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Expression Profiling of Chlorophyll Biosynthesis Pathways in Chlorophyll B-Lacking Mutants of Rice (Oryza sativa L.)

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Abstract : Chloroplast pigments are extremely important during photosynthesis since they play essential roles in light absorption and energy transfer. Therefore, understanding the efficiency of chlorophyll (Chl) biosynthesis could facilitate enhancement in photo-assimilates accumulation, and ultimately, in crop yield. The Chl-deficient mutants have been used extensively to study the Chl biosynthetic pathways and the biogenesis of the photosynthetic apparatus. Rice (Oryza sativa L.) is one of the most leading food crops, serving as staple food for many parts of the world. To author's best knowledge, Chl b–lacking rice has been found; however the molecular mechanism of Chl biosynthesis still remains unclear compared to wild-type rice. In this study, the ultrastructure analysis, photosynthetic properties, and transcriptome profile of wild-type rice (Norin No.8, N8) and its Chl b-lacking mutant (Chlorina 1, C1) were examined. The finding concluded that total Chl content and Chl b content in the C1 leaves were strongly reduced compared to N8 leaves, suggesting that reduction in the total Chl content contributes to leaf color variation at the physiological level. Plastid ultrastructure of C1 possessed abnormal thylakoid membranes with loss of starch granule, large number of vesicles, and numerous plastoglobuli. The C1 rice also exhibited thinner stacked grana, which was caused by a reduction in the number of thylakoid membranes per granum. Thus, the different Chl a/b ratio of C1 may reflect the abnormal plastid development and function. Transcriptional analysis identified 23 differentially expressed genes (DEGs) and 671 transcription factors (TFs) that were involved in Chl metabolism, chloroplast development, cell division, and photosynthesis. The transcriptome profile and DEGs revealed that the gene encoding PsbR (PSII core protein) was down-regulated, therefore suggesting that the lower in light-harvesting complex proteins are responsible for the lower photosynthetic capacity in C1. In addition, expression level of cell division protein (FtsZ) genes were significantly reduced in C1, causing chloroplast division defect. A total of 19 DEGs were identified based on KEGG pathway assignment involving Chl biosynthesis pathway. Among these DEGs, the GluTR gene was down-regulated, whereas the UROD, CPOX, and MgCH genes were up-regulated. Observation through qPCR suggested that later stages of Chl biosynthesis were enhanced in C1, whereas the early stages were inhibited. Plastid structure analysis together with transcriptomic analysis suggested that the Chl a/b ratio was amplified both by the reduction in Chl contents accumulation, owning to abnormal chloroplast development, and by the enhanced conversion of Chl b to Chl a. Moreover, the results indicated the same Chl-cycle pattern in the wild-type and C1 rice, indicating another Chl b degradation pathway. Furthermore, the results demonstrated that normal grana stacking, along with the absence of Chl b and greatly reduced levels of Chl a in C1, provide evidence to support the conclusion that other factors along with LHCII proteins are involved in grana stacking. The findings of this study provide insight into the molecular mechanisms that underlie different Chl a/b ratios in rice.

Keywords : Chl-deficient mutant, grana stacked, photosynthesis, RNA-Seq, transcriptomic analysis
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