

Supplementary Figures

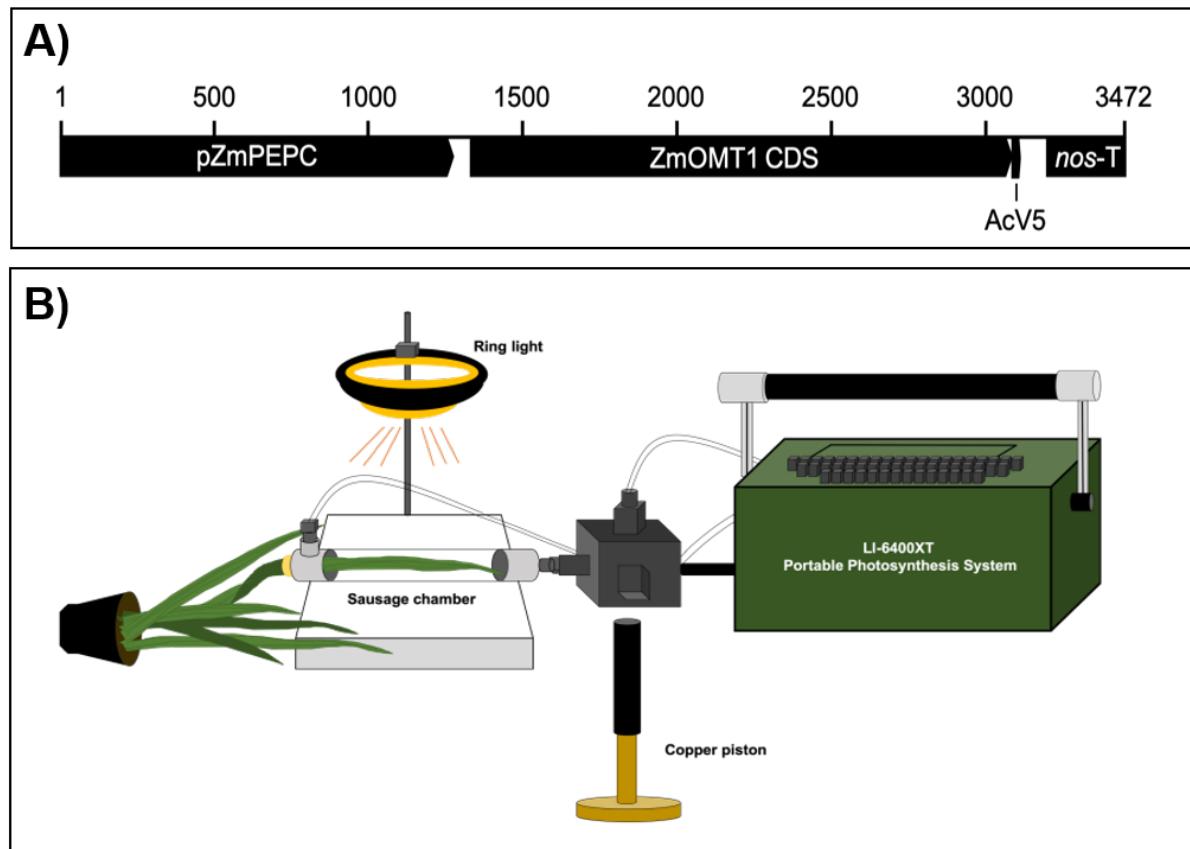


Fig. S1: Schematic of the pSC110:ZmOMT1:AcV5 construct (**A**). Schematic view of the customized gas exchange assembly and its coupling to the LI-COR 6400-Portable Photosynthesis System (**B**).

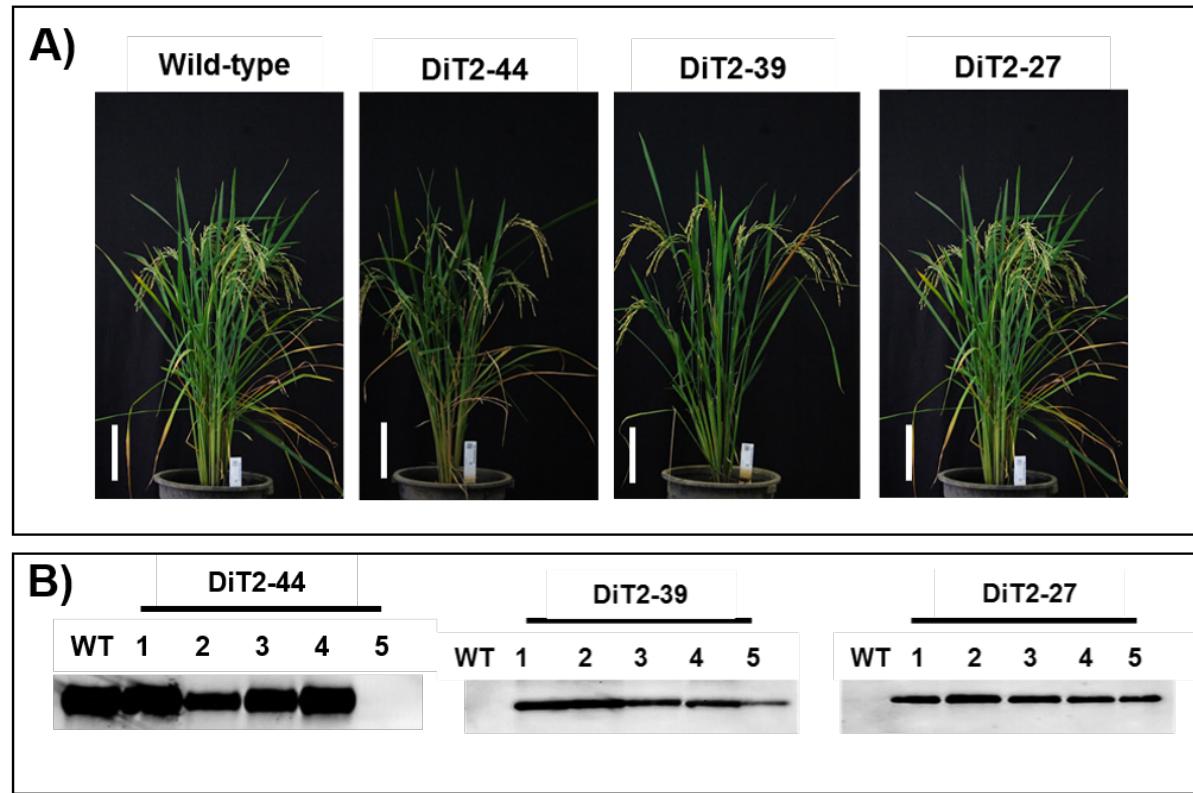


Fig. S2: Representative pictures of wild-type, DiT2-44, DiT2-39 and DiT2-27 lines grown under ambient conditions; 80 days post germination (DPG). The MW of DiT2-AcV5 is 40 kDa. Scale bar: 15 cm (**A**). Western-blot analysis of *ZmDiT2-AcV5* protein expression in rice DiT2-44, DiT2-39 and DiT2-27 leaves at mid-tilling stage. WT, wild-type plants (**B**).

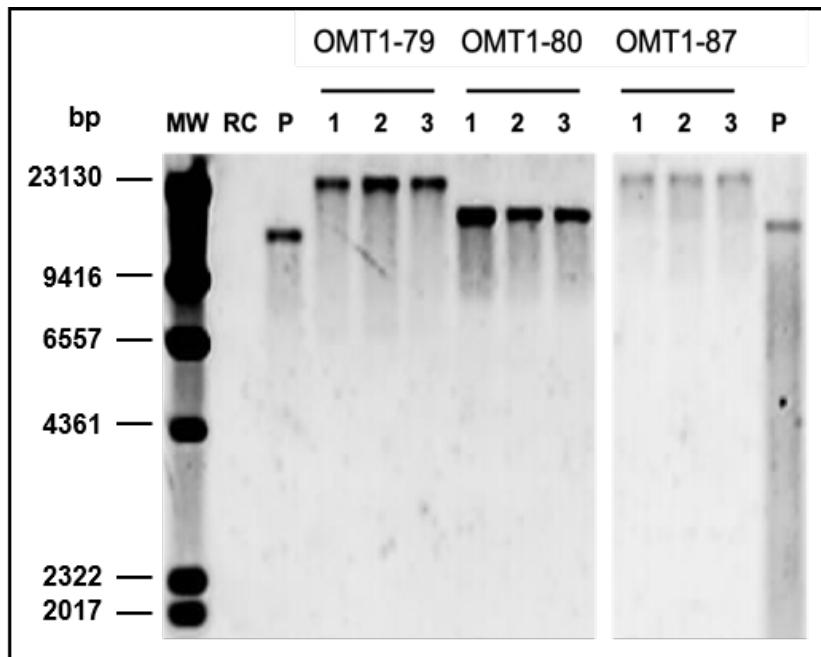


Fig. S3: DNA blots show that the OMT1 lines (OMT1-79, OMT1-80 and OMT1-87) carry a single copy of the *ZmOMT1* CDS and are homozygous at the T₃ generation. Untransformed rice control plants (RC) are negative controls for the transgene. The vector plasmid (P) is a positive control. A DIG-labeled DNA molecular marker indicates molecular weight (MW).

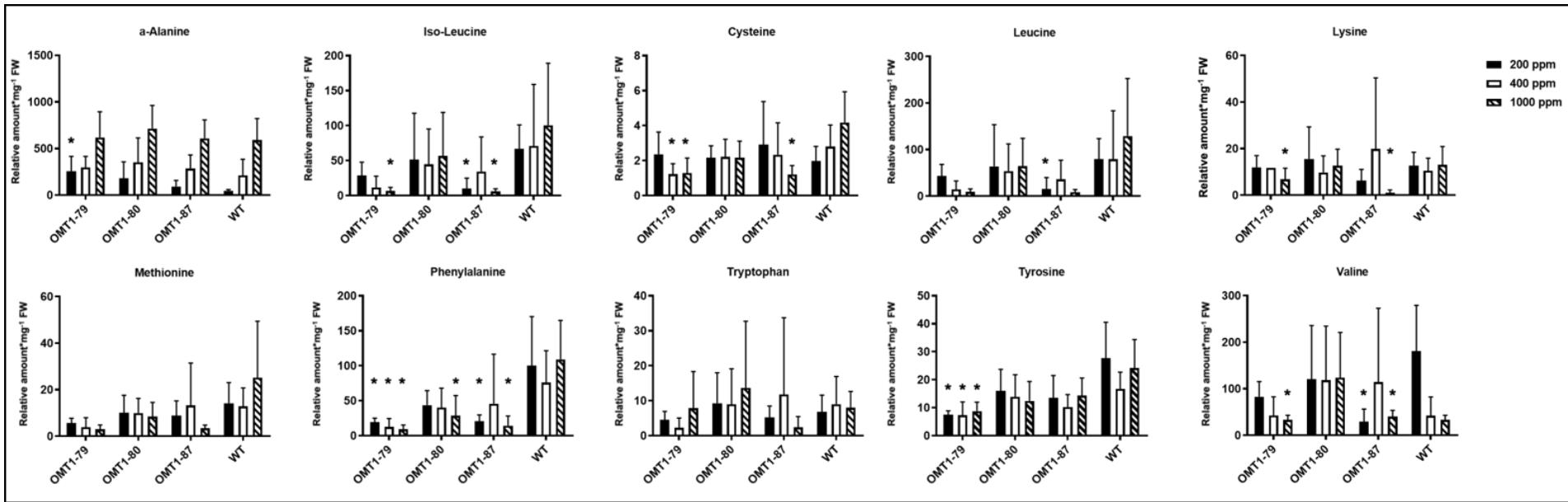


Fig. S4: Relative amount of some individual amino acids in OMT1 lines (OMT1-79, OMT1-80 and OMT1-87) and wild-type (WT) plants under different CO₂ concentrations (200, 400 and 1000 ppm). Value represent the mean ± SEM, n=5, significantly differences are indicated * P ≤ 0.05, Student's t-test

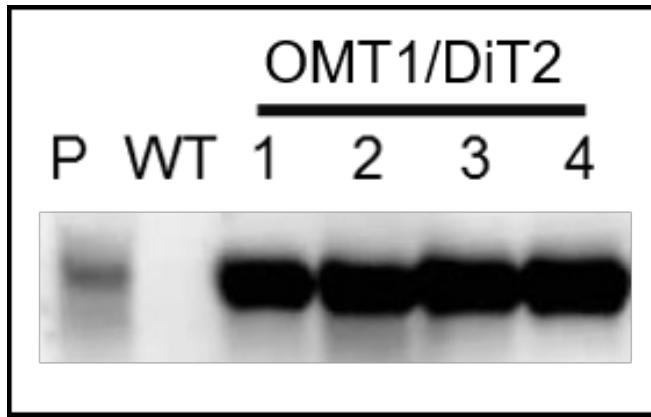
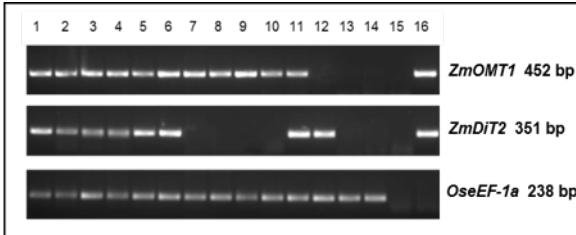


Fig. S5: Western blot analysis of *ZmOMT1* and *ZmDiT2* protein expressions in rice crosses lines of OMT1/ DiT2 (1, 2, 3 and 4). P; OMT1-AcV5 plant (positive control). WT; wild-type plant (negative control).



Well No.	Sample
1	OMT1-79/DiT2-44-1
2	OMT1-79/DiT2-44-2
3	OMT1-45/DiT2-27-1
4	OMT1-45/DiT2-27-2
5	OMT1-80/DiT2-39-1
6	OMT1-80/DiT2-39-2
7	OMT1-79-1
8	OMT1-79-2
9	OMT1-45-1
10	OMT1-80-1
11	OMT1-79/DiT2-44-3
12	DiT2-44-1
13	WT
14	WT
15	Water
16	Plasmids of ZmOMT1 and ZmDiT2

Figure. S6: RT-PCR of *ZmOMT1* and *ZmDiT2* mRNA expressions in OMT1/DiT2 double transgenic lines together with wild-type (WT). RT-PCR of rice housekeeping gene *OseEF-1a* was used as a positive and quality control. The PCR condition were: 95°C for 3 min; 40 cycles of 95°C for 20 sec, 55°C for 30 sec and 72°C for 45 sec; and 72°C for 3 min. RNA was extracted from leaf materials using TRIzol reagent and treated with DNase and the PCR products were electrophoresed on 2% agarose gel.

Supplementary Tables

Table S1. Primers used in this study

Target Gene	Forward Primer	Reverse Primer
ZmPEPC promoter (GRMZM2G083841; base pairs -1212 to +1)	CACCATGGTCGACGC GTCCTCCAC	TCAAGACCAGCCGCTCGCATTTCCAAGACCAAGAC CGATTATCTTC
ZmOMT1 for PCR screening	CGTGGGATAACCCTTA CATGG	CCCGATTATCTTCCACCAGA
OsOMT1 for qPCR	ATGGAATTGGGTCTG CTCCTG	AATCCATACCCCCACCACTG
ZmOMT1 for qRT-PCR	GTGGGGCTATGGGTT TGTCA	TATCTTCCACCAGAACGCCGC
ZmOMT1 for RT-PCR	CGTGGGATAACCCTTA CATGG	CCCGATTATCTTCCACCAGA
ZmDiT2 for RT-PCR	GTTGGAATGGCAGGA CAACT	ACCCAGCCTGAAAACATCTG
OseEF-1a for RT-PCR control	CAACATTGTGGTCAT TGGCC	GCAGTAGTACTTGGTGGTCT
ZmPEPC-promoter for digoxigenin labelled probe	TCCCGAGTTCTAAC CACAG	GTGGCTGAGGCTTCTTTTG
Cloning of ZmDiT2 for overexpression in rice	CACCATGGAGCTCCA CCTCGCCAC	TCAAGACCAGCCGCTCGCATTTCCAAGAGTACAGAC CCAAAAATTCCACCAGATG

Table S2: Vcmax and Jmax based on ACi Data at 21% or 2% O₂ using the PsFit Model.

	Vcmax 25°C (μmol m ⁻² s ⁻¹)	Jmax 25°C (μmol m ⁻² s ⁻¹)	Vcmax 25°C (μmol m ⁻² s ⁻¹)	Jmax 25°C (μmol m ⁻² s ⁻¹)
	21% O ₂		2% O ₂	
Wild-type	93.77 ± 7.82 ^a	176.18 ± 18.19 ^a	121.7 ± 10.91 ^a	154.77 ± 11.58 ^{ab}
OMT1-79	65.06 ± 5.75 ^{bc}	140.78 ± 6.59 ^{ab}	72.41 ± 11.28 ^b	114.52 ± 8.86 ^c
OMT1-80	86.47 ± 12.59 ^{ab}	167.72 ± 20.71 ^a	124.68 ± 25.39 ^a	171.03 ± 20.73 ^a
OMT1-87	58.59 ± 14.15 ^c	122.48 ± 20.16 ^b	100.04 ± 18.48 ^{ab}	126.45 ± 21.21 ^{bc}

Vcmax, maximum rate of Rubisco carboxylation allowed by Rubisco; Jmax, maximum rate of electron transport. Value represent the mean ± SEM, n=5. Different letters within groups indicate that values are statistically different $p \leq 0.05$, Tukey's multiple comparison test. ns indicates non-significant, $p > 0.05$.