

Supplementary Materials

Supplementing the nuclear-encoded PSII subunit D1 induces dramatic metabolic reprogramming in flag Leaves during grain filling in rice

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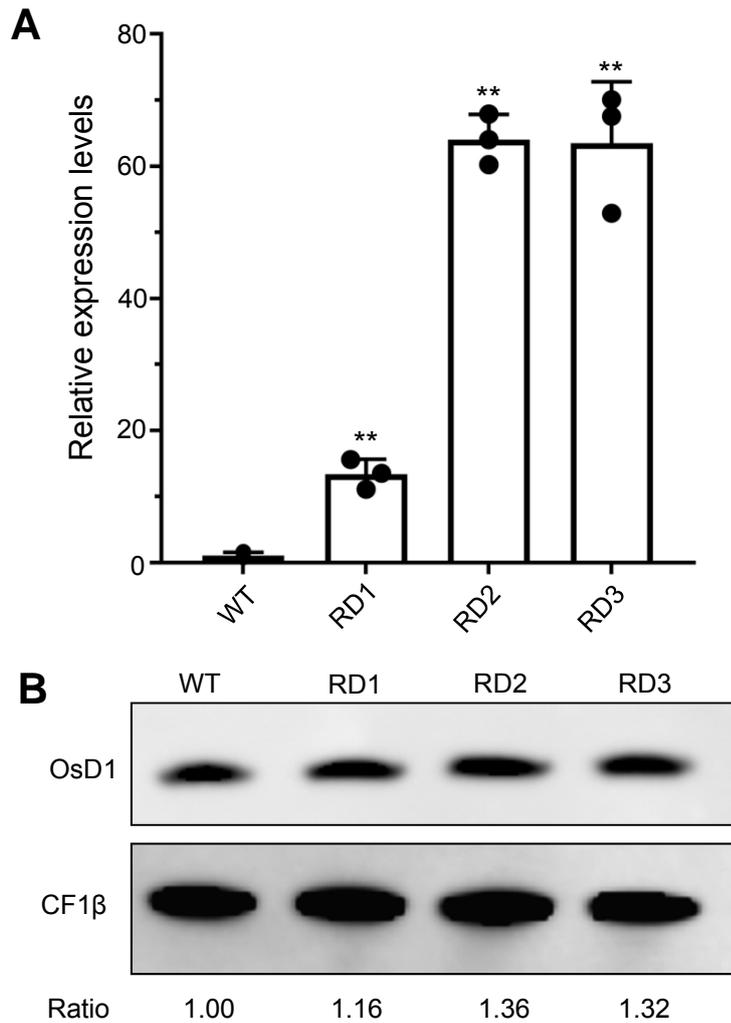
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Supplementary Materials include Supplemental Figures S1-3 and Table S1.

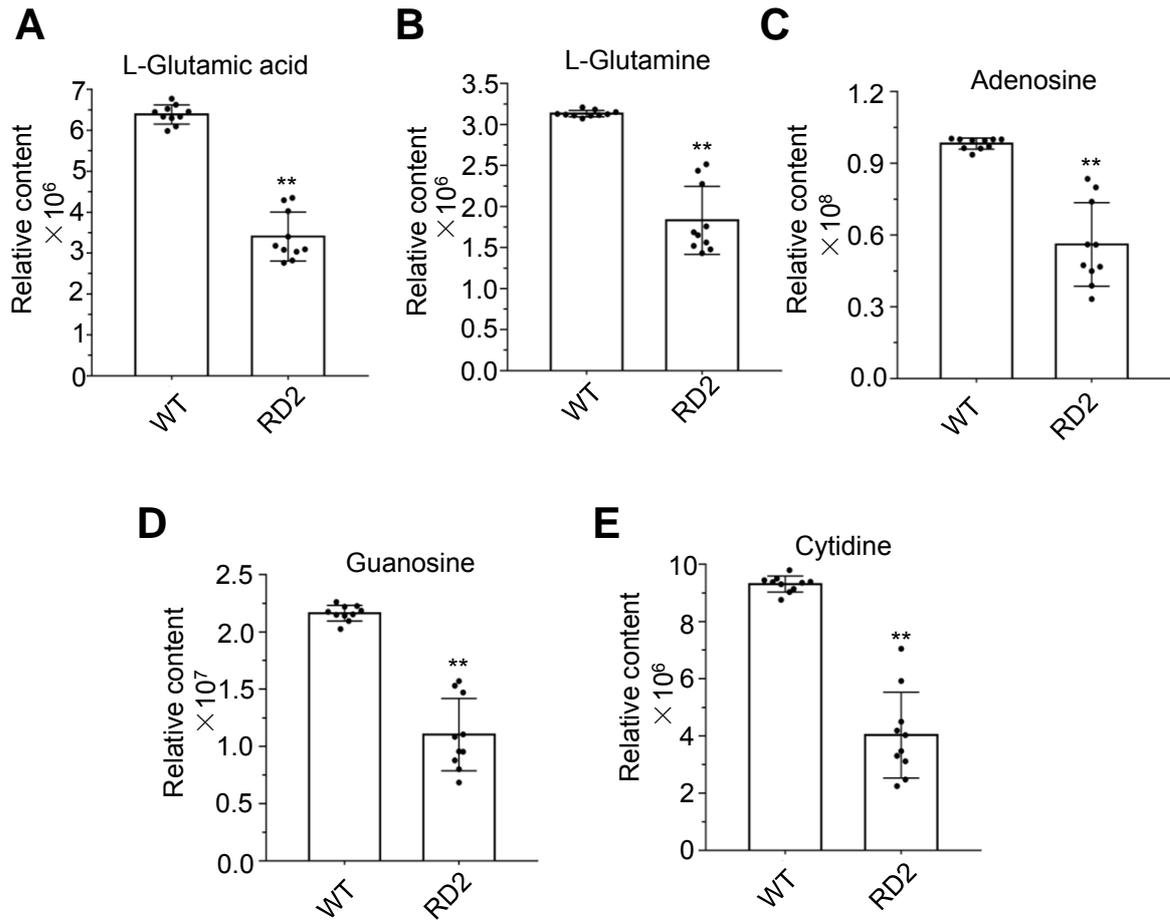
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      *           20           *           40           *           60
AtD1 MTAILERRESESLWGRFCNWITSTENRLYIGWFGVLMIPTLLTATSVFIIAFIAAPPVDI : 60
OsD1 MTAILERRESTSLWGRFCNWITSTENRLYIGWFGVLMIPTLLTATSVFIIAFIAAPPVDI : 60
      *           80           *           100          *           120
AtD1 DGIREPVSGSLLYGNNIISGAIIPTSAAGLHFYPIWEAASVDEWLYNGGPPYELIVLHFL : 120
OsD1 DGIREPVSGSLLYGNNIISGAIIPTSAAGLHFYPIWEAASVDEWLYNGGPPYELIVLHFL : 120
      *           140          *           160          *           180
AtD1 LGVACYMGREWELSFRLGMRPWIAVAYSAPVAAATAVFLIYPIGQGSFSDGMPLGISGTF : 180
OsD1 LGVACYMGREWELSFRLGMRPWIAVAYSAPVAAATAVFLIYPIGQGSFSDGMPLGISGTF : 180
      *           200          *           220          *           240
AtD1 NFMIVFQAEHNILMHPFHMLGVAGVFGGSLFSAMHGSLVTSSLIRETENESANEGYRFG : 240
OsD1 NFMIVFQAEHNILMHPFHMLGVAGVFGGSLFSAMHGSLVTSSLIRETENESANEGYRFG : 240
      *           260          *           280          *           300
AtD1 QEETYNIVA AHGYFGRLIFQYASFNNRSRLHFFLAAPVVG IWF TALGISTMAFNLNGF : 300
OsD1 QEETYNIVA AHGYFGRLIFQYASFNNRSRLHFFLAAPVVG IWF TALGISTMAFNLNGF : 300
      *           320          *           340          *
AtD1 NFNQSVVDSQGRVINTWADIINRANLGMEVMHERNAHNFPLDLAAEVPSTNG : 353
OsD1 NFNQSVVDSQGRVINTWADIINRANLGMEVMHERNAHNFPLDLAAEVPSTNG : 353
      NFNQSVVDSQGRVINTWADIINRANLGMEVMHERNAHNFPLDLAAEVPSTNG
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Supplemental Figure S1. Alignments of derived amino acid sequences of the D1 protein homologs. Amino acid sequences of AtD1 (NP_051039) and OsD1 (AJC09319) were aligned. Identical amino acid residues and conservative changes were depicted in black background.



Supplemental Figure S2. Expressing the rice chloroplast gene *psbA* in the nucleus enhances the D1 abundance. (A) Relative expression levels of *pHsfA2::RbcS^{PTP}-psbA* cDNA were analyzed by qRT-PCR in the flag leaves of wild-type (WT) and the transgenic rice lines of RD1, RD2 and RD3. The specific primers *RbcS-PTP-qRT-F* and *OspsbA-qRT-R*, derived from the plastid-transit peptide encoding sequence *RbcS-PTP* and coding region of the rice *psbA* cDNA, respectively, were used for analysis. Bars indicate the SD. of three biological replicates. ** $P < 0.01$, two-sided Student's *t*-test. Individual values (black-coded dots) and means are shown. (B) Immunodetection of the D1 abundance in thylakoid membranes isolated from the flag leaves of WT and transgenic rice lines of RD1, RD2 and RD3, using the antibody against D1. Equal protein loading was confirmed with the antibody against CF1 β . The signal intensities of western blots were quantified using Image J and representative western blots of the protein expression of OsD1 were normalized to CF1 β .



Supplemental Figure S3. Substantial reduction in the contents of representative amino acids and nucleotides identified in the flag leaves of RD2. Substantial reduction was detected in the contents of representative nucleotides and amino acids such as L-glutamic acid (A), L-glutamine (B), adenosine (C), guanosine (D) and cytidine (E), identified in the flag leaves of the transgenic line RD2 by the LC-MS analysis in comparison with WT (n = 10). ** $P < 0.01$, two-sided Student's *t*-test.

Supplemental Table S1. The primer sequences used in this study

Primer name	Primer sequence (5'-3')
pHsfA2-F	ACGCGTCGACCTTTGCCAATTCCTCTGTCC
pHsfA2-R	CGGGATCCTTTCGTTGTTTATCTCAAAT
RbcS-PTP-F	CGGGATCCATGGCTTCCTCTATGCTCTC
RbcS-PTP-R	TCCCCCGGGTTCGGAATCGGTAAGGTCAG
OsPsbA-F	TCCCCCGGGATGACTGCAATTTTAGAGAG
OsPsbA-R	GGGGTACCTTATCCATTAAGAGATGGAAC
RbcS-PTP-qRT-F	ATGGCTTCCTCTATGCTCTCTTC
OsPsbA-qRT-R	CTCTCTAAAATTGCAGTCAT
ACTIN1-qRT-F	TGGTCGTACCACAGGTATTGTGTT
ACTIN1-qRT-R	AAGGTCGAGACGAAGGATAGCAT