**Fan et al**

**SI Materials and Methods**

**Single site mutation of *OsNRT2.3b* and mRNA synthesis.** A point mutation (H167R) was generated using PCR of two fragments of *OsNRT2.3b* with the mutant site and new restriction site in the primers. *OsNRT2.3b* cDNA in pT7Ts was used as a DNA template and the first PCR fragment (H167RB) was sub-cloned into *Hind*III and *Xba*I of pT7Ts. New plasmid and second PCR fragment (H167R) were digested by *Csp45* I and *Xba* I and ligated into the final plasmid with H167 site mutated *OsNRT2.3b* cDNA (pH167R). The end and mutagenic primers are given (see Supporting Online Materials Table S9), and the mRNA synthesis of pH167R was described as above.

**Oocyte preparation, mRNA injection, $^{15}$N uptake and electrophysiology.** Oocytes preparation, mRNA synthesis and injection, $^{15}$N-nitrate uptake and electrophysiology have been described previously (1, 2). Either 0.5 mM $Na^{15}NO_3$ $^{15}NH_4NO_3$ or $^{15}NH_4Cl$ in ND96 was used for N uptake experiments for 16 h (2). The pH selective microelectrode method was used to measure cytosolic pH (3).

**Expression of *OsNRT2.3a/b-6X-His-tag* and *OsNRT2.3b::GFP* constructs in rice protoplasts.** Rice protoplast preparation and transformation was based on the method in Tang et al 2012 (4). *OsNRT2.3a/OsNRT2.3b* genes were inserted a *6X-His-tag* at 603/513bp site in full-length cDNA and then transferred into the protoplast expression vector pRCS2-ocs-nptII. The *OsNRT2.3b* gene was fused with GFP at the N terminal of the protein. For plasma membrane localization, a working staining solution of 5 μg/ml membrane-selective fluorescent vital dye FM4-64 was used to stain the transfected protoplasts (4). Protoplasts with or without FM4-64 were observed under a 60 × objective. The fluorescence of FM4-64 (a membrane-selective dye) and GFP in the cells was analyzed with a 543 nm helium-neon laser and a 488 nm argon laser, respectively, using a confocal laser scanning microscope LSM410 (Carl Zeiss).

**Flow Cytometry Assay.** Transformed protoplasts were centrifuged at 100 xg for 2 min and most of the supernatant was removed. The protoplasts samples were resuspended with cold W5 solution for the surface signal test of intact protoplasts and Intracellular Fixation and Permeabilization Buffer (Affymetrix eBioscience) for the cytosolic signal test (respectively 5, 6). Anti-6X His tag®antibody fluorescein isothiocyanate (FITC)(ab1206) was gently mixed into the supernatant and incubate the mixture at 28 °C for 1h. The protoplasts samples were again centrifuged at 100 x g for 2 min and then resuspended in cold W5 solution and permeabilization buffer.
Fluorescence percentages of the FITC signal in transfected protoplasts were quantified by flow cytometry (FACSCanto; BD Biosciences, modified using the method in Chou et al. 2012 (7). Approximately 10,000 protoplasts (counted by hemocytometer) were suspended in 500 μl of W5 solution or permeabilization buffer. The FITC was excited with a laser at 488 nm and captured with a FL1-A sensor (the emission wavelength was 505–554 nm. We used 6X-His-tag expressing protoplasts as cellular signal positive controls. Protoplasts with no tag were used for setting a blank control for the whole experiment. The fluorescence index was calculated as the following equation: fluorescence index = fluorescence protoplast numbers / total protoplast numbers. For each pair of samples described here, five independent protoplast transfections were performed, and fluorescence was measured two times for each of five transfections.

**Over-expression vector construction and transgenic plants.** The open reading frames of *OsNRT2.3a, OsNRT2.3b* and H167R were amplified by gene specific primers (see Supporting Information Table S8). The fragment was treated with restriction enzymes and inserted in vectors and sequenced before transformation. Rice (*Oryza sativa*) embryonic calli were transformed using Agrobacterium-mediated methods (8). One copy insertion T0 plants were harvested and grown to generate T1 plants until T7 generation in these experiments (see Tables S3, and S4,

Figs.3 and S7). Homozygous T1 plants were taken for genetic plant production (Tables S3 and S4). Two lines of T7 *OsNRT2.3a* over-expression plants, a-O1 and a-O2, four lines of T7 *OsNRT2.3b* over-expression plants, O1, O2, O4, O8 and two lines of T7 *H167R* over-expression plants, H167R2 and H167R4 were used for further experiments (Tables S3 and S4).

**RT-PCR.** Total RNA from three biological representatives, specifically from the roots of WT and transgenic plants, was isolated using the TRIzol reagent according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA, USA) (4). Plant tissues samples taken for RNA Plant tissues samples taken for RNA extraction were flash frozen at –80 °C in liquid nitrogen immediately on harvesting at 4 weeks seedling stage. RT-PCR analyses were carried with the primers listed in Table S10 (4).

**Antibody production and Western blot.** The full cDNA sequences of *OsNRT2.3a/b* genes were amplified from plasmids of *OsNRT2.3a* (AK109776) and *OsNRT2.3b* (AK072215) by primers, F: GGAATTCTC ACACCCCGGCCGG, R: CGGGATCCAT GTGGGGCGGCATGCTC. The plasmids were kindly provided by Dr. S. Kikuchi (KOME). The PCR fragment was sub-cloned into the bacterial expression vector pGSX (Amersham) at BamH I and EcoR I sites. The amino acid products were purified and their monoclonal-antibodies were synthesized (4). The monoclonal-antibody was selected from 192 individual cell specific reactions to *OsNRT2.3a* (516 aa) or *OsNRT2.3b* (486 aa) protein (4). Plasma membrane protein abstraction from roots and Western blot was done as previously described (4) and repeated twice.

**RNA in situ hybridization.** RNA in situ hybridization was performed as previously described (9). For the *OsNRT2.3b* probe, the binding site is in the *OsNRT2.3b* specific 5' UTR with the sequence CGATGGTTGGGTGCGGCGAGA. The nonsense sequence is GCTACCAACCCACGCC GCTCT. All probes were labelled at 5' end with DIG.

**The $^{15}N$ influx, total P and Fe measurements of transgenic plants.** For the $^{15}N$ influx hydroponic growth conditions were used as described previously in IRRI culture medium at pH 5.5 with 1.25 mM $NH_4NO_3$ as the N supply unless stated otherwise (4). Rice seedlings of WT and over-expression plants were grown in IRRI nutrient solution containing 1.25 mM $NH_4NO_3$ for two months in the greenhouse and then deprived of N for 3 days. The plants were rinsed in 0.1 mM $CaSO_4$ for 1 min, then transferred to a solution containing either 1.25 mM $Ca(^{15}NO_3)_2$ (atom% $^{15}N$: 99.27%) or $(^{15}NH_4)_2SO_4$ (atom% $^{15}N$: 95.7%) or $^{15}NH_4NO_3$ (atom% $^{15}N$: 45%) or $^{15}NH_4{}^{15}NO_3$ (atom% $^{15}N$: 95.5%) for 5 min and finally rinsed again in 0.1 mM $CaSO_4$ for 1 min. Roots were separated from the shoots immediately after the final transfer to $CaSO_4$, and frozen in liquid N. After grinding, an aliquot of the powder was dried to a constant weight at 70 °C. 10 mg powder of each sample was analyzed using the MAT253-Flash EA1112-MS system (Thermo Fisher Scientific, Inc., USA). The whole experiment was repeated twice and each time with five replicates.

The total P and Fe of plants was measured by ICP analysis experiments. The 0.05 g dried crushed plant leaf powder was digested with 5 ml of 98 % $H_2SO_4$ and 3 ml of 30 % hydrogen peroxide. After cooling, the digested sample was diluted to 100 ml with distilled water. The ion concentrations in the solution were measured using the ICP-OES (Perkin Elmer Optima 2000 DV).

**Phloem sap collection.** Rice seedlings were grown in 1.25 mM $NH_4NO_3$ for 8 weeks and then transferred to N treatments (nitrate: 1.25 mM $Ca(NO_3)_2$; ammonium: 1.25 mM $(NH_4)_2SO_4$ for 24 h without nitrification inhibitor. Phloem sap was collected using an insect feeding method with the same plants as above. Each plant was set in a 250 ml flask of IRRI nutrient solution with six plants kept in the insect cage at 26 °C and a 16 h light period. Seven to ten adult brown plant hopper adults were transferred on to each plant at the beginning of the N treatments. Rice phloem dew secreted by the insects was collected at 24 h and 48 h after the N

treatment began (Fig. S12).

**The field experiments of transgenic plants.** Field experiments were conducted in Changxing at the Experimental Station of Zhejiang University with four N（N as urea）application levels as 0, 75, 150 and 300 kg N/ha. Seeds were germinated on 5th May and seedlings of each type were transplanted in flooded plots at 3 rows and 33 plants with 25 cm (row space) x 20 cm (plant space) on 5$^{th}$ June. Plants were maintained flooded throughout their growth period and grown in blocks with a random order for each N application. For the large scale experiments grown at 75 kg N/ha, the plants were transplanted as 10 rows x 128 plants. Three replications were used for all field experiments and the plots with all border rows were finally harvested on the 23th October. The soil nutrient status in this experiment station was total nitrogen (N): 1.00 ± 0.18 mg/g, total phosphorus (P) 0.38 ± 0.08 mg/g, total potassium (K) 39 ± 2.3 mg/g, Olsen P (0.5 mM $NaHCO_3$-extractable P) 23 ± 4.1 mg/kg and soil pH was 6.3 ± 0.47 (n = 6). 60 kg P (as $Ca(H_2PO_4)_2$)/ha and 110 kg K (as $K_2SO_4$)/ha fertilizer was applied to the paddy before transferring. The first N application was carried out before transplanting on 3$^{th}$ June when 20% of the total N fertilizer was mixed into soil. A second application was 40% of the total N was made on 12 June when the rice was at the beginning of the tillering stage. The final application was 40% of the total N was applied on 20 June. The rice growth period after transplanting at Changxing was 120 ± 3 days for WT, a-O1 and a-O2, H167R2, H167R4 lines and 130 ± 2 days at 0-75 kg N/ha level, 135 ± 2 days at 150 kg N/ha level and 140 ± 2 days at 300 kg N/ha level for O1, O2, O4 , and O8 lines. The grain yield was measured at harvest and NUE was defined as grain yield per fertilizer N applied in field experiment.

**RNA preparation and DNA microarray hybridization.** Three replicates of WT (*Nipponbare*), O8 and H167R2 leaves were harvested from the 150 Kg N /ha treatment in the field at Changxing in the Experimental Station at 10:00 am of the 1$^{st}$ August. This was the maximum tillering stage for all plants. Leaf tissues samples taken for RNA extraction were flash frozen at –80 °C in liquid nitrogen immediately on harvesting. RNA extraction, hybridization with Affymetrix rice GeneChip arrays (Santa Clara, CA, USA), data analyses and annotation were as described in previous reports (10).

**Quantitative real-time RT-PCR.** Total RNA from three biological representatives, specifically from leaves of WT and transgenic plants, was isolated using the TRIzol reagent according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA, USA) (4). RT-PCR analyses were conducted using the primers for the photorespiratory genes listed in Table S11 (4).

**Gas exchange and post-illumination $CO_2$ burst measurements.** The rate of light-saturated photosynthesis of flag leaves was measured from 9:00 to 15:00 h using a Li-Cor 6400 portable photosynthesis open system on the plants in the 150 Kg N /ha treatment in the field at the Changxing experiment on the same day as the microarray sampling. The leaf temperature during measurements was 27.0 ± 0.1 °C and light supply was photosynthetic photon flux intensity (PPFD) of 1500 μmol photons $m^{-2}.s^{-1}$ as described previously (11). The ambient $CO_2$ concentration in the cuvette (Ca–c) was adjusted to atmospheric $CO_2$ concentration (Ca) (417 ± 1.0 μmol $CO_2$ $mol^{-1}$), and the relative humidity was maintained at 20%. Data were recorded after equilibration to a steady state (10 min). The measured leaves were labelled, and leaf areas were calculated based on the labelled area. The post-illumination $CO_2$ burst (PIB) was measured at the same labeled leaf under photorespiratory conditions (saturating PPFD of 1,500 μmol photons $m^{-2}.s^{-1}$, Ca–c $CO_2$ concentration of 100 μmol $CO_2$ $mol^{-1}$, relative humidity of 60%–70%) as described previously (12).

**Statistical Analysis of Data.** All the data collected were tabulated and analyzed for significant differences using statistical software (SPSS 13.0; SPSS Inc, Chicago, IL, USA).

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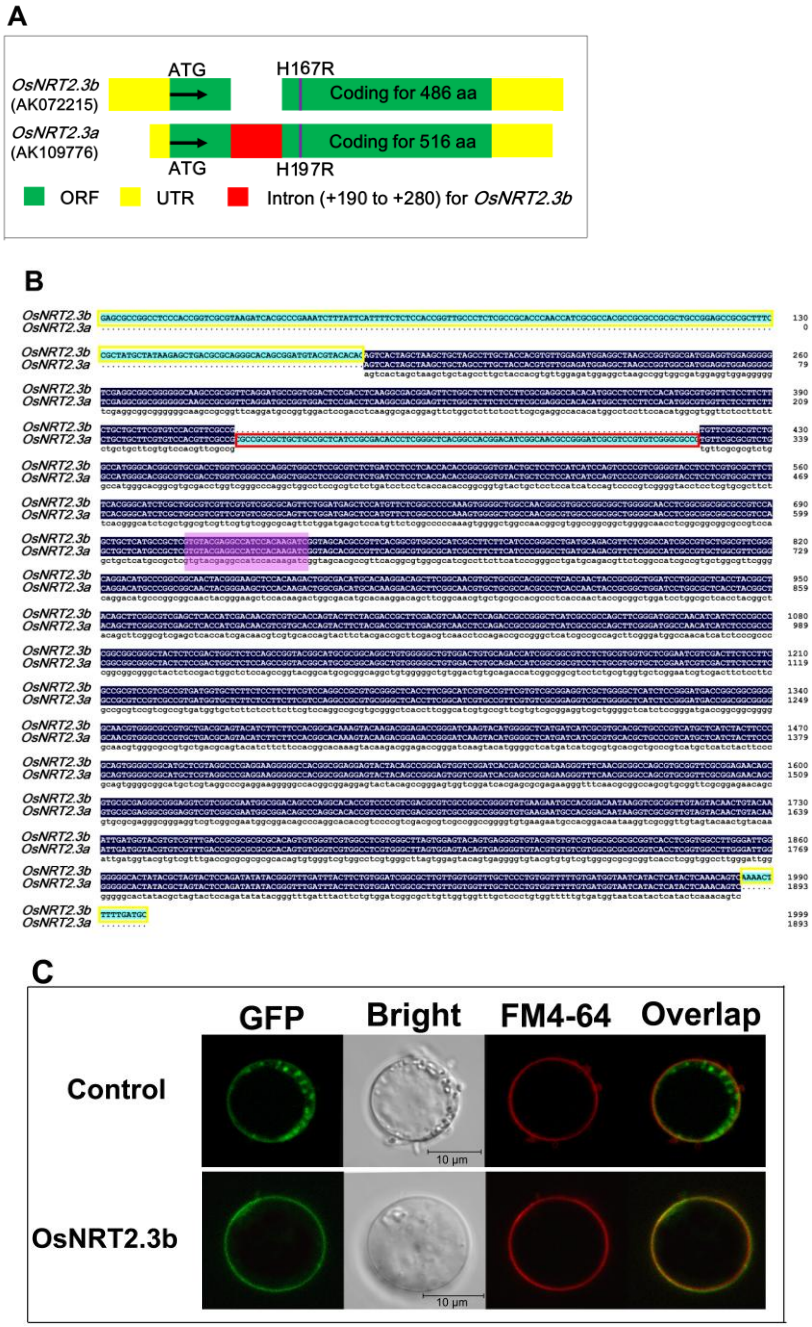


**Fig. S1.** The *OsNRT2.3b/OsNRT2.3a* gene structure and membrane topology of OsNRT2.3b.

A: Comparison of the *OsNRT2.3a* and O*sNRT2.3b* gene structures; B: Comparison of the cDNA sequences of *OsNRT2.3a* and *OsNRT2.3b.* The yellow box indicates different parts in 5'-UTR and 3'-UTR; Gray box marks the ATG location in the sequence; Red box indicates the intron differences in the sequence; Purple box indicates location of the VYEAIHKI motif; C: Representative microscope images of a rice protoplast expressing the NRT2.3b-GFP fusion protein showing plasma membrane localization; GFP: the GFP (green) fluorescence; Bright: A bright-field image; FM4-64: FM4-64FX (red) dye image; Overlap: the overlap of the GFP (green) and FM4-64FX (red) fluorescence. Row 1 is the protoplast expressing GFP used as a control. FM4-64FX is a plasma membrane specific fluorescent dye and the experiment was described previously (4). Bars = 10 μm.

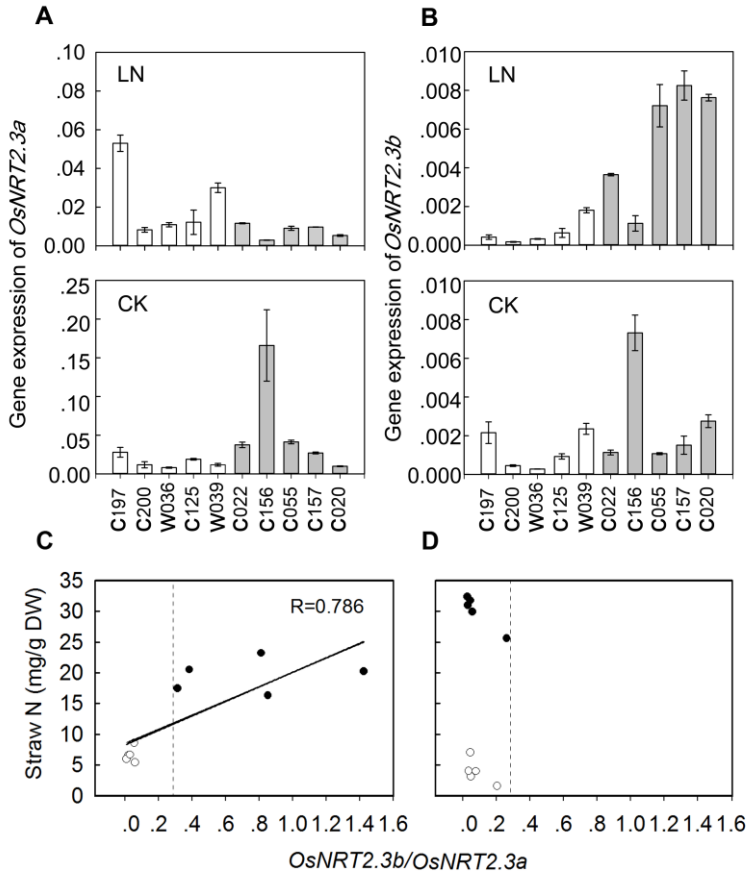


**Fig. S2.** The expression levels and ratio of *OsNRT2.3a*, *OsNRT2.3b,* and N content in straw of two different types of *Indica* rice cultivars from the XP-CLR database as examples for two selection patterns of *OsNRT2.3*.

The expression of *OsNRT2.3a* (A) and *OsNRT2.3b* (B) under low N supply (0.63 mM $NH_4NO_3$, LN) and normal N supply (1.25 mM $NH_4NO_3$, CK). The expression ratio of *OsNRT2.3b/OsNRT2.3a* under low N supply (C) and normal N supply (D). The five *Indica* rice cultivars labeled with an open circle as the examples of one selection type for the *OsNRT2.3* gene from the GWAS database including C197 (Maweinian), C200 (Biwusheng), W039 (Nam Dawk Mai), C125 (Jiefangxian), W036 (Chun 118-33) and the five *Indica* rice cultivars labeled with a closed circle as the examples of the other selection type for the *OsNRT2.3* gene from the GWAS database including C022 (JinnanteB), C156 (ChaoyangyihaoB), C055 (Sanbaili), C157 (L301B), C020 (Guangluai-4-1). The rice plants were grown hydroponically in IRRI nutrient solution with different N treatments for 4 weeks before harvesting for mRNA expression and total N measurement in the rice straw. The *OsNRT2.3b* and *OsNRT2.3a* expression data were first normalised to the *OsActin* control. As *OsNRT2.3b* is mainly expressed in the shoot and *OsNRT2.3a* is mainly expressed in the root, the expression ratio of *OsNRT2.3b/OsNRT2.3a* is equal to the real time PCR normalised value of *OsNRT2.3b* in the straw divided by the real time PCR normalised value of *OsNRT2.3a* in the root.

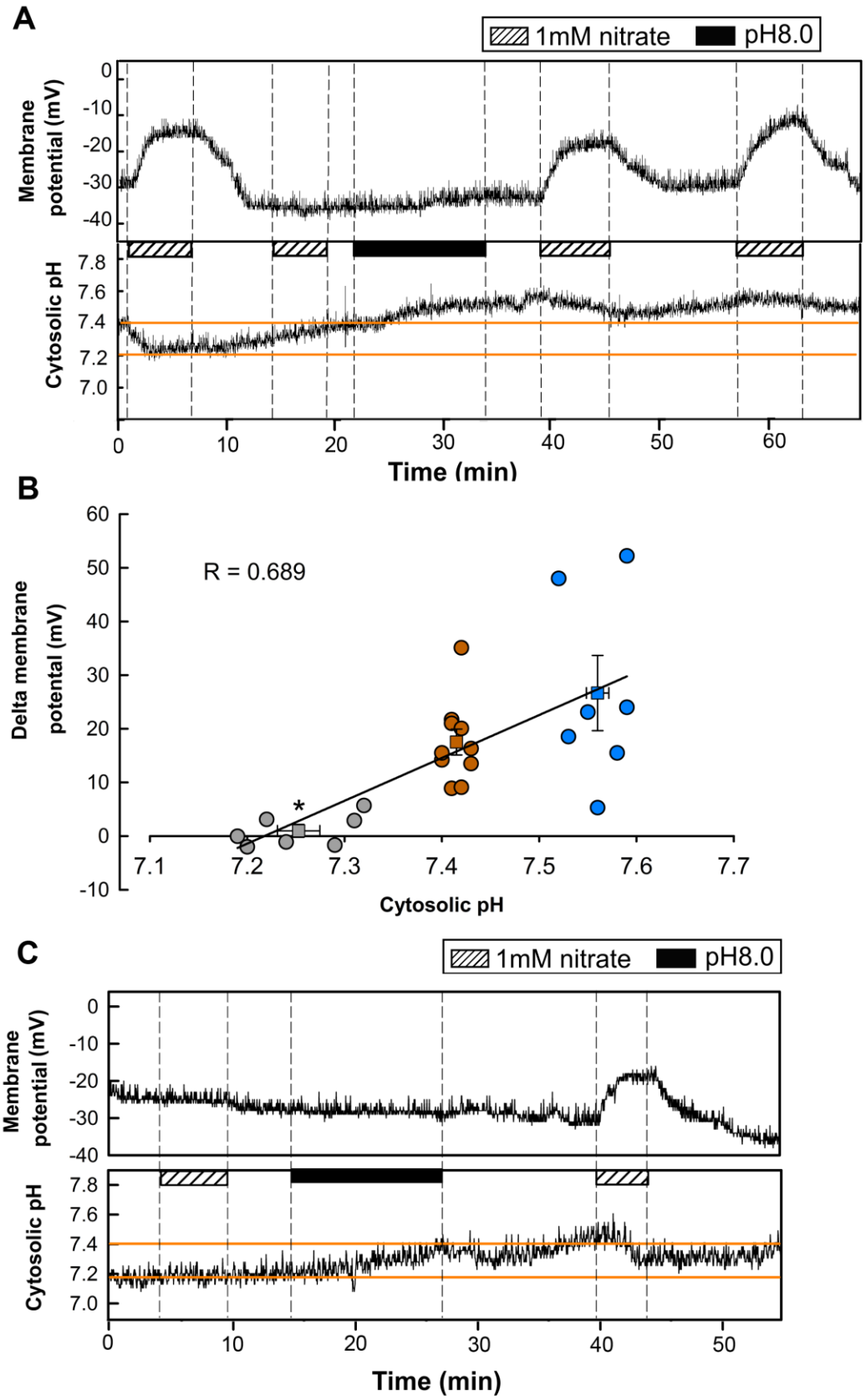


**Fig. S3.** The functional analysis of OsNRT2.3b in *Xenopus* oocytes.

A: A double barreled pH electrode recording of cytosolic pH from an *OsNRT2.3b* injected oocyte, treated with 1 mM nitrate (shaded bar) in pH 7.4 and washing with pH 8.0 saline (black bar). All the solution pH was 7.4 except pH 8.0 washing solution; B： The relationship between cytosolic pH and delta membrane potential caused by nitrate transport at pH7.4. Brown dots present the data collected at the first nitrate treatment; Gray dots collected at the second nitrate treatment; Blue dots collected the nitrate treatments after washing with pH 8.0 solution; Squares are the means with bi-direction SE. The symbol * indicates a significant difference among these three data sets at the 5 % level tested with a one-way ANOVA analysis; C: A double barreled pH electrode recording of cytosolic pH from an *OsNRT2.3b* injected oocyte, treated with 1 mM nitrate (shaded bar) in pH 7.0 and washing with pH 8.0 saline (black bar). In order to reduce the cytosolic pH, the cells were incubated in pH 7.0 for 2 hours before the recording. During the recording the bathing solution was pH 7.0 except for the pH 8.0 washing solution.

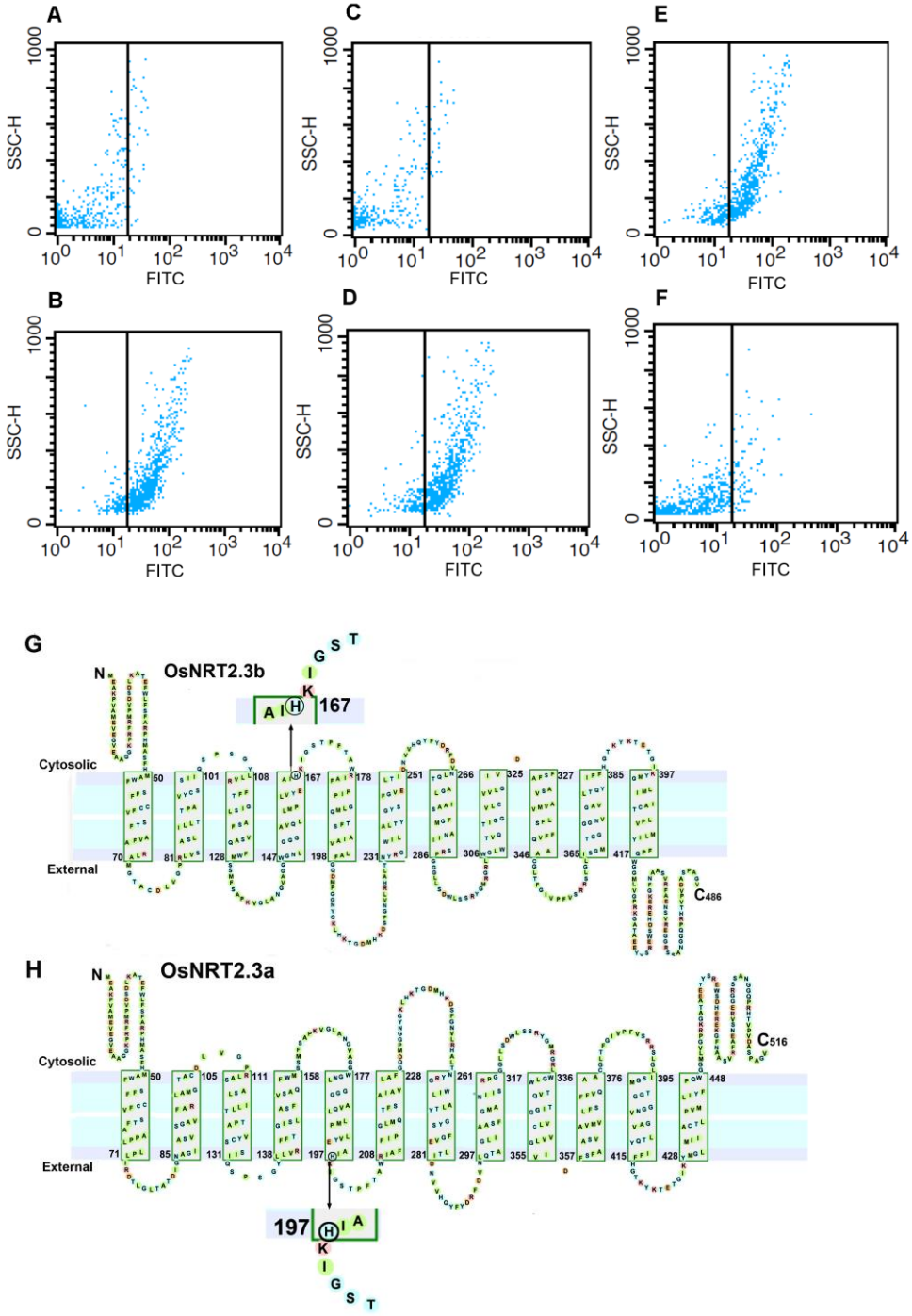


**Fig. S4.** The membrane orientation of the OsNRT2.3a and OsNRT2.3b pH sensing motifs in rice protoplasts determined by flow cytometry.

6X-His-tag expressing protoplasts were used as positive controls. A, C, E within only W5 solution for external side signal; B, D, F with permeabilization buffer in W5 solution for the cytosolic face signal; A, B: 6 His-Tag; C, D: 6 His-Tag insertion at H167 of OsNRT2.3b; E, F: 6 His-Tag insertion at H197 of OsNRT2.3a. G, H: Consensus transmembrane (TM) secondary structure of OsNRT2.3b and OsNRT2.3a modified on the prediction by TMPred (www.ch.embnet.org/software/TMPRED_form.html)

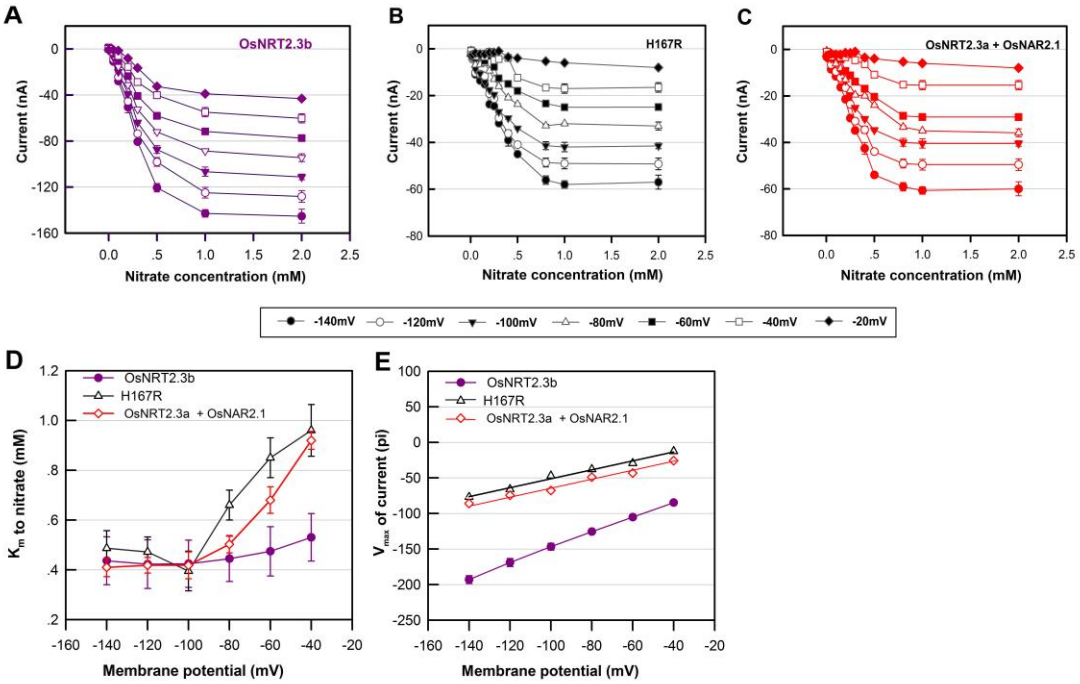


**Fig. S5.** Nitrate influx kinetics of oocytes injected with *OsNRT2.3b*, *OsNRT2.3a* + *OsNAR2.1* and H167R mRNA.

A: Current–voltage difference curves for the oocytes expressing the rice nitrate transporter *OsNRT2.3b*; B: Current–voltage difference curves for oocytes expressing the single site mutated rice nitrate transporter H167R; C: Current–voltage difference curves for oocytes expressing *OsNRT2.3a* + *OsNAR2.1*; D: The $K_m$ of *OsNRT2.3b*, H167R and *OsNRT2.3a* + *OsNAR2.1* for nitrate; E: The $V_{max}$ of *OsNRT2.3b*, H167R and *OsNRT2.3a* + *OsNAR2.1* for current. Curves of the $V_{max}$ and membrane potential were fitted with linear equations. As for *OsNRT2.3b,* the equation is Y = 1.07X - 40.55 ($R^2$ = 0.999); for H167R, Y = 0.63X + 11.73 ($R^2$ = 0.987); for *OsNRT2.3a* + *OsNAR2.1,* Y = 0.59X - 4.61 ($R^2$ = 0.982). The relationship between applied nitrate concentrations and currents elicited from mRNA-injected oocytes treated with different concentrations of nitrate were fitted with the Michaelis-Menten equation. The nitrate-elicited currents of were recorded in oocyte using two-electrode voltage clamp (pClamp 10.2, Axon). The oocytes were incubated in nitrate-free MBS and then treated with MBS containing 0.005, 0.02, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5, 0.8, 1, 2 mM sodium nitrate. Membrane potential of oocytes was pulsed from +20 to −140 mV with 20 mV incremental steps. The currents were recorded to obtain current–voltage curves (2).

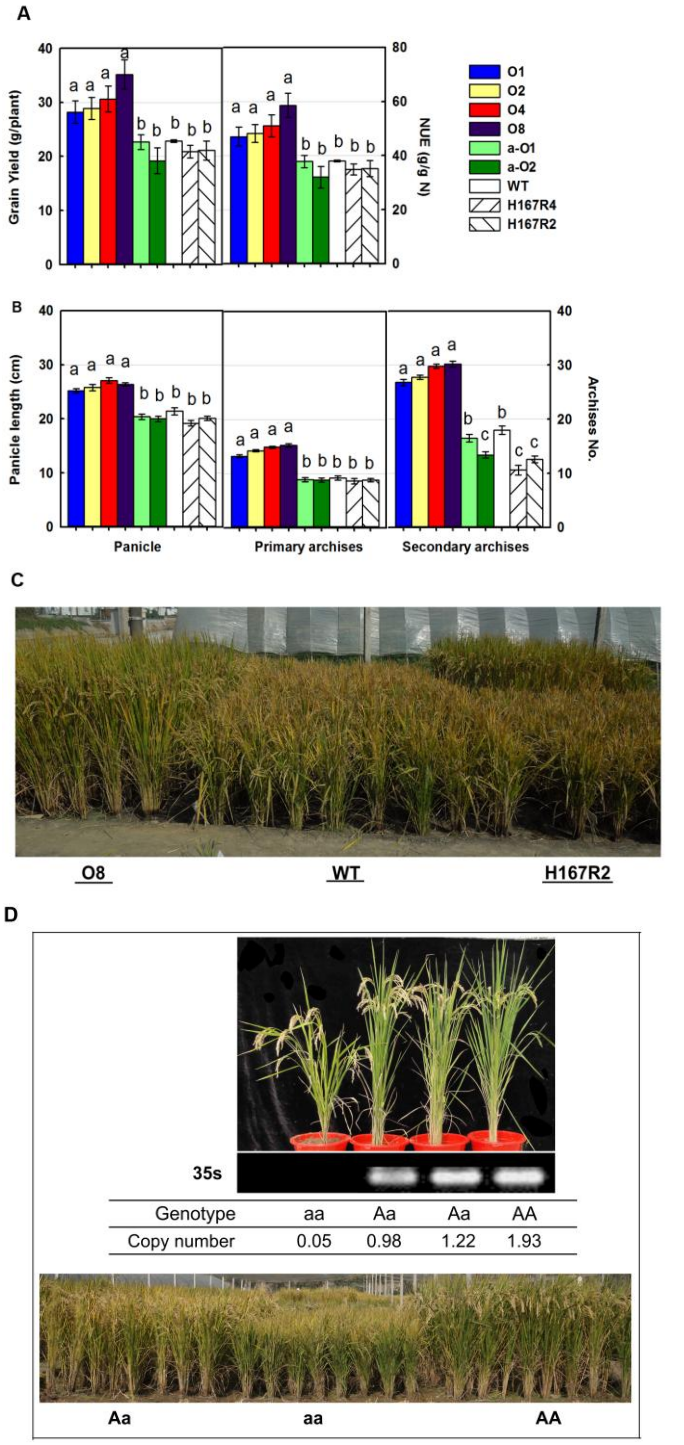


**Fig. S6.** The *Nipponbare* phenotype of *OsNRT2.3b, OsNRT2.3a*, and H167R over-expression plants.

A: The grain yield and NUE of *OsNRT2.3b, OsNRT2.3a* and H167R mutant over-expression lines in pot; B: The panicle characteristics of *OsNRT2.3b, OsNRT2.3a* and H167R mutant over-expression lines in pot; a,b,c letters indicate a significant difference between WT and the over-expressors at the 5 % level tested with a one-way ANOVA analysis. C: The field plot experiment of *OsNRT2.3b* and H167R in their T7 generations was performed in an experimental farm of Nanjing Agricultural University; D: The B1F2 generation phenotype of *Nipponbare* (♀) × O8 T5 (♂). Their molecular test with

35s PCR and copy number (as described in Table S2). The plot growth conditions for the B1F4 generation phenotyping of *Nipponbare* (♀) × O8 T5 (♂), and the field experiment was as described above.

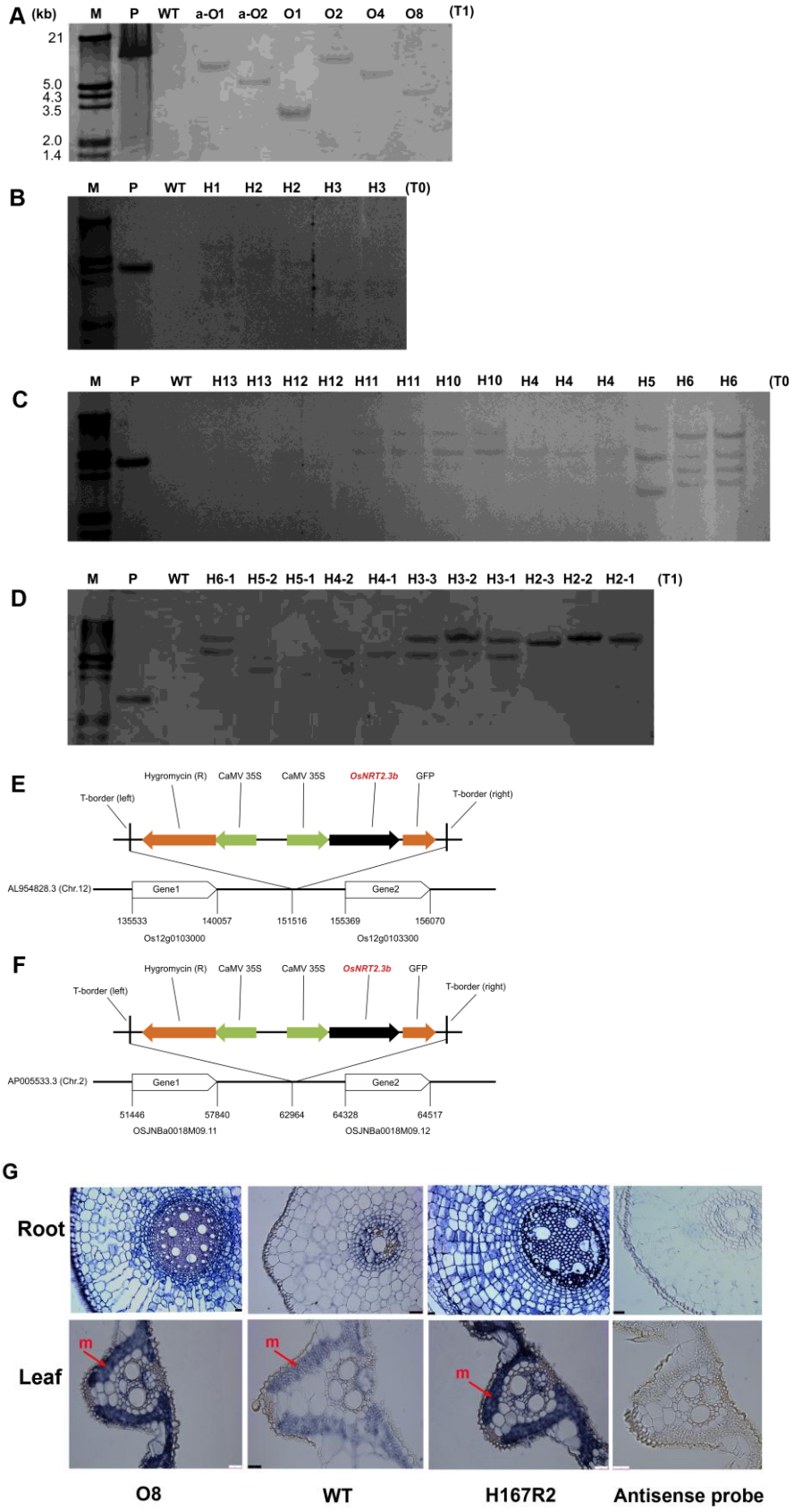


**Fig. S7.** Molecular identification of *OsNRT2.3b*, *OsNRT2.3a* and H167R over-expression plants.

A: Southern blot to identify the copy number in genome of *OsNRT2.3a* and *OsNRT2.3b* over-expression plants T1 DNA Southern blot, M: marker; P: positive control; a-O1 and a-O2, *OsNRT2.3a* over-expression lines; O1, O2, O4, O8, *OsNRT2.3b* over-expression lines; B,C: H167R over-expression plant T0 DNA Southern blot, Hn, H167R over-expression lines; D: H167R over-expression plant T1 DNA Southern blot. Total genomic DNA was isolated from 10 g of leaf sample using the CTAB method. Ten micrograms of genomic DNA of T0 plants digested by *BamH* I and *Hind*III (TaKaRa, Tokyo) and T1 DNA digested by *Kpn*I and *Hind*III (TaKaRa, Tokyo) were separated on 0.7% agarose gel then transferred to Nytran N membranes (Schleicher and Schuell, Germany). Probes for the hygromycin resistance gene were prepared with a DIG-High Prime DNA Labeling and Detection Starter Kit I (Roche Diagnostics, Germany) using part of the open reading frame (ORF) of each homolog which was amplified by the following primers: F: 5'-CGTCTGCTGCTCCATACAAG-3' and R: 5'-GAAGTGCTTGACATTGGGGA G-3'. E Tail PCR result and characterization of 35S-OsNRT2.3b T-DNA insertion sites in the genome of O4 plants. O4. T-DNA insertion site is in the non-coding region of chromosome 12 between Os12g0103000 and Os12g0103300; F: Tail PCR result and characterization of 35S-OsNRT2.3b T-DNA insertion sites in the genome of O8 in Nipponbare over-expression plants. O8. T-DNA insertion site is in the non-coding region of chromosome 2 between OSJNBa0018M09.11 and OSJNB10018M09.12. The tail PCR primer was discussed in Table S11; G :The *RNA in situ* hybridization in roots and leaves of O8, WT and H167R2 lines. m: mesophyll cells.

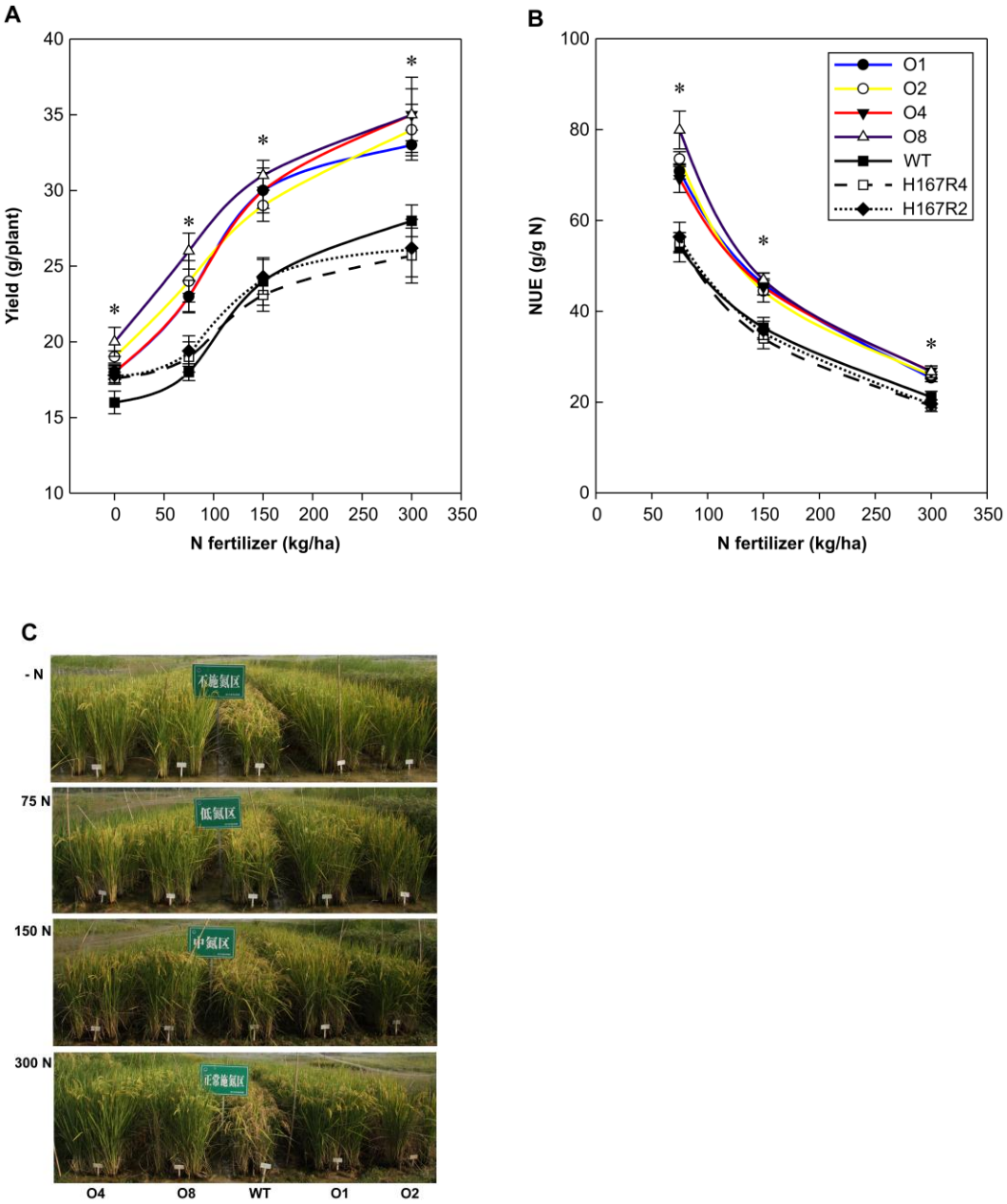


**Fig. S8.** The grain yield and NUE of T7 *OsNRT2.3b* over-expression lines grown in field plots under different N fertilizer supplies.

A: Grain yield in different N application in plot experiments; B: The NUE under different N applications in plot experiments. NUE: nitrogen use efficiency = g-grain yield/g-applied fertilizer N. Values are mean ± S.E (n = 3); C The growth of WT and T7 *OsNRT2.3b* over-expression lines in 0, 75, 150, 300 kg N/ha supplies condition in plot experiments. The symbol ***** above bars indicates a significant difference ($p < 0.05$) between the transgenic lines and WT at the same N fertilizer application rate estimated by ANOVA (one-way). The N application experiments were performed in plots at Changxing Experimental Station, Zhejiang University (May-Oct. 2012). In total 21 plots in each N block were used. Four blocks separated by walls were supplied with 0, 75, 150, 300 kg N/ha independently.

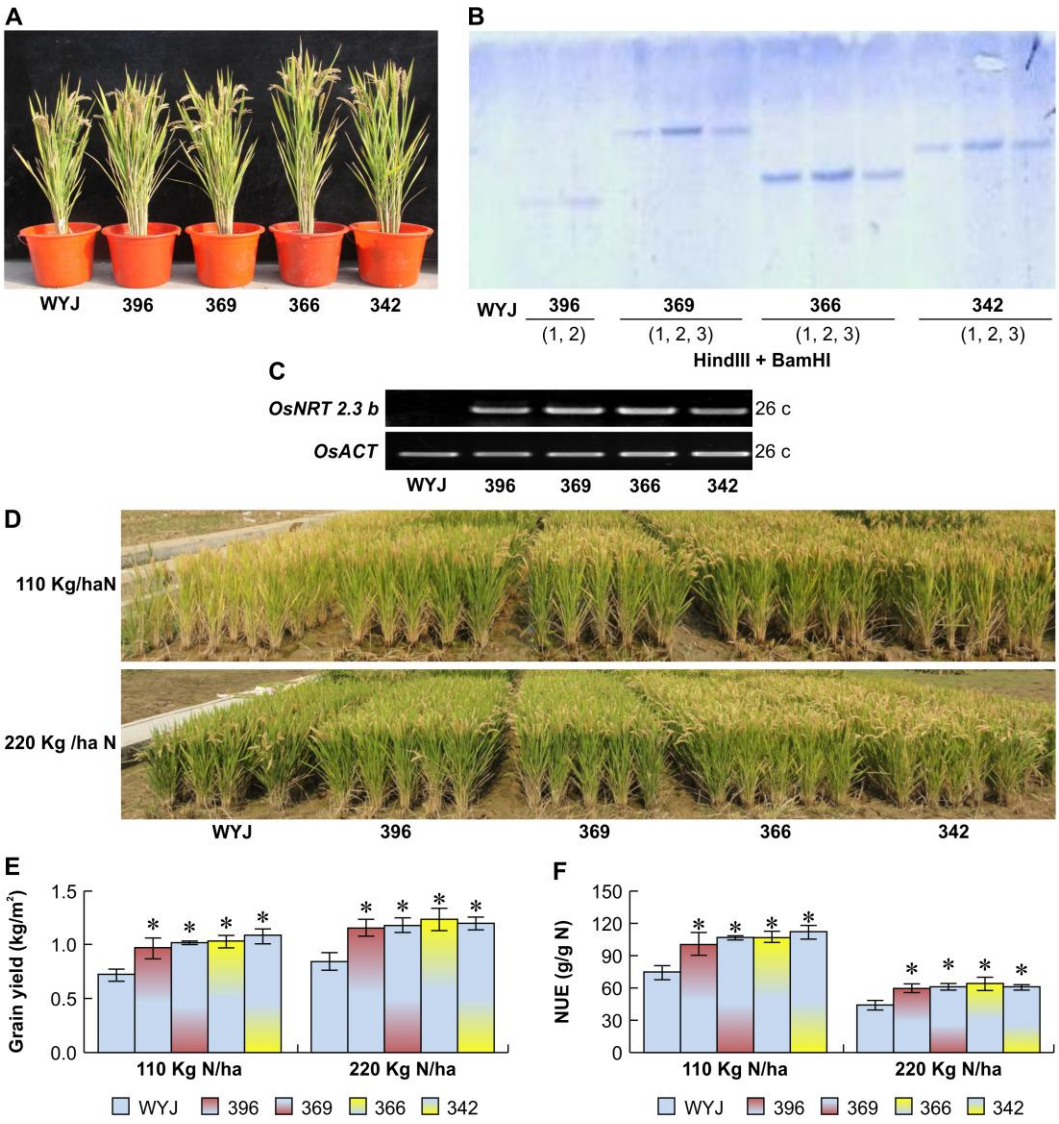


**Fig. S9.** The phenotype of *OsNRT2.3b* over-expression lines in the WYJ7 cultivar background.

A: The T5 pot experiment was performed in Nanjing in 2011 (Table S5). B: Southern blot of T1 plants. The T1 lines of 396-2, 369-1, 366-1 and 342-1 over-expressed *OsNRT2.3b* in comparison to their wild type (WYJ7 cultivar as WT) background. Then the 396-2, 369-1, 366-1 and 342-1 lines with one copy insertion were renamed as 396, 369, 366 and 342 for the further experiments; C: RT-PCR with primers (Table S10), 26 cycles were set for this PCR. D: T5 plot experiments at the Experimental Station of Zhejiang University (May 2011-Oct. 2011) with two N application levels 110 and 220 kg N/ha. Seeds were put to germinate on 5th May 2011 and 100 seedlings were transferred to the paddy field as 5 rows × 20 plants on 5th June and arranged randomly. Fertilizers were applied as listed in Table S5. The picture was taken on 10th Oct before harvest. The soil nutrient status before fertilizer addition was: total N 1.68 ± 0.21 mg/g, total P 0.48 ± 0.18 mg/g, total K 46.47 ± 2.85 mg/g, 0.5 mM $NaHCO_3$-extractable P 38 ± 2.1 mg/kg, soil pH 6.43 ± 0.28 (n= 6); e and f: The grain yield and NUE in plot experiment. Values are mean ± S.E (n = 3). The symbol * indicates a significant difference between WT and the over-expressers at

the 5 % level tested with a one-way ANOVA analysis.

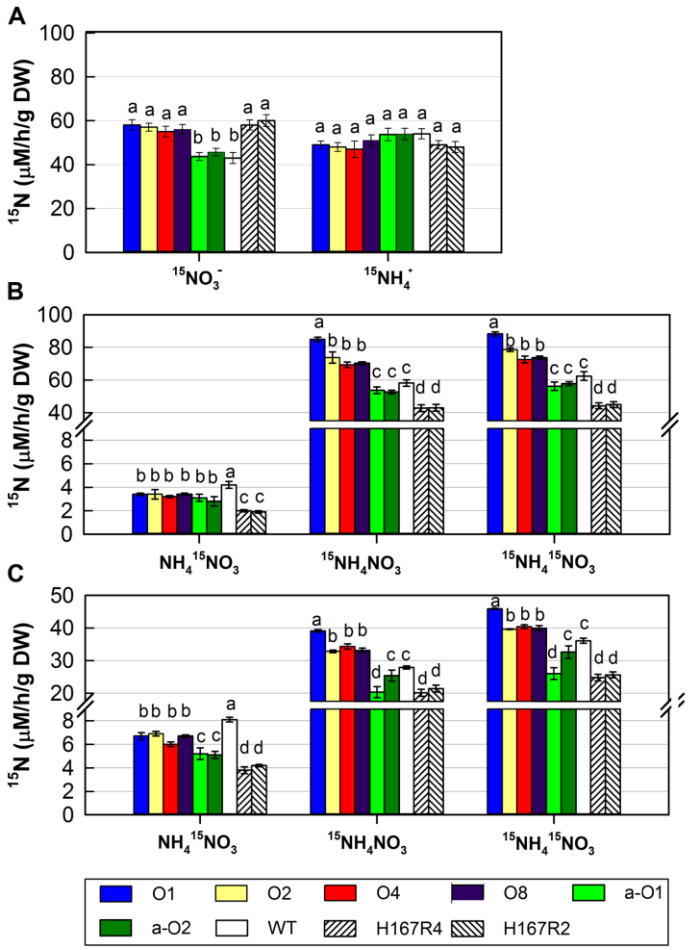


**Fig. S10.** The effects of *OsNRT2.3b*, *OsNRT2.3a* and H167R over-expression on the root influx of $^{15}NO_3^-$ and $^{15}NH_4^+$ at pH 6 and 4 for 5 min.

A: The root $^{15}N$ influx rate at 2.5 mM $NO_3^-$ (supplied as $Ca(NO_3)_2$) or $NH_4^+$ (supplied as $NH_4Cl$) at pH 6.0; B: The $^{15}N$ influx in $NH_4{}^{15}NO_3$, $^{15}NH_4NO_3$ and $^{15}NH_4{}^{15}NO_3$ supply at pH 6 ; C: The $^{15}N$ influx in $NH_4{}^{15}NO_3$, $^{15}NH_4NO_3$ and $^{15}NH_4{}^{15}NO_3$ supply at pH 4. The $^{15}N$ influx was measured after 5 min incubation. Values are mean ± S.E (n = 5). a, b, c, d letters above bars indicating significant differences ($p < 0.05$) between the transgenic lines and WT under the same treatment conditions and these were statistically tested by one-way ANOVA.

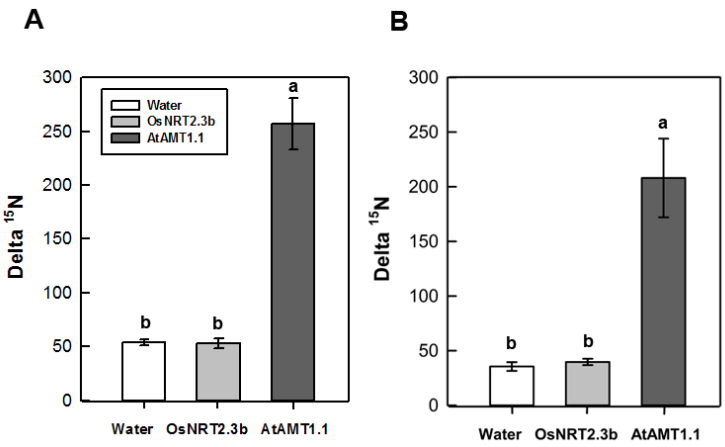


**Fig. S11.** OsNRT2.3b does not transport $^{15}NH_4^+$ in oocytes.

The oocytes were injected with water or *OsNRT2.3b* mRNA or positive control *AtATM1.1* mRNA; 0.5 mM $^{15}NH_4Cl$ (A) or 0.5 mM $^{15}NH_4NO_3$ (B) with atom% $^{15}N$ 98 % was added into ND96 solution and the oocytes were incubated overnight (16 h). Values are mean ± S.E (n = 15). a, b letters above bars indicating significant difference ($p < 0.05$) between the mRNA and water injected cells under the same treatment conditions and these were statistically tested by one-way ANOVA.

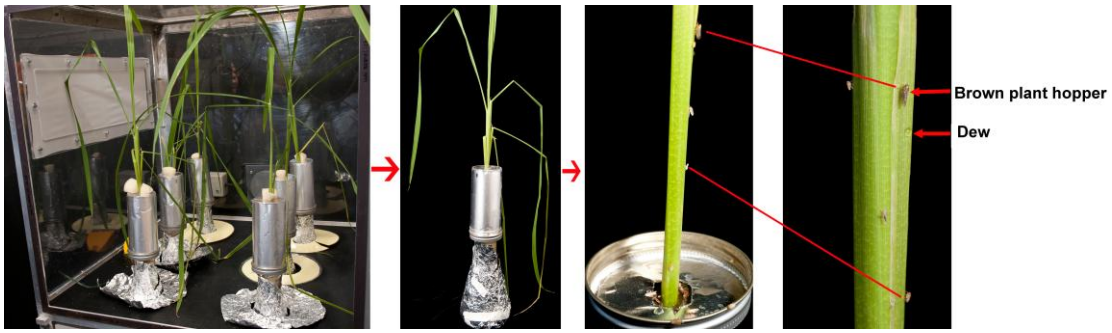


**Fig. S12.** The method for phloem sap sampling using the Brown Plant Hopper (*Nilapavata lugens*).

Rice seedlings were grown hydroponically in 1.25 mM $NH_4NO_3$ for 8 weeks and then transferred to N treatments (N: 2.5 mM $NO_3^-$; A: 2.5 mM $NH_4^+$). Each plant was placed in a 250 ml flask of IRRI nutrient solution with six plants kept in the insect cage at 26 °C and a 16 h light period. Seven to ten brown plant hopper adults were transferred on to each plant at the beginning of the N treatments. Rice phloem dew secreted by the insects feeding on the shoot was collected at 24 and 48 h after the N treatments began. Phloem sap pH was measured using a pH selective microelectrode (3); Five replicates were used for this experiment.

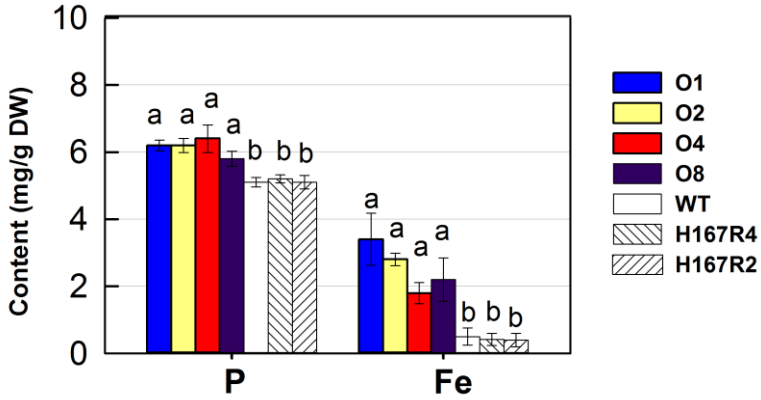


**Fig. S13.** The effect of *OsNRT2.3b* and H167R over-expression on total P and Fe content in rice leaves.

The total P and Fe content in leaves was measured by ICP analysis. A weighed sample of the dried crushed plant material powder (0.05 g) was digested with 5 ml of 98 % $H_2SO_4$ and 3 ml of 30 % hydrogen peroxide. After cooling, the digested sample was diluted to 100 ml with distilled water. The ion concentrations in the solution were measured using the ICP- OES (Perkin Elmer Optima 2000 DV). Values are mean ± S.E (n = 4). a, b letters above bars indicating significant difference ($p < 0.05$) between the transgenic lines and WT under the same treatment conditions and these were statistically tested by one-way ANOVA.

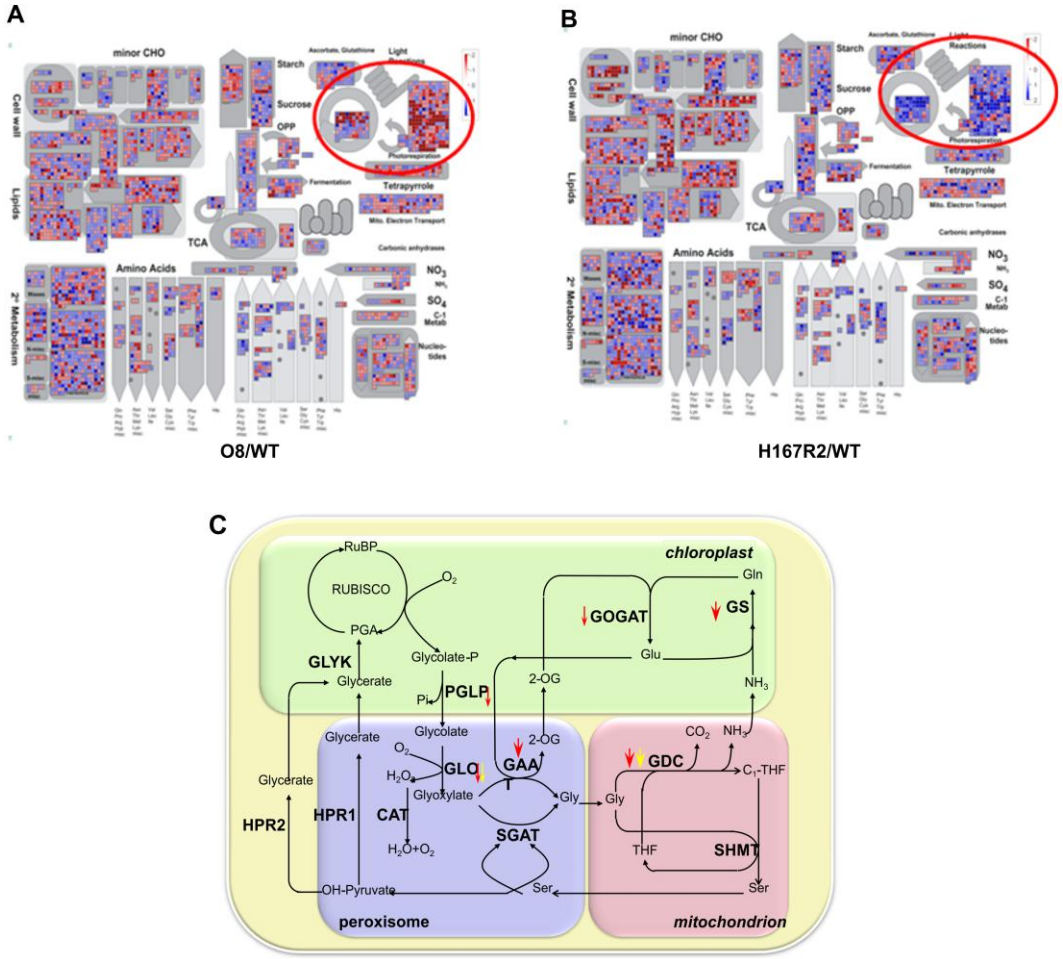


**Fig. S14.** Genome-wide expression analysis of *OsNRT2.3b* over-expression line O8 and H167R2 by gene chip microarray compared with WT.

A: T7 O8 microarray data compared with WT; B: the H167R2 microarray data compared with WT; C: the photorespiratory genes expression in O8 leaves compared with WT checked by microarray (yellow arrow) and qRT-PCR (red arrow, Table S7). The size of the arrows indicates the level of change in expression in O8 compared with WT. Three replicates of WT (*Nipponbare*), O8 and H167R2 leaves were harvested from 150 kg N /ha treatment in a field of Changxing experiment station at 10:00 am of the 1$^{st}$ August. This was the maximum tillering stage for all plants. Total RNA were extracted and analyzed by AFFYMETRIX microarray (Gene Tech, Shanghai) and submitted to NCBI. The experimental details are addressed in SI Appendix. The figures were drafted using the MapMan software (3.5.1R2 version).

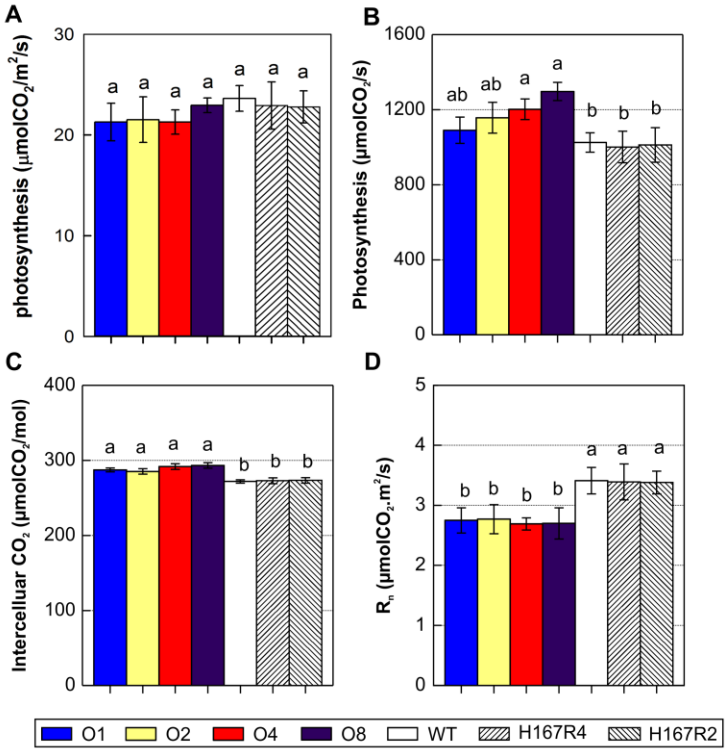


**Fig. S15.** The photosynthesis, intercellular $CO_2$ concentration and photorespiration in plants over-expressing *OsNRT2.3b* compared with WT.

A: The photosynthesis per unit of leaf area; B: The photosynthesis of a leaf was calculated as the net photosynthesis multiplied by the measured leaf area; C: Intercellular $CO_2$ concentration; D: The net dark respiration ($R_n$) was reached during $CO_2$ PIB recording at stable recording stage from 100 to 200 seconds after shutting off the light, according to Supplemental Figure S4 of Kebeish *et al.*, 2007 (12). The net photosynthesis, intercellular $CO_2$ concentration and photorespiration were measured on the same the plants for microarray analysis using a Li-Cor 6400 infrared gas analyzer as described before (11). Values are mean ± S.E (n = 4). a, b letters indicates significant differences between WT and over-expression plants at the 5 % level with one-way ANOVA analysis.

**Table S1. Consensus transmembrane (TM) secondary structure of OsNRT2.3a/OsNRT2.3b predicted.**

**For OsNRT2.3a**

| Program | TM | | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alom_v2 | 7 | N-in | 54 | 74 | | | 110 | 130 | 139 | 159 | | |
| DAS-Tnfilter | 8 | | 52 | 69 | | | 114 | 126 | 138 | 152 | | |
| HmmTop_v2 | 12 | N-in | 49 | 69 | 85 | 105 | 111 | 131 | 145 | 165 | 172 | 192 |
| Minnou | 12 | | 43 | 67 | 80 | 103 | 111 | 129 | 134 | 163 | 179 | 182 |
| Phobius | 11 | N-out | 50 | 70 | 81 | 101 | 111 | 131 | 137 | 157 | 176 | 196 |
| PredTmr_v1 | 9 | | 52 | 72 | | | 111 | 131 | 142 | 162 | 172 | 192 |
| Scampi | 8 | N-out | 49 | 69 | | | 101 | 127 | 138 | 158 | | |
| SosuiG_v1.1 : | 11 | | 51 | 71 | | | 111 | 131 | 136 | 156 | | 180 |
| SVMtm_v3 | 11 | | 47 | 67 | 85 | 105 | 109 | 129 | 139 | 159 | | |
| TmHMM_v2 | 10 | N-in | 57 | 71 | | | 109 | 129 | 138 | 158 | 175 | 195 |
| TMMOD | 11 | N-out | 52 | 72 | 83 | 103 | 111 | 131 | 138 | 158 | 174 | 194 |
| TmPred | 11 | N-in | 48 | 68 | 84 | 104 | 114 | 134 | 140 | 160 | 168 | 189 |
| TopPred_v2 | | | 45 | 65 | 85 | 105 | 112 | 132 | 142 | 162 | 173 | 193 |
| Topcon | 11 | N-in | 48 | 68 | 83 | 103 | 112 | 132 | 138 | 158 | | 180 |

**For OsNRT2.3b**

| Program | TM | | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alom_v2 | 7 | N-in | 50 | 66 | 85 | 101 | 108 | 124 | | | 179 | 195 |
| DAS-Tnfilter | 8 | | 52 | 70 | 83 | 96 | 108 | 122 | | | 181 | 195 |
| HmmTop_v2 | 11 | N-out | 50 | 69 | 82 | 101 | 116 | 134 | 143 | 162 | 179 | 198 |
| Minnou | 12 | | 46 | 71 | 82 | 117 | 122 | 131 | 136 | 166 | 175 | 198 |
| Phobius | 9 | N-out | 50 | 70 | 82 | 101 | 107 | 127 | 139 | 159 | 179 | 199 |
| PredTmr_v1 | 9 | | 50 | 67 | 82 | 101 | 112 | 132 | 143 | 162 | 179 | 198 |
| Scampi | 7 | N-in | 50 | 70 | 77 | 97 | 108 | 128 | | | 174 | 194 |
| SosuiG_v1.1 : | 11 | | 51 | 73 | 80 | 102 | 105 | 127 | 149 | 171 | 176 | 198 |
| SVMtm_v3 | 10 | | 49 | 70 | 82 | 96 | 111 | 127 | | | 176 | 197 |
| TmHMM_v2 | 10 | N-in | 50 | 72 | 82 | 100 | 107 | 129 | 144 | 166 | 179 | 198 |
| TMMOD | 10 | N-in | 50 | 70 | 82 | 101 | 108 | 128 | 143 | 166 | 179 | 199 |
| TmPred | 10 | N-in | 48 | 67 | 81 | 106 | 115 | 131 | 145 | 162 | 179 | 198 |
| TopPred_v2 | 10 | N-in | 47 | 67 | 82 | 102 | 112 | 132 | 143 | 163 | 179 | 199 |
| Topcon | 10 | N-in | 48 | 68 | 81 | 101 | 107 | 127 | | | 179 | 199 |

**Note**:14 The pH-sensing motif VYEAIHKI is around residue 197 for OsNRT2.3a and 167 for OsNRT2.3b.

**Table S2 NRT2 nitrate transporters which have the AE motifs.**

| Plant | Id number | Motif1 | Pos.1 | Motif 2 | Pos. 2 |
|---|---|---|---|---|---|
| Arabidopsis (AtNRT2.7) | AT5G14570 | FFVGFSLANF | 136 | EILIGGLGN | 259 |
| A. lyrata | 350602 | FFVGFSLANF | 136 | EILIGGLGN | 259 |
| Shepherd's purse | Carubv1000893 2m.g | FFVGFSLANF | 136 | EILIGGLGN | 251 |
| | Carubv10011028m | FSVASPI | 24 | LWIIQTAGG | 355 |
| Poplar | Potri.009G008500 | FSVASPI | 24 | LWIIQTAGG | 355 |
| | Potri.009G008600 | FSVASPI | 20 | LWIIQTAGG | 351 |
| Cucumber | Cucsa.268720 | WRIAFFVPGF | 213 | LWILQTLGGVF | 344 |
| Apple | MDP0000239537 | FSVASPI | 24 | LWIIQTAGG | 335 |
| Peach | ppa004104m.g | FSVASPI | 24 | LWIIQTAGG | 355 |
| Soybean | Glyma13g39850 | WRIAFFVPGF | 224 | LWILQTLGGVF | 355 |
| | Glyma12g30050 | WRIAFFVPGF | 224 | LWILQTLGGVF | 355 |
| Purple false brome | Bradi2g47640 | FFIPGVMQTF | 209 | LWVVQTIGG | 336 |
| Barley | MLOC_75087.1 | FFIPGVMQTF | 209 | LWVVQTIGG | 336 |

**Table S3. Copy numbers of *OsNRT2.3b* and *OsNRT2.3a* over-expressing T0 and T1 plants in the *Nipponbare* background.**

| *NRT2.3b*-OE T0 lines | Relative quantity | Absolute quantity | Copy numbers | Genotype |
|---|---|---|---|---|
| WT-1 | | 0.05 | 0 | aa |
| bUbi1 | 0.64 | 0.26 | 1 | Aa |
| bUbi2 | 1.16 | 0.43 | 1 | Aa |
| b35s1 | 4.30 | 1.67 | >2 | AaBb |
| b35s2 | 1.70 | 0.63 | >1 | Aa |
| b35s3 | 10.87 | 4.50 | >4 | AaBbCcDd |
| b35s4 | 2.13 | 0.74 | 2 | AaBb |
| b35s5 | 4.38 | 1.73 | >2 | AaBb |
| b35s6 | 1.14 | 0.40 | 1 | Aa |
| b35s7 | 4.43 | 1.61 | >2 | AaBb |

| *NRT2.3b*-OE T1 lines | Relative quantity | Absolute quantity | Copy numbers | Genotype |
|---|---|---|---|---|
| WT | | 1.23 | | |
| bUbi1-1**(O1)** | 2.40 | 3.09 | 1 | AA |
| bUbi1-2 | 0.74 | 0.86 | 1 | Aa |
| bUbi2-1**(O2)** | 1.17 | 1.41 | 1 | AA |
| bUbi2-2 | 0.33 | 0.36 | 1 | Aa |
| b35S2-1 | 0.96 | 0.94 | 1 | Aa |
| b35S2-2**(O4)** | 1.65 | 2.01 | 1 | AA |
| b35S2-3 | 0.48 | 0.50 | 1 | Aa |
| b35S6-1 | 0.64 | 0.68 | 1 | Aa |
| b35S6-2 | 0.71 | 0.81 | 1 | Aa |
| b35S6-3 | 0.02 | 0.03 | 0 | aa |
| b35S6-4**(O8)** | 2.14 | 2.40 | 1 | AA |

| *NRT2.3a*-OE T0 lines | Relative quantity | Absolute quantity | Copy numbers | Genotype |
|---|---|---|---|---|
| WT-1 | | 0.05 | 0 | aa |
| aUbi40 | 2.44 | 2.26 | 2 | AaBb |
| aUbi41 | 4.16 | 4.43 | 6 | AaBbCcDd |
| aUbi44 | 4.30 | 2.67 | >2 | AaBb |
| aUbi45 | 1.70 | 1.63 | >1 | Aa |
| aUbi48 | 1.07 | 0.80 | 1 | Aa |
| aUbi49 | 2.13 | 0.74 | 2 | AaBb |
| aUbi56 | 3.74 | 2.85 | >2 | AaBb |
| aUbi66 | 3.10 | 2.45 | >2 | AaBb |
| aUbi69 | 0.98 | 0.73 | 1 | Aa |
| aUbi70 | 4.43 | 1.61 | >2 | AaBb |

| *NRT2.3a*-OE T1 lines | Relative quantity | Absolute quantity | Copy numbers | Genotype |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| WT | | 1.23 | | |
| aUbi48-1**(a-O1)** | 2.12 | 2.09 | 1 | AA |
| aUbi48-2 | 0.94 | 0.82 | 1 | Aa |
| aUbi48-3 | 1.32 | 1.61 | 1 | AA |
| aUbi69-1 | 0.84 | 0.78 | 1 | Aa |
| aUbi69-2 | 0.93 | 0.85 | 1 | Aa |
| aUbi69-3 | 0.62 | 0.73 | 1 | Aa |
| aUbi69-4**(a-O2)** | 2.01 | 2.11 | 1 | AA |

**Note**: The relative quantification was calculated by comparison with sucrose phosphate synthase (SPS), a single-copy of the rice gene (13,14). The absolute quantification was calculated according to the method described previously (15). Both methods were used to calculate the copy numbers in over-expression plants. One copy insertion T0 plants (Aa) were harvested and grown to generate T1 plants. T1 plants (AA) were taken as being homozygous; bUbi means OsNRT2.3b Ubi promoter over-expression line; b35S means OsNRT2.3b 35S promoter over-expression line; aUbi means OsNRT2.3a Ubi promoter over-expression line. The bUbi1-1, bUbi2-1, b35s2-2 and b35s6-4 lines were renamed as O1, O2, O4 and O8 ; aUbi48-1 and aUbi69-4 lines were renamed as a-O1 and a-O2 for the further experiments. Southern blot analysis (Fig. S7A) confirmed this result.

**Table S4. Copy numbers of *OsNRT2.3b* and H167R mutant over-expressing T0 and T1 plants in the *Nipponbare* background.**

| *H167R*-OE T0 lines | Copy numbers | Genotype |
|---|---|---|
| WT-1 | 0 | aa |
| H1 | 2 | AaBb |
| H2 | 1 | Aa |
| H3 | 2 | AaBb |
| H4 | 1 | Aa |
| H5 | 3 | AaBbCc |
| H6 | 4 | AaBbCcDd |
| H10 | 2 | Aa |
| H11 | 3 | AaBbCc |
| H12 | 0 | aa |
| H13 | 0 | aa |

| *H167R*-OE T1 lines | Copy numbers | Genotype |
|---|---|---|
| WT | | |
| H2-1**(H167R2)** | 1 | AA |
| H2-2 | 1 | AA |
| H2-3 | 1 | AA |
| H4-1**(H167R4)** | 1 | AA |
| H4-2 | 1 | AA |

**Note**: Southern blot results summary for the copy number of the T0 and T1 over-expression lines.

**Table S5. Summary of field experiments for the over-expression of *OsNRT2.3b***

| Year | Cultivar | Promoter | Location | Generation | Lines No. | Repeat |
|---|---|---|---|---|---|---|
| 2009-4-2009-10 | WYJ/ Nipponbare | 35s/Ubi | Changxing /Nanjing (subtropical) | T1 | 9 | 3 |
| 2009-12-2010-5 | WYJ/ Nipponbare | 35s/Ubi | Hainan (tropical) | T2 | 8/4 | 3 |
| 2010-4-2010-10 | WYJ/ Nipponbare | 35s/Ubi | Changxing /Nanjing (subtropical) | T3 | 4/4 | 3 |
| 2010-12-2011-5 | WYJ/ Nipponbare | 35s/Ubi | Hainan (tropical) | T4 | 4/4 | 3 |
| 2011-4-2011-10 | WYJ/ Nipponbare | 35s/Ubi | Changxing /Nanjing (subtropical) | T5 | 4/4 | 3 |
| 2011-12-2012-5 | WYJ/ Nipponbare | 35s/Ubi | Hainan (tropical) | T6 | 4/4 | 3 |
| 2012-5-2012-10 | WYJ/ Nipponbare | 35s/Ubi | Changxing /Nanjing (subtropical) | T7 | 4/4 | 3 |
| 2012-12-2013-5 | WYJ/ Nipponbare | 35s/Ubi | Hainan (tropical) | T8 | 4/4 | 3 |
| 2013-5-2013-10 | WYJ/ Nipponbare | 35s/Ubi | Changxing /Nanjing (subtropical) | T9 | 4/4 | 3 |

**Table S6. The agronomic traits of *OsNRT2.3b* and H167R T7 over-expression plants and WT at four different N fertilizer treatments.**

| | 0 kg N | 75 kg N | 150 kg N | 300 kg N |
|---|---|---|---|---|
| Genotypes | | Dry weight (g/plant) | | |
| O1 | 35.0±2.2a | 40.5±2.3a | 57.1±2.3a | 60.8±5.4b |
| O2 | 37.5±1.7a | 40.5±3.1a | 59.4±3.4a | 64.3±3.5b |
| O4 | 37.1±1.9a | 40.1±3.6a | 57.9±4.1a | 63.4±5.7b |
| O8 | 38.4±3.1a | 40.6±2.8a | 60.6±4.8a | 65.5±5.2b |
| WT | 21.2±0.6b | 21.9±1.1b | 37.2±1.0b | 47.2±4.0c |
| H167R4 | 37.3±3.6a | 40.3±4.6a | 59.6±5.2a | 82.7±4.0a |
| H167R2 | 39.8±4.0a | 41.6±3.9a | 62.7±5.0a | 89.9±4.0a |
| | | Effective tillering No. | | |
| O1 | 8.1±0.9b | 8.5±0.9b | 10.2±1.5b | 9.3±1.3b |
| O2 | 8.1±1.1b | 8.6±1.0b | 9.2±1.8b | 9.2±1.2b |
| O4 | 8.2±1.2b | 8.4±13b | 9.3±1.9b | 9.5±1.2b |
| O8 | 8.3±1.1b | 8.6±1.1b | 9.4±1.7b | 9.2±1.1b |
| WT | 9.3±0.9b | 9.3±0.8b | 11.0±1.5b | 11.3±1.1b |
| H167R4 | 14.3±1.2a | 15.2±1.8a | 16.6±1.9a | 17.1±1.2a |
| H167R2 | 14.1±1.1a | 15.3±2.4a | 16.1±1.7a | 17.8±2.1a |
| | | Seed No./ panicle | | |
| O1 | 140 ±8.0a | 159 ±9.5a | 142 ±8.0a | 154 ±7.0a |
| O2 | 148±9.1a | 164±10.1a | 165±9.1a | 167±11.1a |
| O4 | 148±8.6a | 160±9.1a | 165±9.6a | 163±12.6a |
| O8 | 152±8.9a | 174±11.4a | 170±8.9a | 180±13.9a |
| WT | 116±4.4b | 119±7.1b | 117±6.4b | 120±6.4b |
| H167R4 | 77±7.1c | 78±8.1c | 80±5.7c | 83±5.1c |
| H167R2 | 78±7.2c | 79±5.3c | 83±7.9c | 85±4.2c |
| | | Weight/1000 seeds | | |
| O1 | 23.2±0.2a | 24.2±0.4a | 24.2±0.3a | 25.0±0.3a |
| O2 | 23.0±0.3a | 24.3±0.2a | 24.1±0.4a | 25.2±0.6a |
| O4 | 22.9±0.3a | 24.2±0.3a | 24.1±0.5a | 25.0±0.8a |
| O8 | 23.0±0.3a | 24.3±0.4a | 24.1±0.4a | 25.0±0.8a |
| WT | 23.2±0.2a | 24.4±0.2a | 24.6±0.2a | 25.0±0.6a |
| H167R4 | 25.7±0.2a | 25.4±0.2a | 25.8±1.2a | 25.7±1.2a |
| H167R2 | 25.7±0.2a | 25.7±0.2a | 25.8±0.9a | 25.7±1.2a |
| | | Seed setting rate (%) | | |
| O1 | 72.0±2.0a | 78.0±1.5a | 88.0±2.9a | 95.1±3.0a |
| O2 | 73.0±1.9a | 78.8±2.9a | 88.3±2.4a | 94.5±2.9a |
| O4 | 72.0±2.6a | 78.8±2.3a | 89.0±2.2a | 93.5±1.6a |
| O8 | 74.0±2.8a | 79.5±2.4a | 88.0±2.6a | 92.9±3.1a |
| WT | 64.9±2.4b | 68.1±2.2b | 78.9±2.8b | 83.3±2.2b |
| H167R4 | 62.9±5.9b | 62.7±6.2b | 69.5±6.8bc | 67.7±4.9c |
| H167R2 | 62.1±3.8b | 62.8±4.2b | 68.4±5.8c | 70.3±5.2c |

| | | | | |
|---|---|---|---|---|
| | | Grain weight (g/plant) | | |
| O1 | 18.9±1.4b | 25.5±1.5a | 31.8±1.3a | 34.0±2.0a |
| O2 | 20.1±0.9a | 27.1±2.1a | 32.5±1.8a | 36.7±2.0a |
| O4 | 20.0±1.0a | 25.6±2.0a | 33.0±2.3a | 36.1±2.3a |
| O8 | 21.5±1.7a | 28.8±2.0a | 33.9±2.7a | 38.2±2.4a |
| WT | 16.2±0.4c | 18.6±0.9b | 24.7±0.8b | 28.2±1.0b |
| H167R4 | 17.6±0.5b | 19.0±1.0b | 23.1±1.1b | 25.7±1.9b |
| H167R2 | 17.8±0.4b | 19.4±1.0b | 24.3±1.1b | 26.2±1.8b |
| | | NUE (g/g N) | | |
| O1 | | 70.8±1.6a | 46.0±2.3a | 25.3±0.8a |
| O2 | | 73.5±1.6a | 44.4±2.3a | 25.9±0.8a |
| O4 | | 69.2±3.2a | 45.4±1.8a | 26.8±0.7a |
| O8 | | 79.9±4.1a | 46.9±1.6a | 26.7±1.2a |
| WT | | 54.1±3.2b | 36.4±1.5b | 21.1±1.8b |
| H167R4 | | 55.1±2.2b | 34.0±2.2b | 19.2±1.3b |
| H167R2 | | 56.4±3.2b | 35.8±1.9b | 19.6±0.8b |

**Note**: Ten replicate plants of each plot were sampled for this agronomic analysis and three plot replications are shown in Fig. S8C. Values are mean ± S.E. (n = 30). Small letters (a, b, c) indicate a significant difference at 5 % levels when compared with WT.

**Table S7. The qRT-PCR of photorespiration related genes in *OsNRT2.3b* over -expression line O4 and O8 plants compared with WT.**

| Accession No. | Annotation | WT | O4 | O8 | P –value |
|---|---|---|---|---|---|
| AK067732.1 | GGAT | 10 ±0.0 | 2.6 ±0.2 | 3.2 ±0.2 | 0.00 |
| AK104854.1 | PGLP | 10±0.0 | 3.3±0.1 | 5.4±0.2 | 0.00 |
| AK065491.1 | GS2 | 10±0.0 | 2.9±0.2 | 4.3±0.5 | 0.01 |
| AK068130.1 | Fd-GOGAT | 10±0.0 | 3.1±0.3 | 5.7±0.8 | 0.02 |
| AK098878 | GLO | 10±0.0 | 3.2±0.2 | 4.1±0.4 | 0.01 |
| AK062851 | GDC | 10±0.0 | 3.5±0.3 | 4.1±0.3 | 0.01 |

**Note**: The same tissue samples as used for microarray analysis with three replications. The qRT-PCR primer was discussed in Table S12.

**Table S8. The primers used for over-expression constructs**

| Promoter | Vector | | Primers | Enzyme |
|---|---|---|---|---|
| CaMV-35S | pCAMBIA1302 | F | atCCATGGAGATCTCAGGGCACAGCGGATG | BglⅡ |
| | | R | atCCATGGAGATCT ACACCCCGGCCGG | BglⅡ |
| Ubiquitin | pTCK303 | F | caACTAGTGCTACCACGTGTTGGAGATG | SpeI |
| | | R | gaACTAGTGAGCAAACCACCAACAAGC | SpeI |

**Table S9. The primers used for H167R site mutant of *OsNRT2.3b***

| Mutation | Mutagenic primer | Codon change | New restriction site |
|---|---|---|---|
| H167R-F | **GCCATT CGA AAGATCGGTAGCACGC** (original sequence: GCCA**TC CAC A**AGATC GGTAGCACGC)[a] | **CAC (H) – CGA (R)** | Csp45 I (**TTCGAA**) |
| H167R-R | GCATTCTAGATTCGAATGGCCTCGTACACG | | Xba I ( **TCTAGA**) |
| H167RB-F | T7 | | XbaI (**TCTAGA**) |
| H167RB-R | GCATTCTAGA**TT CGA A**TGGCCTCGTACACG | | Csp45I (**TTCGAA**) |

[a] The product of A**TT** and A**TC** is the same amino acid, isoleucine.

**Table S10. The primers used for RT-PCR of *OsNRT2.3* gene**

| Genes | Primers |
|---|---|
| *OsNRT2.3a* (AK109776) | F: 5'- GCTCATCCGCGACACCCT -3'<br>R:5'- GTCGAAGCGGTCG TAGAA -3' |
| *OsNRT2.3b* (AK072215) | F:5'- CGTTCGCCGTGTT -3'<br>R:5'- TCGAAGCGGTCGTAG AAG -3' |
| *OsActin* (NM_197297) | F:5'-TTATGGTTGGGATGGGACA-3'<br>R:5'-AGCACGGCTTGAATAGCG-3' |

**Table S11. The tail PCR primers and reaction programs for the identification of the insertion location of *OsNRT2.3b* over-expression line O4 and O8 in *Nipponbare* over-expression plants.**

| | Tail PCR Primers |
|---|---|
| SP1 | GCATGACGTTATTTATGAGATGGG |
| SP2 | AATATAGCGCGCAAACTAGG |
| SP3 | GCGGTGTCATCTATGTTAC |
| AD1 | TGWGNAGWANCASAGA |
| AD2 | WAGTGNAGWANCANAGA |
| AD3 | STTGNTASTNCTNTGC |
| AD4 | WCAGNTGWTNGTNCTG |

REACTION PROGRAM (Control Method: CALCULATED):

| TAIL 1° | TAIL 2° | TAIL 3° |
|---|---|---|
| 1=4° for 2 min. | 1=95° for 2 min. | 1=95° for 2 min. |
| 2=92° for 2 min. | 2=94° for 10 sec. | 2=94° for 10 sec. |
| 3=95° for 1 min. | 3=66° for 1 min. | 3=60° for 1 min. |
| 4=94° for 30 sec. | 4=72° for 2 min. | 4=72° for 2 min. |
| 5=66° for 1 min. | 5=94° for 10 sec. | 5=Go to step 2, for 19 more cycles |
| 6=72° for 2 min. | 6=66° for 1 min. | 6=72° for 5 min. |
| 7=Go to step 4 for 5 more cycles | 7=72° for 2 min. | 7=4° forever |
| 8=94° for 30 sec. | 8=94° for 10 sec. | 8=END |
| 9=30° for 3 min. | 9=44° for 1 min. | |
| 10=Ramp for 72° at 0.2°/sec, 72° for 2 min. 30 sec. | 10=72° for 2 min. | |
| 11=94° for 5 sec. | 11=Go to step 2, for 12 more cycles | |
| 12=66° for 1 min. | 12=72° for 5 min. | |
| 13=72° for 2 min. | 13=4° forever | |
| 14=94° for 5 sec. | 14=END | |
| 15=66° for 1 min. | | |
| 16=72° for 2 min. | | |
| 17=94° for 5 sec. | | |
| 18=44° for 1 min. | | |
| 19=72° for 2 min. | | |
| 20=Go to step 12, for 15 more cycles | | |
| 21=72° for 5 min. | | |
| 22=4° forever | | |
| 23=END | | |

**Table S12. The primers used for real time qRT-PCR of photorespiratory genes**

| Gene | Accession No. | Primers |
|---|---|---|
| GGAT | AK067732.1 | AGGAGAGCACGCCTGATGAC |
| | | AGGCAGTAGAAAACATCGGC |
| PGLP | AK104854.1 | GTTGGAGCAGTTGTTGTGGG |
| | | CAACCATTGACCCTCCAC |
| GS2 | AK065491.1 | GAAGGTATGAAGAACTTGGACG |
| | | TAACTGGCGAATGGAAGGTG |
| Fd-GOGAT | AK068130.1 | GTGCCGAGTTTGAACGAG |
| | | ACACCTTCCTGTTGTCTAATC |
| GLO | AK098878 | ACGACGCCAGAGAGCACGCC |
| | | GGATTAAGAGCATGAACGACCC |
| GDC | AK062851 | ATCAAGGTGAAGCCGAGCAG |
| | | AGGGAGAAATTAGGTCGGAGTG |
| *OsActin* | AB047313 | TTATGGTTGGGATGGGACA |
| | | AGCACGGCTTGAATAGCG |