Supplementary Material

Diurnal regulation of rice N uptake ability under interrupted N supply

Md. Mehedi Hasan[A], Gibrilla Dumbuya[A,B], Habtamu Assega Alemayehu[C,D], Uzuki Matsushima[D], Maya Matsunami[D], and Hiroyuki Shimono[D,E,]*

[A]United Graduate School of Agricultural Sciences, Iwate University, 3-18-8, Ueda, 020-8550, Morioka, Iwate, Japan.

[B]Sierra Leone Agricultural Research Institute/Njala Agricultural Research Centre, P.O. Box 540, Freetown, Sierra Leone.

[C]Ethiopian Institute of Agricultural Research/Fogera National Rice Research and Training Centre, P.O. Box 1937, Fogera, Ethiopia.

[D]Faculty of Agriculture, Iwate University, 3-18-8, Ueda, 020-8550, Morioka, Iwate, Japan.

[E]Agri-Innovation Center, Iwate University, 3-18-8, Ueda, 020-8550, Morioka, Iwate, Japan.

[*]Correspondence to: Hiroyuki Shimono Faculty of Agriculture, Iwate University, 3-18-8, Ueda, Morioka, Iwate 020-8550, Japan and Agri-Innovation Center, Iwate University, 3-18-8, Ueda, Morioka, Iwate 020-8550, Japan Email: shimn@iwate-u.ac.jp

Supplementary Table S1. Primer information.

Name of gene	Primer ID	Sequence		Reference
		FW	RV	
Aquaporins	*OsPIP1;1*	CGCAATCGTGATGTCCTGTT	ACGATTGAGTTGTTCAGGGTTC	Matsunami et al. (2018)
	OsPIP1;2	CTGCATCAGCTATTATCACTGTCAA	AAGCAGGCAGCGGGAAA	Hayashi et al. (2015)
	OsPIP1;3	ACGTGTGTTATTACCGTCTGGTG	CGCACACACAAGTACCATTTCTC	
	OsPIP2;1	CCGCTGGTCGTTTTGTTTC	TACAGGCTAAACACATGAGACATCC	
	OsPIP2;4	ATTGTTTGGGCTCTTTCTCCTTC	TGCAAACACTGAAACATACACCAC	
	OsPIP2;5	ATGGGAAGAACAGAGCACAGG	ACTTGCTTTTGATAGCGCACAC	
Ammonium transporters	*OsAMT1;1*	AATGCTTTTGCCGCTCTCTC	CTTCCCACCTTCACACCACA	Matsunami et al. (2018)
	OsAMT1;2	GCGGGTTCATGCTCAAGTC	TAGCTCAGCTCCTCCGGTTC	
	OsAMT1;3	GTCCGTTTGGGGAAAGTGT	AAAAGAAAGCCACAACCACCA	
	OsAMT2;1	GAGAGACCAGACCACTATAGTCTA	ATGTCGGCCTTTATCGTGTTG	
	OsAMT2;2	GCAAGATGGAGTAGCAGATATTAC	TAGGACACATTGCATACTGACTG	Newly designated
	OsAMT3;3	CACTGCCTGGAATATTGTTATCAC	GTGTACAGCATCATCTCCAATAAG	
Enzymes	*OsGS1;1*	GCTCCGGTTGCTCCATTG	TTACTTCTCCCAGCACAAATGC	Matsunami et al. (2018)
	OsGS1;2	CCGAGTGTCATTTTGTGTACTCTGA	CCGTCCCTACGGAAAAAGC	
	OsGOGAT1	CGGGCTCAATAGGGAAAAGAG	CAGAACACACCAAAATTTCCAATT	
	OsAS1	TCGCAGGCGAAGAGGGCTCACGTCCTC	AGCGGGGAGACGATGGCGAGACGCTGC	Ohashi et al. (2015)
	OsG6PDH1	CCAATGCAGGCTCTGTCTAG	TTCCAAGATCACCAACATTACAG	Kano et al. (2013)
Reference gene	*UBC*	CCGTTTGTAGAGCCATAATTGCA	AGGTTGCCTGAGTCACAGTTAAGTG	Matsunami et al. (2018)

Hayashi et al. (2015) Aquaporins in developing rice grains. Bioscience, Biotechnology and Biochemistry, 79, 1422–1429. Kano et al. (2013) The rare sugar d-allose acts as a triggering molecule of rice defence via ROS generation. Journal of Experimental Botany, 64, 4939–4951. Ohashi et al. (2015) Asparagine synthetase1, but not asparagine synthetase2, is responsible for the biosynthesis of asparagine following the supply of ammonium to rice roots. Plant and Cell Physiology, 56, 769–778.Okamura et al. (2020) Root sampling and RNA extraction methods for field-based gene expression analysis of soybeans. Plant Production Science. (in press)

Supplementary Table S2. Nitrogen (N) content, N concentration, N uptake rate (*NUR*), water uptake rate (*WUR*), and growth parameters at 25 days after treatment in independent repeated experiment. CT_{full} and CT_{half} represent controls in which the full-concentration nutrient solution was supplied for the whole day and half the day, respectively; DF and NF represent nutrient supply only during the 12-hour day and night, respectively.

Treatment	Whole plant			Root				Leaf area	Specific leaf N content	Daily *NUR*		Daily *WUR*	
	N content	Dry weight	N concentration	N content	Dry weight	N concentration	Biomass distribution			Per whole-plant dry weight	Per root dry weight	Per whole-plant dry weight	Per root dry weight
	(mg/plant)	(g plant^{-1})	(mg g^{-1} DW)	(mg plant^{-1})	(g plant^{-1})	(mg g^{-1} DW)	(%)	(cm^2/plant)	(g m^{-2})	(mg N/plant h^{-1})	(mg N g^{-1} DW h^{-1})	(g plant^{-1} h^{-1})	(g g^{-1} DW h^{-1})
CT_{full}	125 ±3 a	3.90 ±0.10 a	32.2 ±0.3 a	15.8 ±1.0 a	0.71 ±0.04 b	22.3 ±0.2 a	18.1 ±0.6 b	419 ±14 a	1.65 ±0.05 a	0.26 ±0.02 a	0.37 ±0.01 a	2.78 ±0.12 a	3.94 ±0.14 a
CT_{half}	106 ±1 b	3.84 ±0.10 a	27.6 ±0.7 b	15.5 ±0.5 a	0.80 ±0.03 a	19.3 ±0.4 b	20.9 ±0.2 a	421 ±10 a	1.50 ±0.03 b	0.20 ±0.01 b	0.24 ±0.01 c	2.67 ±0.14 a	3.33 ±0.12 b
	(0.84)	(0.98)	(0.86)	(0.98)	(1.13)	(0.87)	(1.15)	(1.01)	(0.91)	(0.75)	(0.66)	(0.96)	(0.84)
DF	82 ±2 c	3.25 ±0.09 b	25.4 ±0.4 c	10.4 ±0.4 b	0.58 ±0.02 a	18.2 ±0.5 c	17.7 ±0.3 b	341 ±8 b	1.41 ±0.02 b	0.15 ±0.01 c	0.25 ±0.01 bc	2.11 ±0.08 b	3.67 ±0.08 a
	(0.66)	(0.83)	(0.79)	(0.66)	(0.81)	(0.82)	(0.98)	(0.82)	(0.86)	(0.56)	(0.69)	(0.76)	(0.93)
NF	66 ±2 d	2.89 ±0.08 c	22.9 ±0.2 d	9.3 ±0.4 b	0.64 ±0.02 bc	14.7 ±0.2 d	22.0 ±0.4 a	308 ±12 c	1.26 ±0.01 c	0.18 ±0.01 bc	0.28 ±0.02 b	1.85 ±0.04 b	2.92 ±0.08 c
	(0.53)	(0.74)	(0.71)	(0.59)	(0.89)	(0.66)	(1.21)	(0.74)	(0.77)	(0.68)	(0.76)	(0.66)	(0.74)
ANOVA	***	***	***	***	***	***	***	***	***	***	***	***	***

Values are means ± standard errors (*n* = 6). DW, dry weight; RDW, root dry weight; Significance: ***$P < 0.001$. Numbers in parentheses indicate the relative value (the actual value divided by the CT_{full} value). Values of a variable labeled with different letters differ significantly among the treatments (ANOVA followed by LSD tests, $P < 0.05$).

Supplementary Table S3. Nitrogen (N) content, N concentration, N uptake rate (*NUR*), water uptake rate (*WUR*), and growth parameters at 23 days after treatment in Experiment 3. CT_{full} and CT_{half} represent controls in which the full-concentration nutrient solution was supplied for the whole day and half the day, respectively; DF and NF represent nutrient supply only during the 12-hour day and night, respectively.

Treatment	Whole plant			Root				Leaf area	Specific leaf N content	Daily *NUR*		Daily *WUR*	
	N content	Dry weight	N concentration	N content	Dry weight	N concentration	Biomass distribution			Per whole-plant DW	Per root dry weight	Per whole-plant dry weight	Per root dry weight
	(mg/plant)	(g plant⁻¹)	(mg g⁻¹ DW)	(mg plant⁻¹)	(g plant⁻¹)	(mg g⁻¹ DW)	(%)	(cm²/plant)	(g m⁻²)	(mg N/plant h⁻¹)	(mg N g⁻¹ DW h⁻¹)	(g plant⁻¹ h⁻¹)	(g g⁻¹ DW h⁻¹)
CT_{full}	28.3 ±0.3 a	0.98 ±0.04 ab	28.9 ±0.3 a	4.8 ±0.1 a	0.20 ±0.04 a	23.6 ±0.4 a	21.0 ±0.6 a	133 ±2 ab	1.23 ±0.02 ab	0.05	0.20	0.89	4.31
CT_{half}	29.1 ±0.4 a	1.02 ±0.05 a	28.5 ±0.4 a	4.1 ±0.0 b	0.20 ±0.03 a	20.9 ±0.1 bc	19.3 ±0.5 b	140 ±5 a	1.25 ±0.04 a	0.04	0.16	0.92	4.54
	(1.03)	(1.04)	(0.99)	(0.85)	(0.96)	(0.88)	(0.92)	(1.06)	(1.01)	(0.90)	(0.78)	(1.04)	(1.06)
DF	24.4 ±0.2 b	0.96 ±0.05 ab	25.4 ±0.2 c	3.7 ±0.1 c	0.19 ±0.02 a	19.4 ±0.4 c	19.9 ±0.3 ab	132 ±3 ab	1.13 ±0.03 bc	0.04 ±0.00	0.20 ±0.01	0.81	4.25
	(0.86)	(0.98)	(0.88)	(0.77)	(0.93)	(0.82)	(0.95)	(0.99)	(0.92)	(0.78)	(0.99)	(0.91)	(0.99)
NF	23.2 ±0.3 b	0.86 ±0.02 b	22.9 ±0.3 b	3.9 ±0.1 bc	0.18 ±0.02 a	21.3 ±0.5 b	21.4 ±0.6 a	126 ±3 b	1.13 ±0.01 c	0.04 ±0.00	0.15 ±0.01	0.86	4.23
	(0.82)	(0.88)	(0.94)	(0.81)	(0.90)	(0.90)	(1.02)	(0.95)	(0.92)	(0.78)	(0.71)	(0.97)	(0.98)
ANOVA	***	ns	**	**	ns	**	*	ns	ns				

Values are means ± standard errors (*n* = 4-8). DW, dry weight; RDW, root dry weight; Significance: **$P < 0.01$, *$P < 0.05$; ns, not significant. Numbers in parentheses indicate the relative value (the actual value divided by the CT_{full} value). Values of a variable labeled with different letters differ significantly among the treatments (ANOVA followed by LSD tests, $P < 0.05$).