

CHARACTERIZATION OF THE RICE RNA BINDING PROTEIN OSTUDOR-SN

Tong Sunny Gao, Kelly Doroshenk, Thomas W. Okita

Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

Introduction

Optimal growth and development is dependent upon the strict coordination of gene expression events within the cell. The highly conserved RNA binding protein Tudor-SN is involved in multiple aspects of nuclear and cytoplasmic RNA processing and is found in most higher eukaryotes and microorganisms. The multi-functionality of this protein is likely due to its modular domain structure. In rice, *OsTudor-SN* plays a role in the cytoplasmic transport, localization, translation and/or stability of storage protein RNAs, which are specifically targeted to distinct subdomains of the endoplasmic reticulum resulting in asymmetric protein accumulation (Crofts et al., 2004; Figure 1). *OsTudor-SN* interacts with both prolamine and glutelin RNAs and co-localizes with prolamine RNA transport particles. Downregulation of *OsTudor-SN* by RNAi leads to a reduction in prolamine RNA and protein, as well as glutelin protein (Wang et al., 2008). We sought to investigate the specific functions of *OsTudor-SN* by characterizing three genetic mutants with distinct domain mutations: EM1084, TCM1239 and PMT64. The effect of the mutations on *OsTudor-SN* protein expression in developing rice seed was assessed, as was overall protein expression patterns. In addition, storage protein gene expression was investigated. Our preliminary results suggest specific domains of Tudor-SN may be responsible for distinct activities during gene expression that are ultimately critical for optimal plant development.

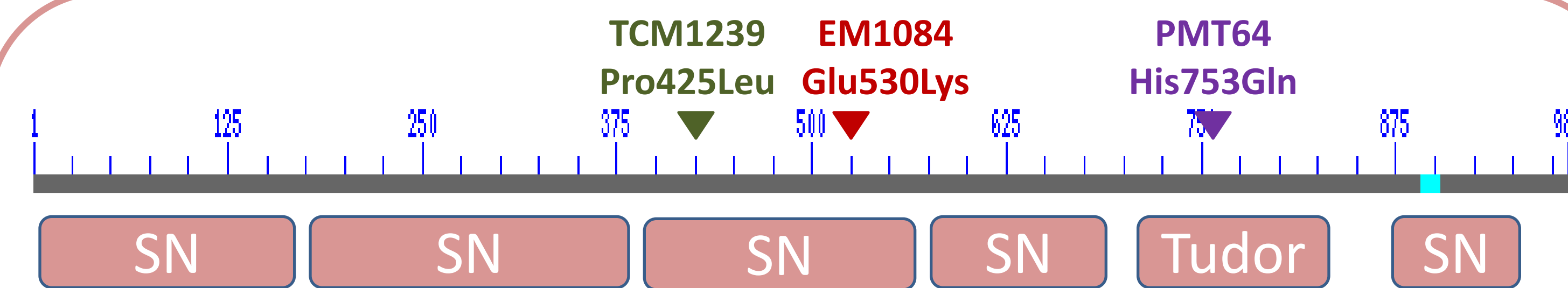


Figure 2. *OsTudorSN* domain structure and location of point mutations in genetic mutants. *OsTudor-SN* has 4 nucleic acid and protein binding Staphylococcus nuclease (SN) domains, a protein interacting Tudor domain, and a partial SN domain. This modular structure likely contributes to Tudor-SN's multi-functionality. Mutant lines EM1084 and TCM1239 contain independent mutations within the 3rd SN domain, while PMT64 has a mutation within the Tudor domain.

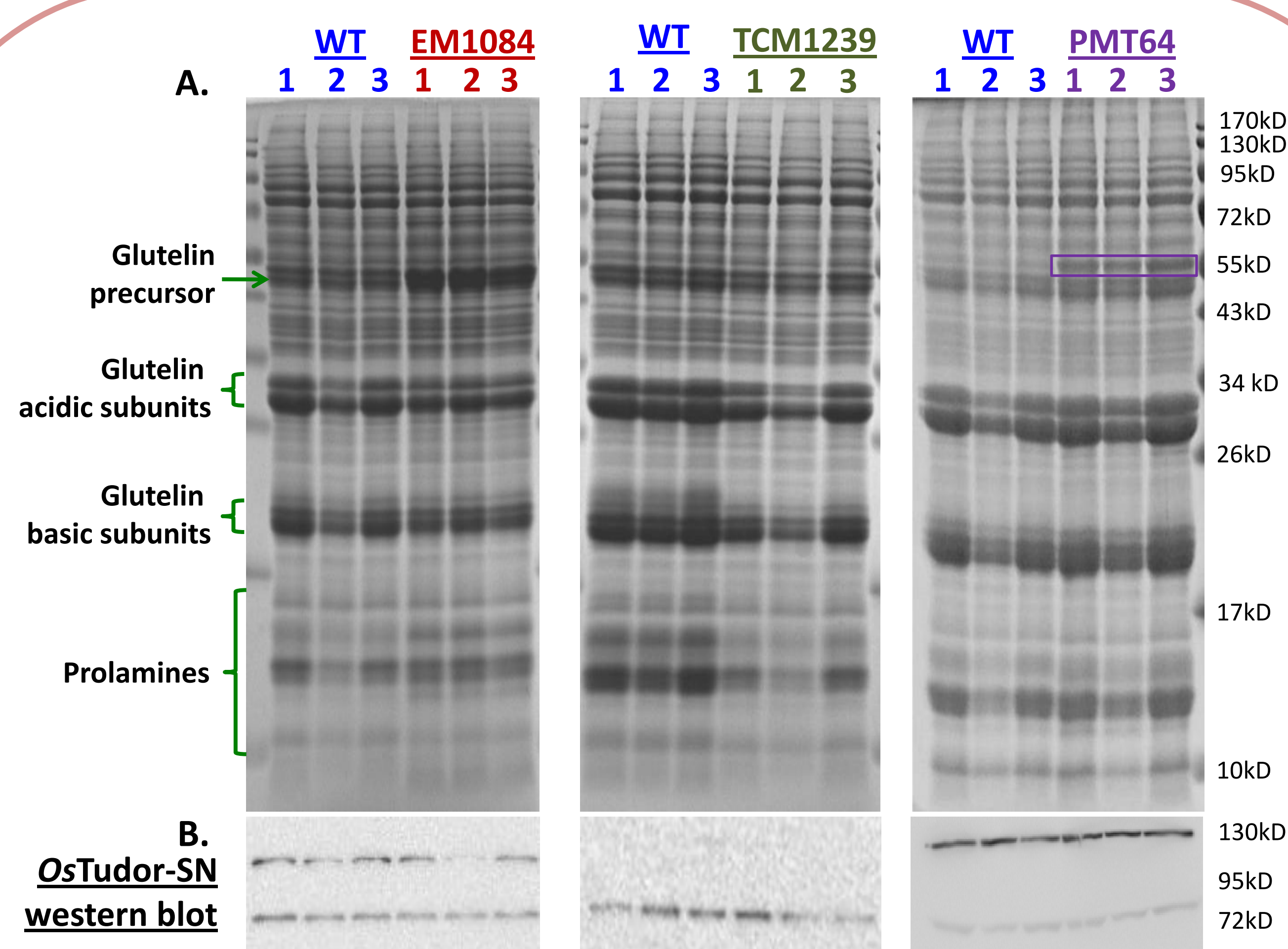


Figure 3. Analysis of protein expression profiles in wildtype (WT) and *OsTudor-SN* genetic mutants. Protein extracts from individual developing rice seeds (10-12 DAF) were made in triplicate and examined by SDS-PAGE. A: Coomassie blue stained SDS-PAGE gel. Compared to WT, EM1084 accumulates increased levels of glutelin precursor, which is normally processed into acidic and basic subunits after transport to PB-II. TCM1239 appears similar to WT, but may accumulate slightly less glutelin and prolamine. In PMT64, glutelin precursor levels are slightly elevated and a novel, highly accumulating 55kD protein is evident. B: Western blot using antibody to *OsTudor-SN*. The upper band is full length *OsTudor-SN*, while the lower is proteolytically truncated protein. All three mutants express similar amounts of *OsTudor-SN* compared to WT.

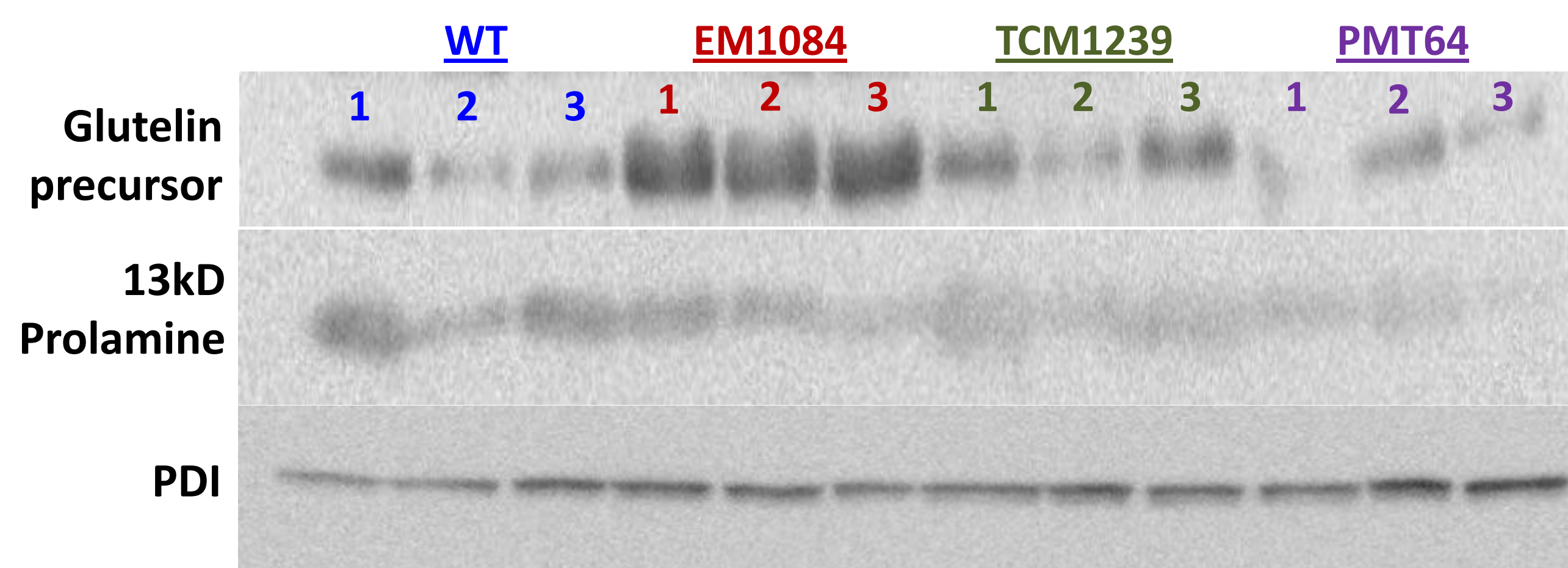


Figure 4. Western blot analysis of glutelin and prolamine protein expression in WT and *OsTudor-SN* genetic mutants using seed extracts described in Fig. 3. Compared to WT, EM1084 has higher amounts of glutelin precursor as in Fig. 3, while TCM1239 and PMT64 appear to have slightly less 13kD prolamine. PDI serves as a loading control.

References

Crofts AJ, Washida H, Okita TW, Ogawa M, Kumamaru T, Satoh H. 2004. *Plant Physiol*, 136:3414-3419.
Wang C, Washida H, Crofts AJ, Hamada S, Katsube-Tanaka T, Kim D, Choi SB, Modi M, Singh S, Okita TW. 2008. *Plant J*, 55:443-454.

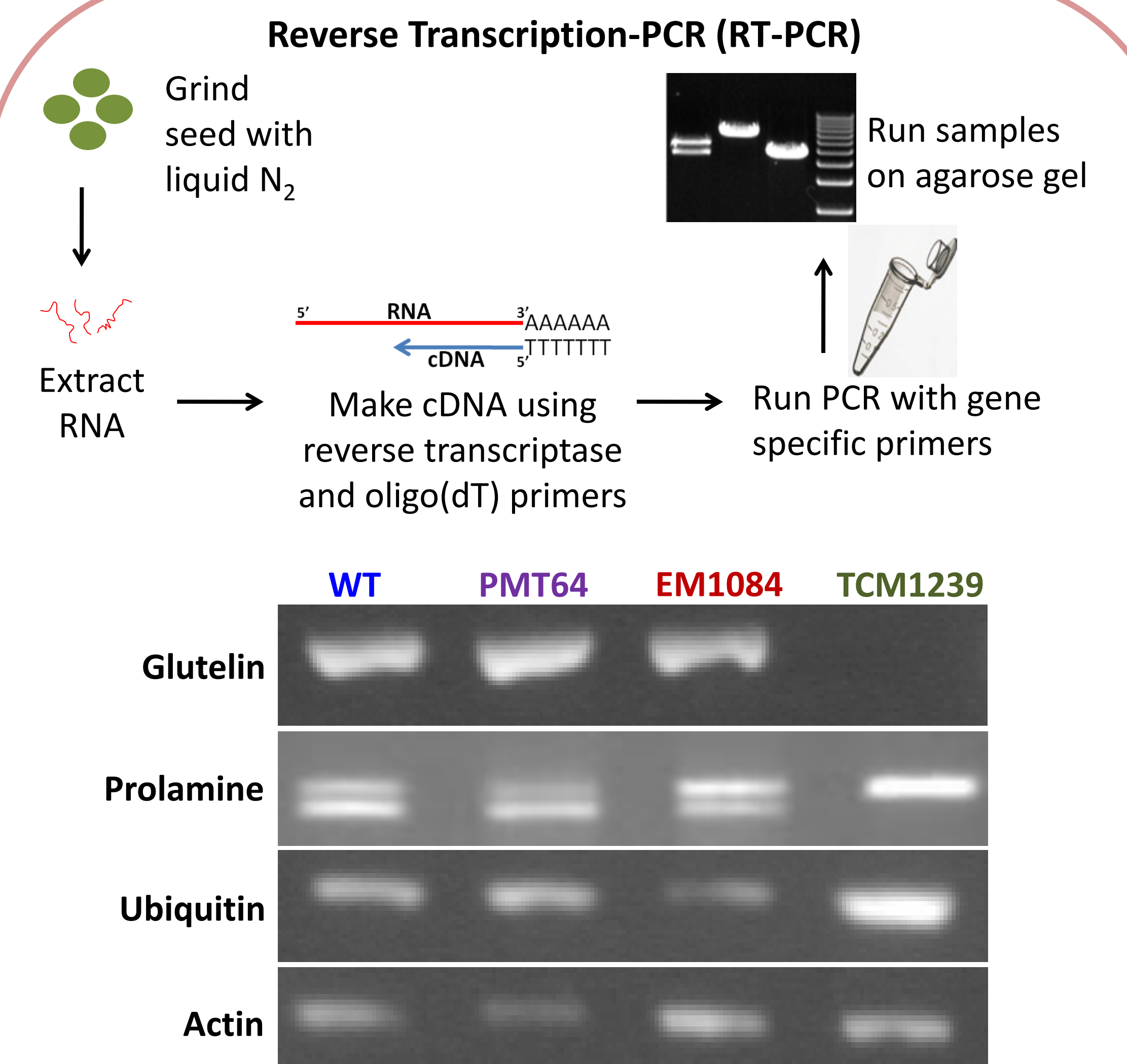


Figure 5. Analysis of prolamine and glutelin transcript levels in WT and *OsTudor-SN* genetic mutants. RNA extracted from 10 DAF seeds was subjected to reverse transcription PCR. The resulting cDNA and gene specific primers were used to assess transcript abundance. Compared to WT, PMT64 and EM1084 have similar amounts of glutelin transcript, while TCM1239 interestingly had no detectable glutelin. Prolamine transcript levels were also similar, although PMT64 may have slightly less. Ubiquitin and actin were used as positive loading controls.

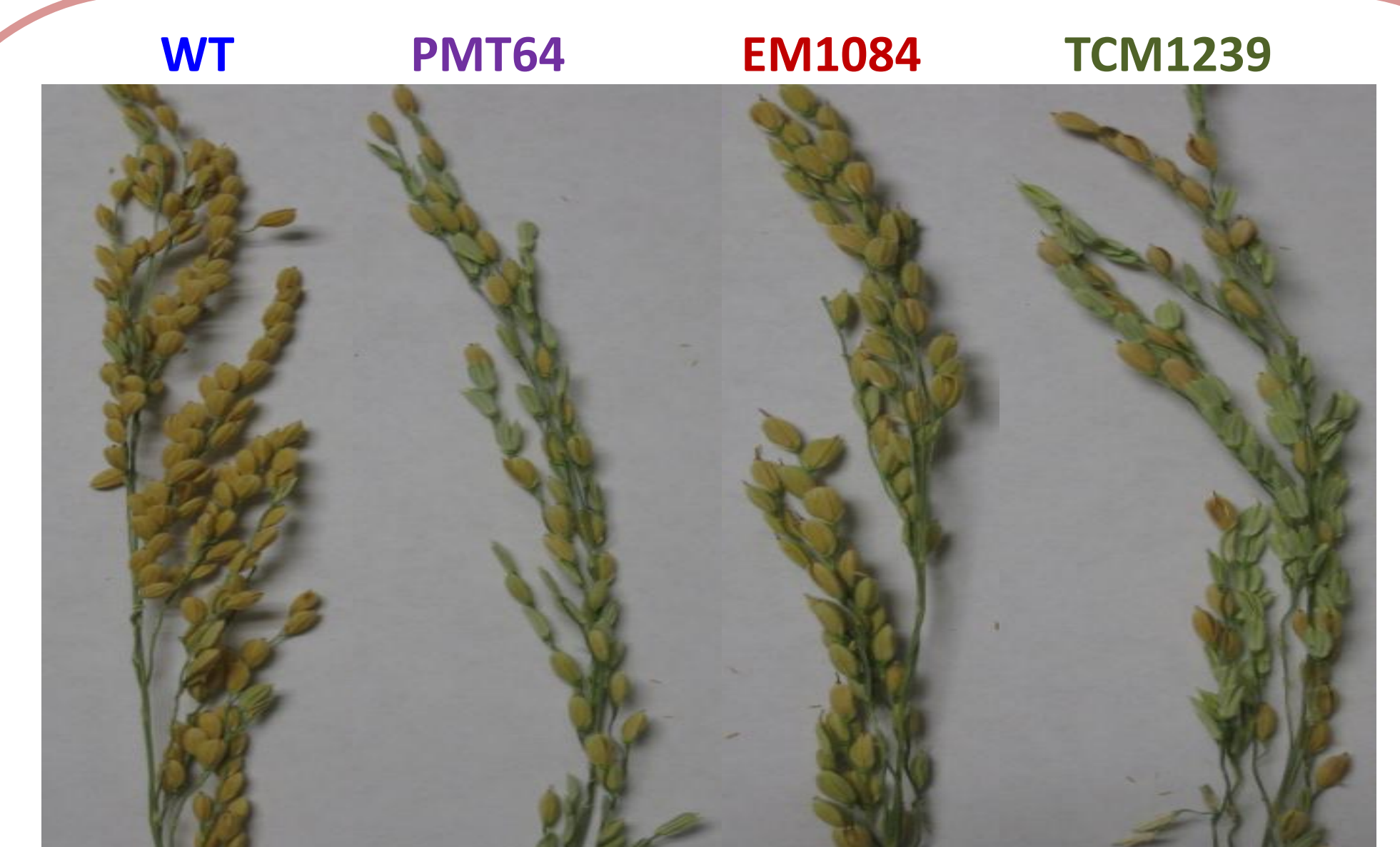


Figure 6. Phenotype of mature seeds from WT and *OsTudor-SN* genetic mutants. WT panicles possess numerous filled and developed mature seeds (yellow), while the mutants display varying degrees of reduced seed set (as indicated by green unfilled seed coats). In addition, mutant plants grow slower than WT, with EM1084 and TCM1239 having an extensive delay to flowering.

Conclusions

- Analysis of *OsTudor-SN* mutants reveal varying effects on storage protein gene expression, suggesting different domains of *OsTudor-SN* are involved in distinct molecular processes.
- Significant accumulation of glutelin precursor in EM1084 indicates improper sorting to PB-II, where it is proteolytically cleaved. A characteristic shared by rice mutants defective in glutelin RNA localization, this suggests the SN domain of *OsTudor-SN* may play a role in proper RNA sorting.
- Defects in plant growth and seed development in the mutants indicate *OsTudor-SN* is required for optimal plant development.

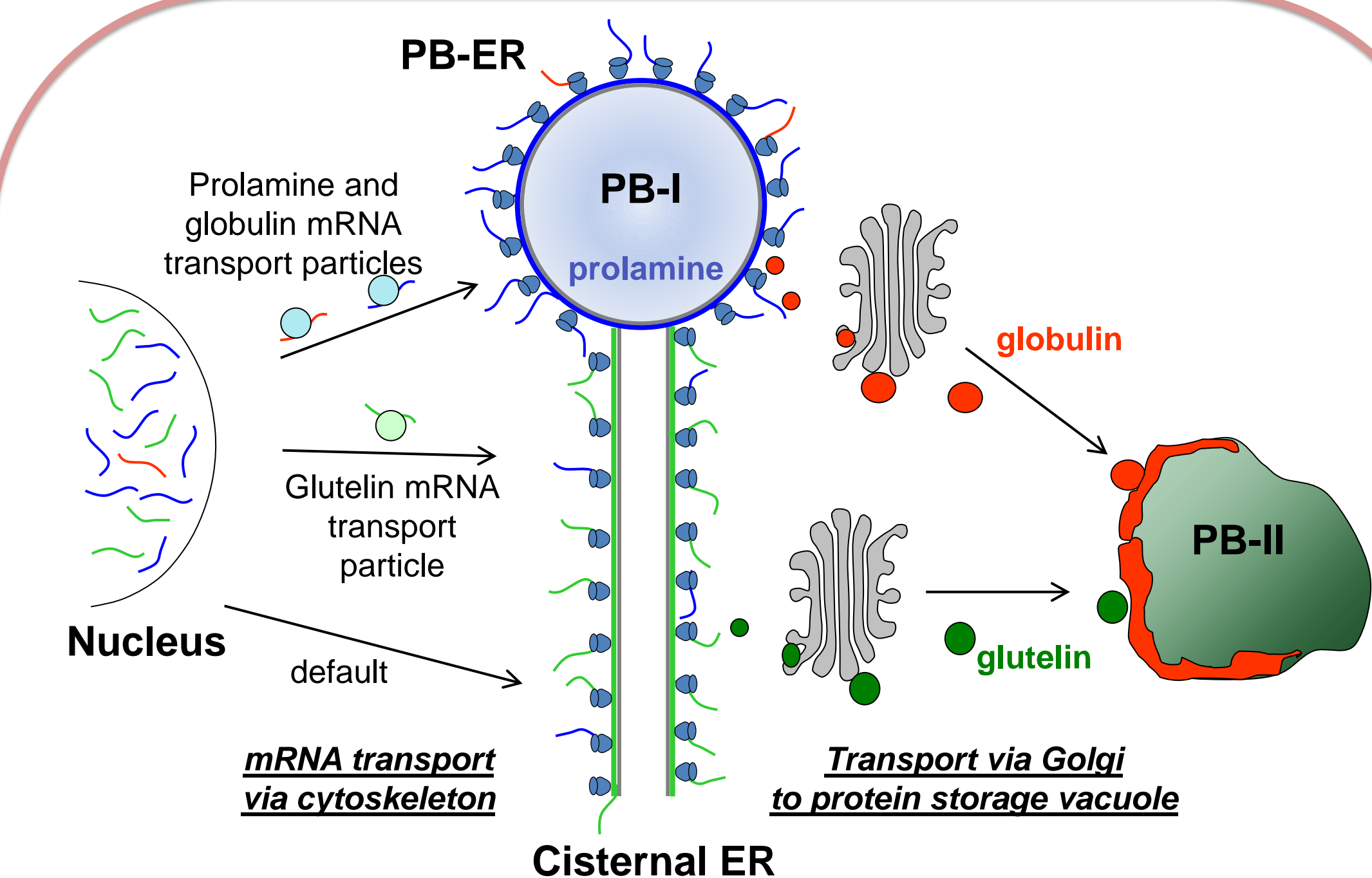


Figure 1. Targeted transport of storage protein mRNAs in developing rice seed endosperm directs protein synthesis and localization to different subdomains within the cell. Prolamine and globulin mRNA are transported via the actin cytoskeleton to protein body ER (PB-ER), while glutelin mRNA is targeted to the cisternal ER. After translation, prolamine polypeptides accumulate within the ER in intracisternal inclusion granules known as protein body type I (PB-I), while both globulin and glutelin are transported via the Golgi to protein storage vacuoles (PB-II). Evidence suggests *OsTudor-SN* may play a role in storage protein RNA transport, localization, translation, and/or stability.

Acknowledgements

This work was generously supported by National Science Foundation Grant DBI-0605016.