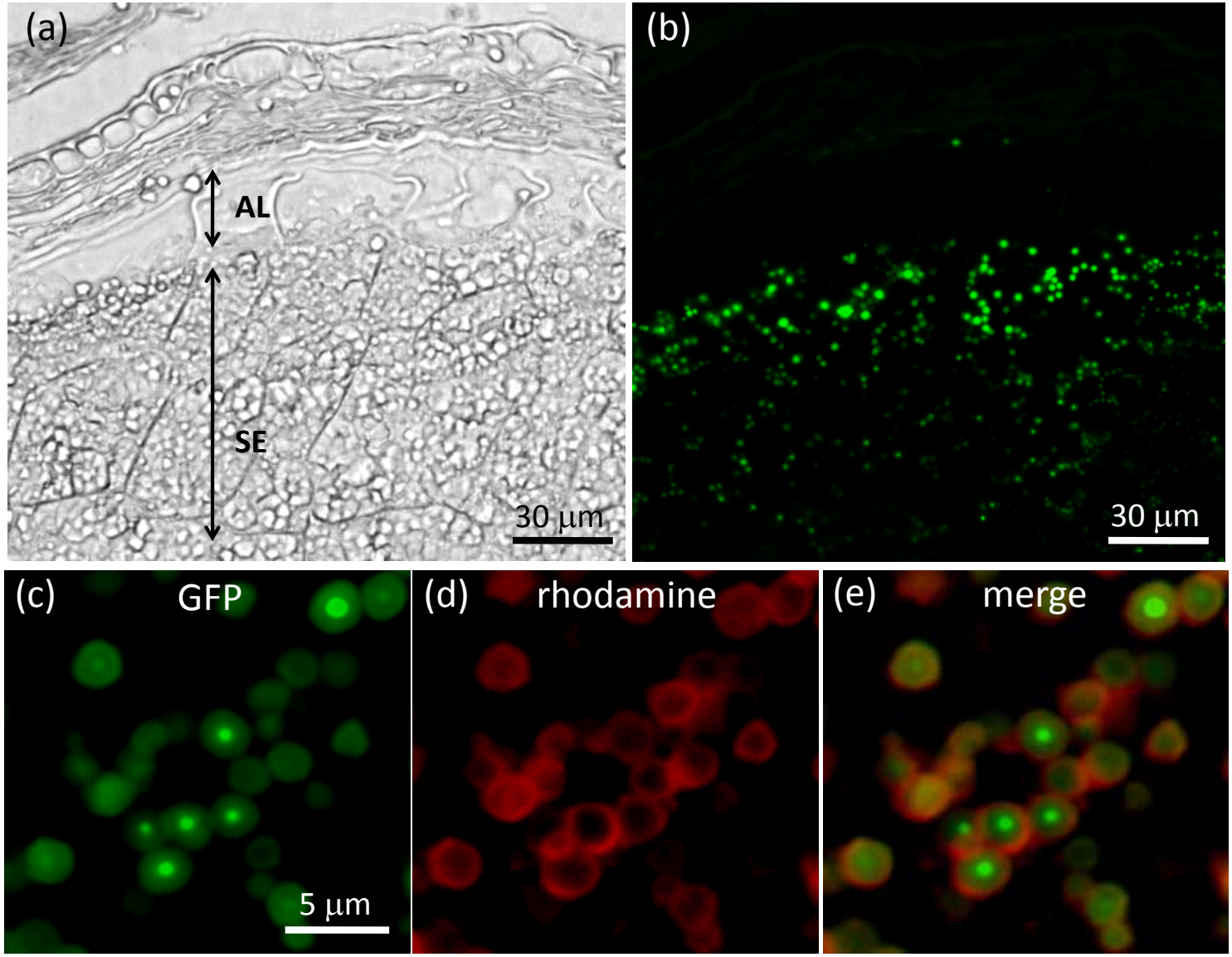

Figure 4. Fluorescence images of endosperm cells of RM1-GFP plants.

(a,b) Light transmission (a) and fluorescence (b) images of mature seed section of RM1-GFP plant using fluorescence microscope. AL, aleurone cells; SE, starchy endosperm cells. (c-e) Mature seed section of RM1-GFP plant was stained with rhodamine B, which specifically binds prolamins in PB-I. (c) and (d) indicates GFP fluorescence (green) and PB-Is (red), respectively. (e) is merged image of (c) and (d).

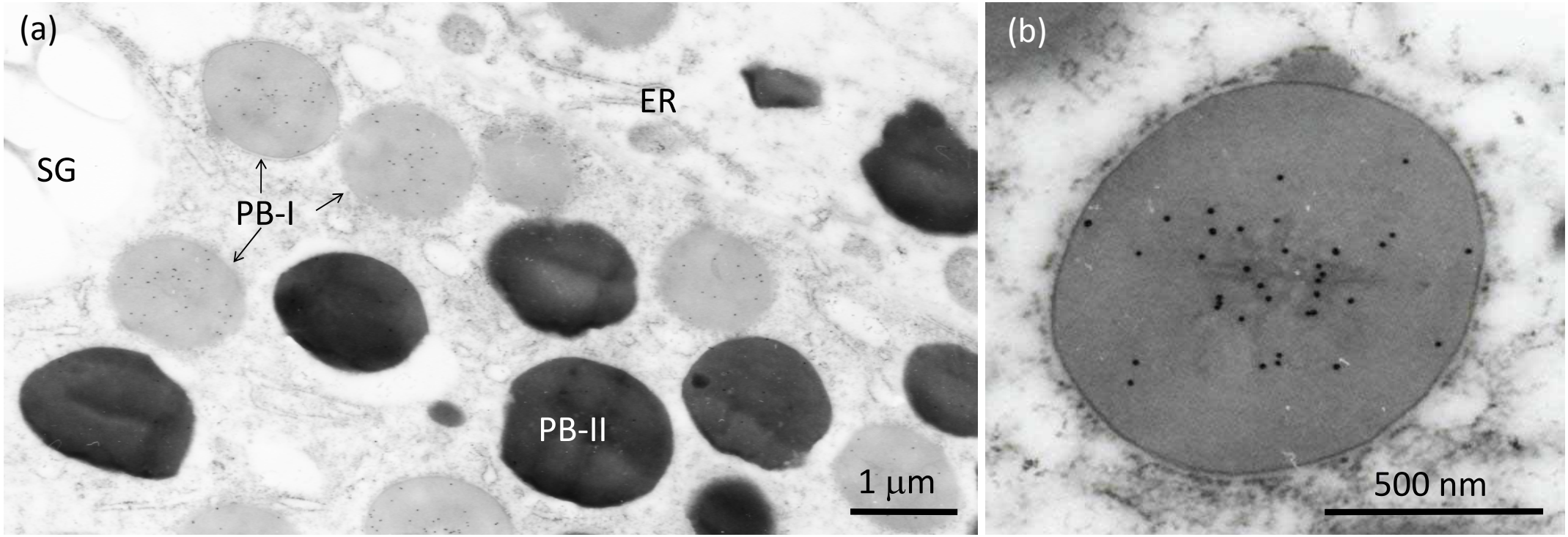

Figure 5. Accumulation of RM1-GFP proteins in PB-I of starchy endosperm cells.

(a) Immunoelectron micrographs of starchy endosperm cells of RM1-GFP plants at 12 days after flowering detected with anti-GFP antibody, showing that RM1-GFP proteins were localized in PB-I (arrows). SG, starch granule; ER, endoplasmic reticulum; PB-I, protein body type I; PB-II, protein body type II. (b) Magnified image of PB-I.

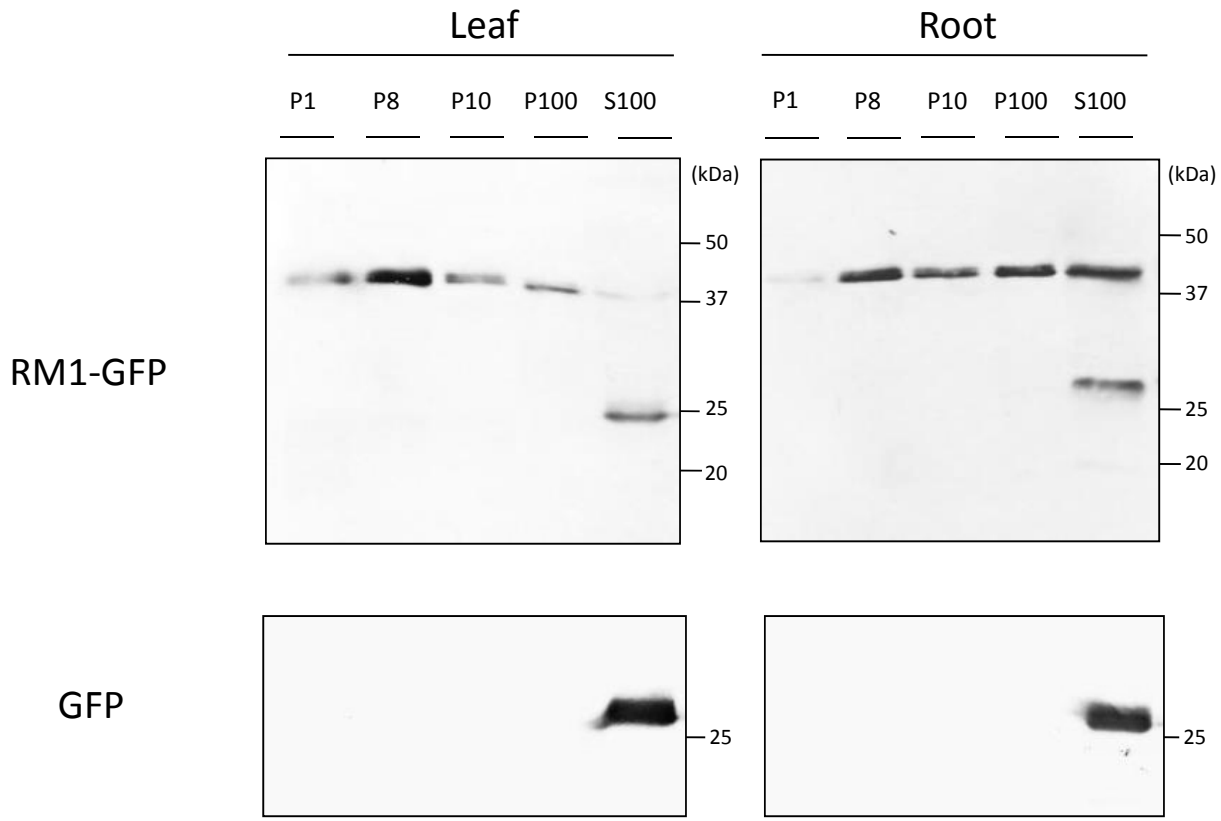

Figure 6. Subcellular distribution of RM1-GFP proteins in leaves and roots.

Homogenates from leaves and roots of RM1-GFP plants and GFP plants were subjected to differential centrifugation to obtain the 1000*g* pellet (P1), the 8000x*g* pellet (P8), the 10,000x*g* pellet (P10), the 100,000x*g* pellet (P100) and the 100,000x*g* supernatant (S100). Each fraction was subjected to immunoblotting with anti-GFP antibody.

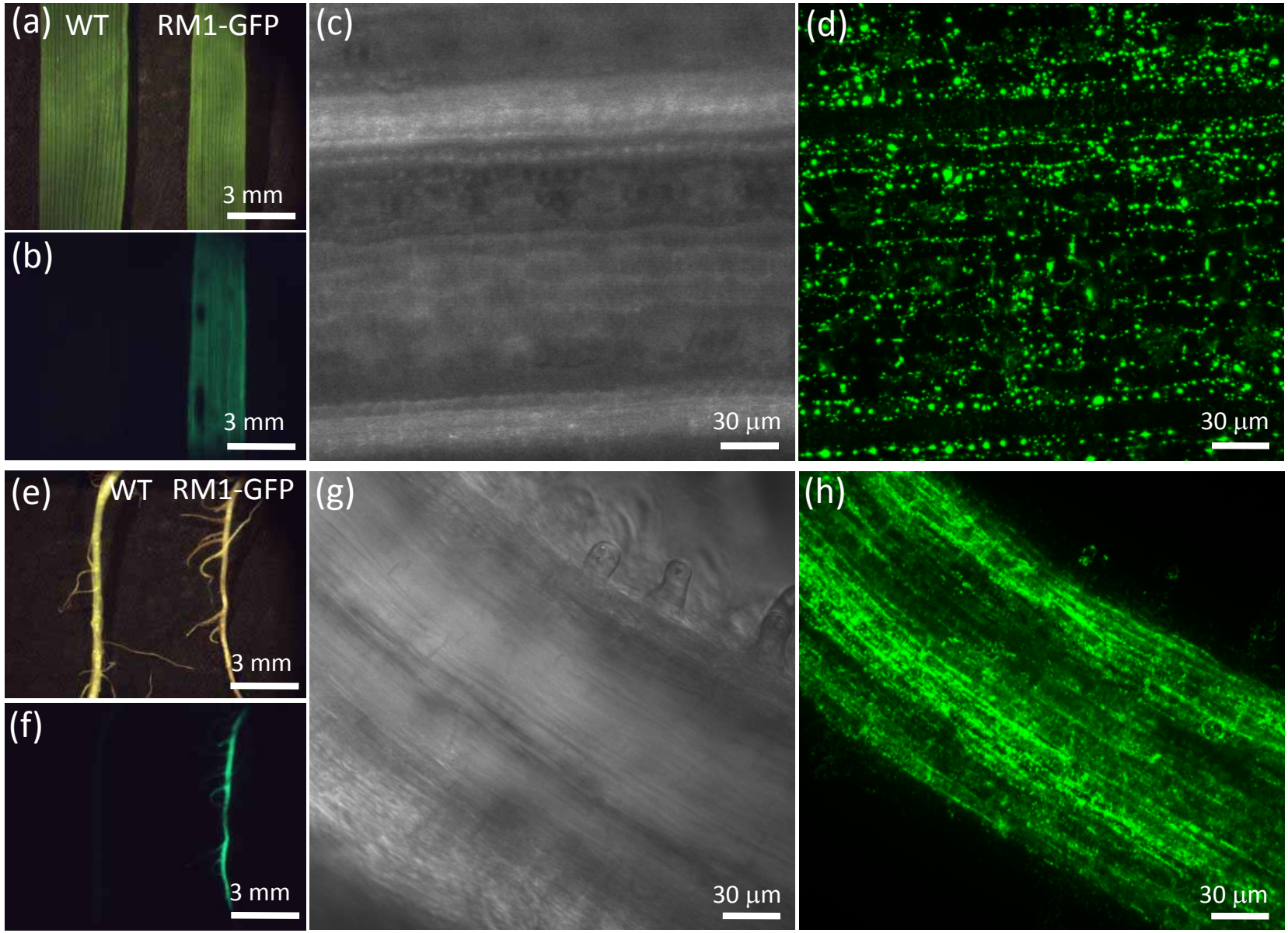

Figure 7. Fluorescence images of leaves and roots of RM1-GFP plants.

Light transmission (a,c,e,g) and fluorescence (b,d,f,h) images of leaves (a-d) and roots (e-h) of RM1-GFP plants using fluorescent stereomicroscope (a,b,e,f) and confocal laser scanning microscope (c,d,g,h).

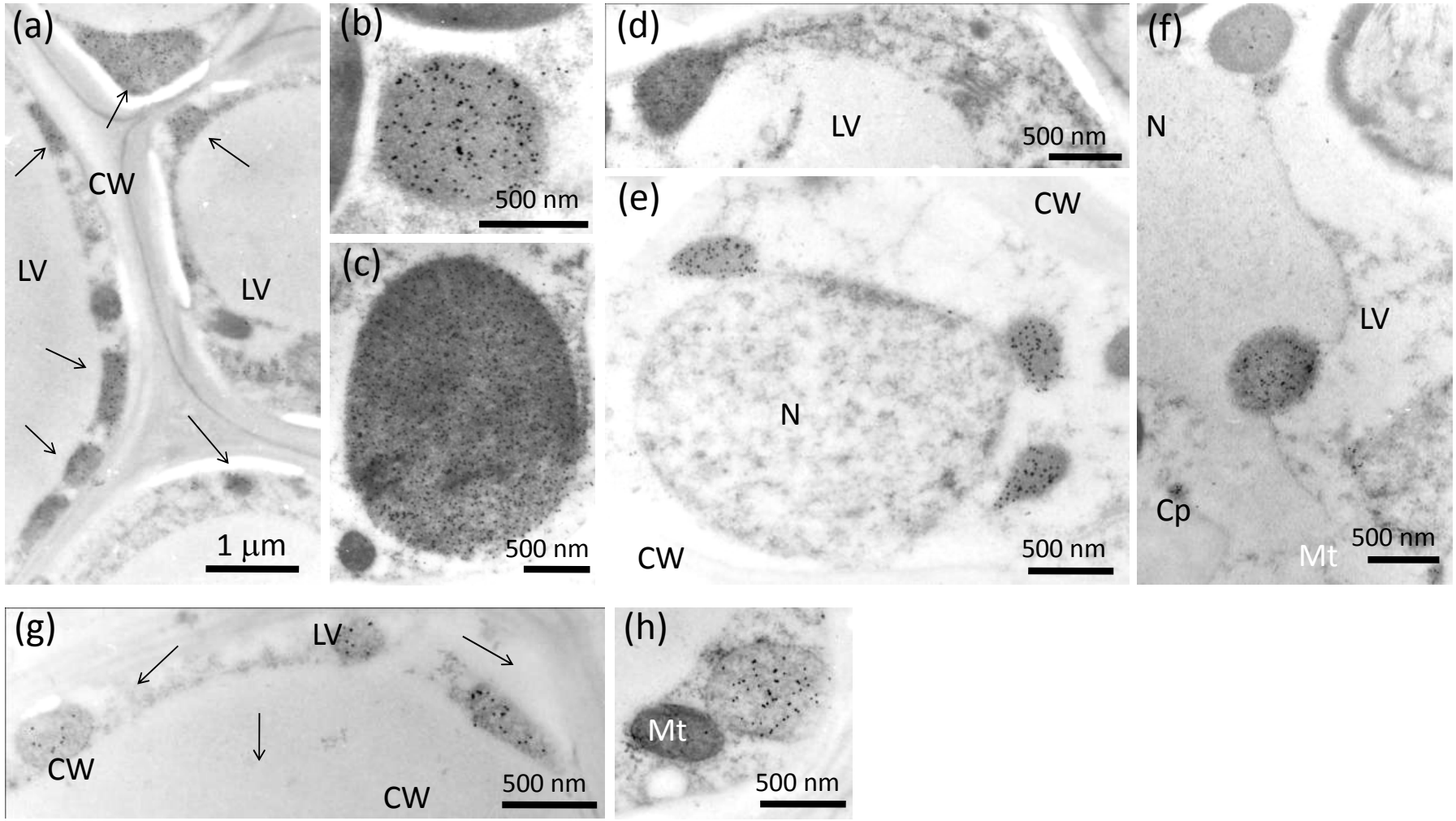

Figure 8. Accumulation of RM1-GFP proteins in PB-like structures of leaf and root cells.

Immunoelectron micrographs of high-pressure frozen/freeze-substituted leaf cells (a-f) and root cells (g,h) of RM1-GFP plants with anti-GFP antibody. Numerous PB-like structures (arrows) are visible in the leaf and root cells. Examination of several leaf and root sections of WT and GFP plants showed that these organelles labeled with anti-GFP antibodies were not present in untransfomed plants and *GFP* plants (data not shown). LV, lytic vacuole; CW, cell wall; N, nucleus; Cp, chloroplast; Mt, mitochondrion.

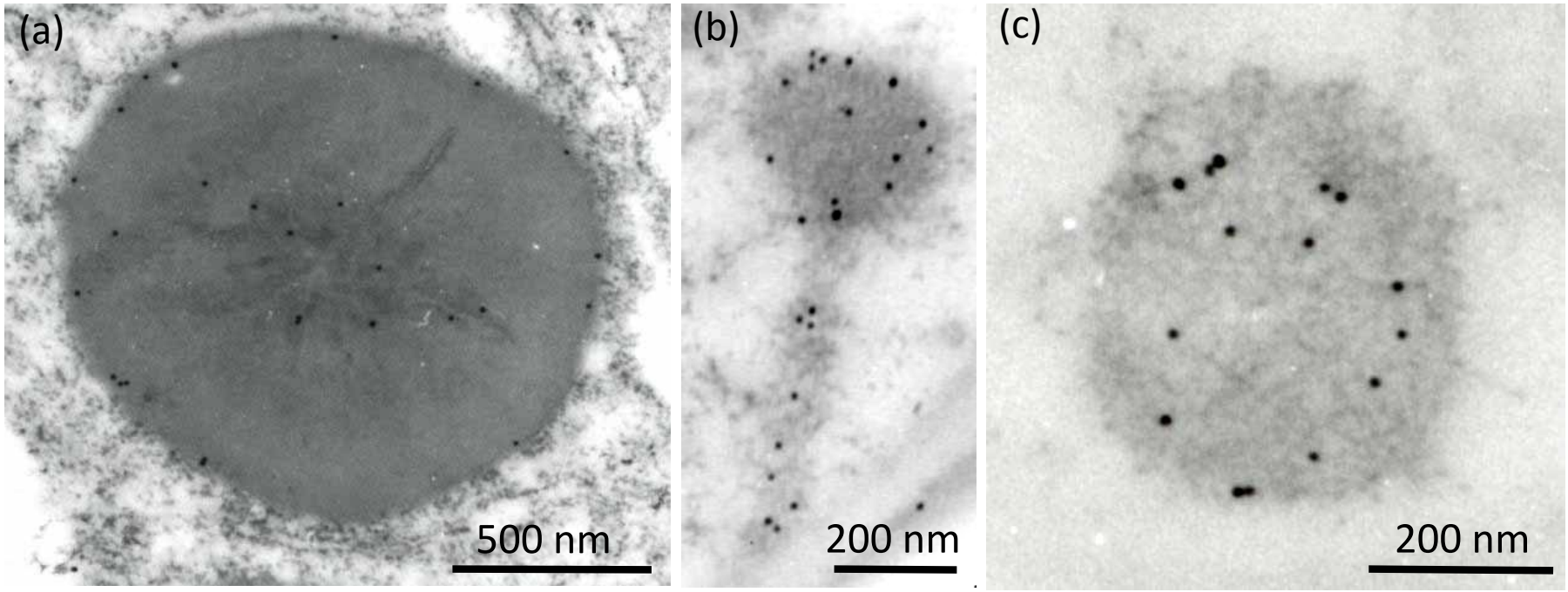

Figure 9. Association of an ER-resident molecular chaperone, BiP, with PB-I and PB-like structures.

Immunoelectron micrographs of PB-I in starchy endosperm cell of seed (a) and PB-like structures in leaf cell (b) and root cell (c) of RM1-GFP plants with anti-pumkin BiP antibody.

Conclusion

- RM1-GFP fusion proteins accumulated not only in the seeds but also in the leaves and roots of the transgenic rice plants.
- RM1-GFP fusion proteins accumulated in PB-I in the endosperm cells of RM1-GFP rice seeds, whereas the most of the fusion proteins accumulated in ER in the leaf and root cells.
- The accumulation of RM1-GFP fusion proteins induced the formation of protein granules in ER lumen which is similar to PB-I.

The accumulation of 13 kDa prolamin does not depend on tissues.

The coding sequence of 13 kDa prolamin contains the information for the accumulation in ER.

Abstract

Prolamins, one group of the rice seed storage proteins, are synthesized on rough endoplasmic reticulum (rER) and deposited in ER-derived protein body type I (PB-I). It is not fully understood how prolamins are retained within ER since they do not contain the well-characterized KDEL/HDEL ER retention motif. In order to elucidate whether prolamin would accumulate in vegetative tissues, transgenic rice plants constitutively expressing the 13 kDa prolamin fused to green fluorescent protein (RM1-GFP) were produced and the subcellular localization of RM1-GFP proteins in seeds, leaves and roots was investigated. RM1-GFP proteins accumulated not only in the seeds but also in the leaves and roots. The subcellular fractionation experiments showed that RM1-GFP protein accumulated in ER in the leaves and roots. Microscopic observation of GFP fluorescence and immunocytochemical analysis revealed that RM1-GFP proteins specifically accumulated in PB-I in the endosperm of the seeds, whereas they were present in PB-like structures in the leaves and roots. Our results show that the accumulation of 13 kDa prolamin does not depend on tissues, suggesting that the coding sequence of 13 kDa prolamin contains the information for accumulation in ER.