

Table S1. Primers used to amplify the *OsNRT2.1* open reading frame.

Primer name	Forward primer (5'to3')	Reverse primer (5'to3')
<i>OsNRT2.1</i>	CCAAGGTACCATGGACTCGTCGACGGTGGGC	GGCCACTAGTTTAGGCCGTGCTCCGGCGAG

Table S2. Primers used to amplify the *OsNAR2.1* and *Ubiquitin* promoters.

Primer name	Forward primer (5'to3')	Reverse primer (5'to3')
<i>OsNAR2.1</i> Promoter	GAGGCGCGCCTGCTGACAAACCAAACCGACT	CATTAATTAACCCACCTCTCCCACCTCAC
<i>Ubiquitin</i> Promoter	GGCGCGCCGTCGACCTGCAGGTCGAC	TTAATTAAGTGCAGTGCAGCGTGACCC

Table S3. Primers used to detect *OsActin*, *OsNAR2.1*, and *OsNRT2.1* gene expression.

Gene name	Accession No.	Forward primer (5'to3')	Reverse primer (5'to3')
<i>OsActin</i>	AB047313	GGAACTGGTATGGTCAAGGC	AGTCTCATGGATAACCGCAG
<i>OsNAR2.1</i>	AP004023	GTCGTCGAGAAGCGCAAGA	GTCCACTGAAGCTGCGAACTT
<i>OsNRT2.1</i>	AB008519	AGCACCATGTTCAACAGCAAGA	CGAAGTAGGCCAGCCTCCAC

Table S4. Methods of NUE calculations

Components:	Expressed as:	Definition:
Agronomic nitrogen use efficiency	ANUE	G_w / N_s
Nitrogen recovery efficiency	NRE	$RTNA / N_s \times 100\%$
Physiological nitrogen use efficiency	PNUE	$G_w / TNAM$
Nitrogen harvest index	NHI	$GNAM / TNAM \times 100\%$
Dry matter translocation	DMT	$DMA - (DMM - \text{Grain Yield})$
Dry matter translocation efficiency	DMTE	$DMT / DMA \times 100\%$
Contribution of pre-anthesis assimilates to grain yield	CPAY	$DMT / \text{Grain Yield} \times 100\%$
Harvest index	HI	$\text{Grain Yield} / DMM \times 100\%$
Post-anthesis N uptake	PANU	$TNAM - TNAA$
Nitrogen translocation	NT	$TNAA - (TNAM - GNAM)$
Nitrogen translocation efficiency	NTE	$NT / TNAA \times 100\%$
Contribution of pre-anthesis N to grain nitrogen accumulation	CPNGN	$NT / GNAM \times 100\%$

Note: G_w = grain yield – grain yield of zero-N plot; $RTNA$ = $TNAM$ of N-treated plot – $TNAM$ of zero-N plot; N_s = N supply; $TNAM$ = total N accumulation at maturity; $GNAM$ = grain N accumulation at maturity.

Table S5. Real-time quantitative RT-PCR analysis of endogenous *OsNRT2.1* and *OsNAR2.1* expression in various transgenic lines and wild-type (WT) plants.

<i>OsNRT2.1</i>		<i>pUBi:OsNRT2.1</i>				<i>pOsNAR2.1:OsNRT2.1</i>			
Organs in plant	WT	OE1	OE2	OE3	I-fold	O6	O7	O8	I-fold
Seed	1.0	4.4	4.9	6.4	4.2	1.1	1.0	1.0	0
PL	1.2	5.6	7.7	10.3	5.5	1.4	1.4	1.3	0.1
Leaf blade I	2.2	22.8	18.2	26.5	9.0	2.7	2.9	2.5	0.2
Leaf blade II	2.9	29.2	30.6	32.7	9.4	2.7	2.7	3.3	0
Leaf blade III	3.2	27.9	31.9	40.8	9.5	3.4	3.5	3.5	0.1
Leaf sheath I	7.2	42.8	49.3	63.5	6.2	17.1	17.5	17.8	1.4
Leaf sheath II	6.8	45.7	49.9	63.1	6.7	15.6	15.7	15.1	1.3
Leaf sheath III	6.0	43.9	47.3	61.0	7.5	12.2	12.8	11.4	1
Inter node I	3.1	23.2	28	32.2	8	6.7	6.6	6.4	1.1
Inter node II	3.3	29.2	33.8	36.7	9.2	8.4	9.0	8.7	1.7
Inter node III	3.7	35.9	45.2	46.2	10.6	8.8	10.8	11.0	1.8
Root	21.1	98.7	110.7	151.2	4.7	52.5	55.8	58.7	1.6
<i>OsNAR2.1</i>		<i>pUBi:OsNRT2.1</i>				<i>pOsNAR2.1:OsNRT2.1</i>			
Organs in plant	WT	OE1	OE2	OE3	I-fold	O6	O7	O8	I-fold
Seed	0.3	0.6	0.7	0.8	1.8	0.3	0.2	0.2	0
PL	0.3	0.9	1.2	1.4	2.6	0.4	0.4	0.3	0.1
Leaf blade I	0.3	1.8	1.6	1.8	5.3	0.3	0.3	0.3	0.1
Leaf blade II	0.3	1.6	2	2.1	4.6	0.3	0.4	0.5	0.2
Leaf blade III	0.3	1.3	1.5	2.2	4.6	0.3	0.4	0.3	0.1
Leaf sheath I	2.1	9.3	9.5	10.5	3.6	6.7	6.7	6.8	2.2
Leaf sheath II	2.0	9.6	9.8	10.1	3.9	7.0	7.0	6.7	2.4
Leaf sheath III	1.8	7.4	7.8	8.7	3.3	5.5	5.5	5.3	1.9
Inter node I	0.7	4.3	4.66	5.3	5.8	2.4	2.4	2.4	2.4
Inter node II	0.7	4.4	4.3	5.4	5.4	2.9	2.9	2.9	2.9
Inter node III	0.8	5.3	6.2	6.7	6.2	3.0	3.0	3.2	2.7
Root	4.9	15.5	17.2	23.1	2.8	12.7	13.1	14.2	1.7

Note: I-fold: The average increase fold compared with WT. PL means palea and lemma. RNA was extracted from 14 days after pollination. We defined the *OsNRT21* expression of WT was set equal to 1

Figure S1. Diagram of *pUbi:OsNRT2.1* and *pOsNAR2.1:OsNRT2.1* constructs.

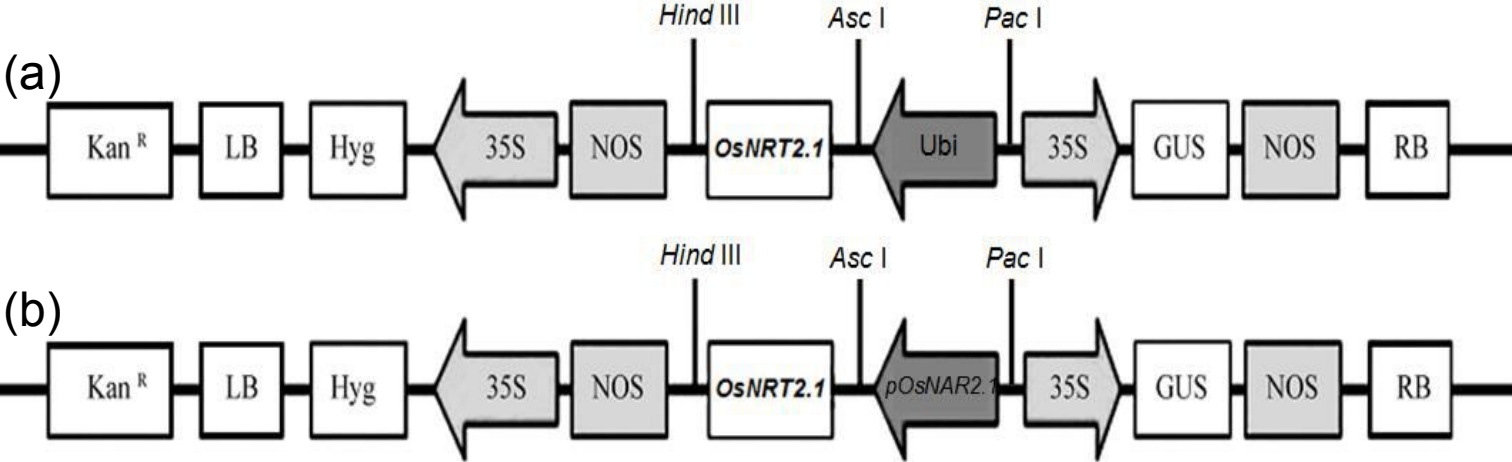


Figure S2. Characterization of T0 generation transgenic lines.

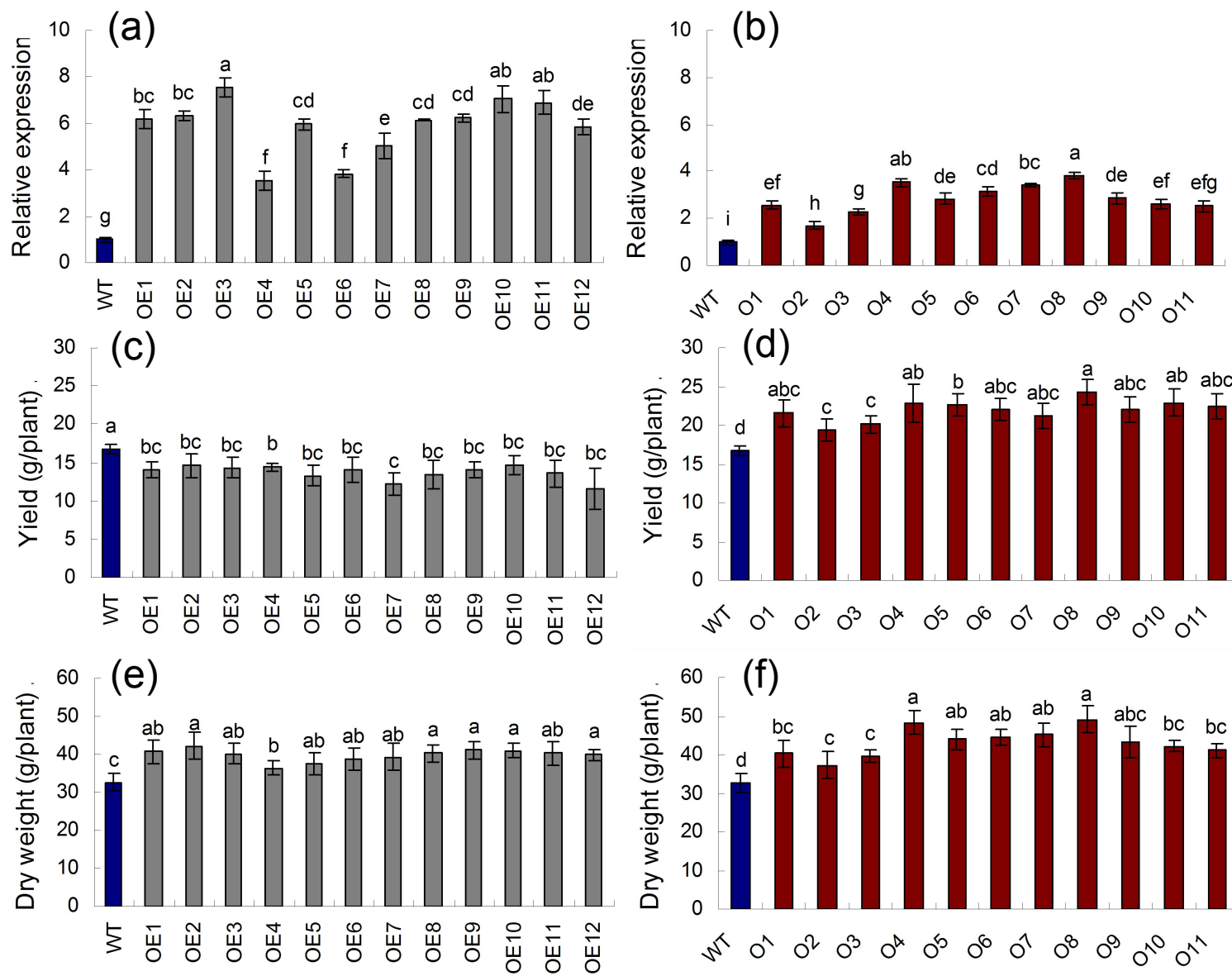


Figure S3. Grain yield and dry weight of WT and T1 generation transgenic plants.

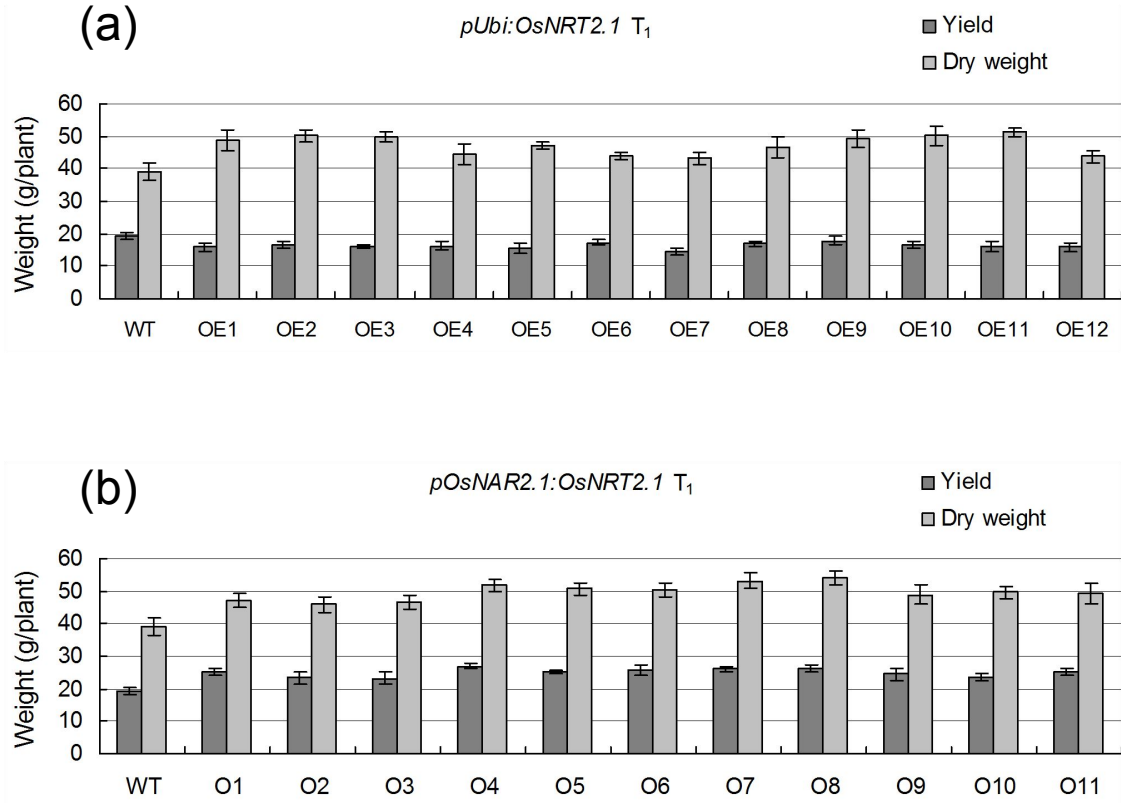


Figure S4. Southern blot analysis of transgene copy number.

M P O4-2 O5-5 O6-4 O7-6 O8-3 O9-9 OE1-2 OE2-5 OE3-4 OE4-2 OE5-1

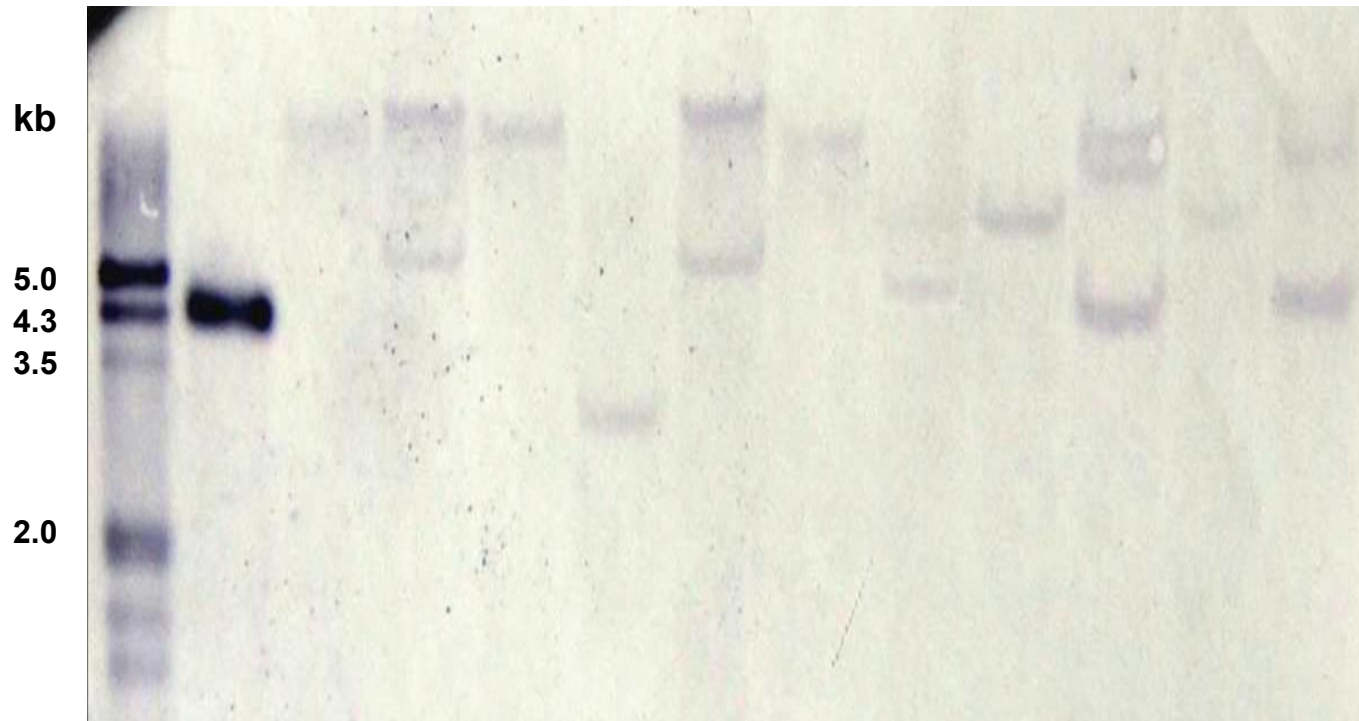


Figure S5. Grain yield, dry weight and ANUE of WT and T4 generation transgenic plants under low and normal N supplies .

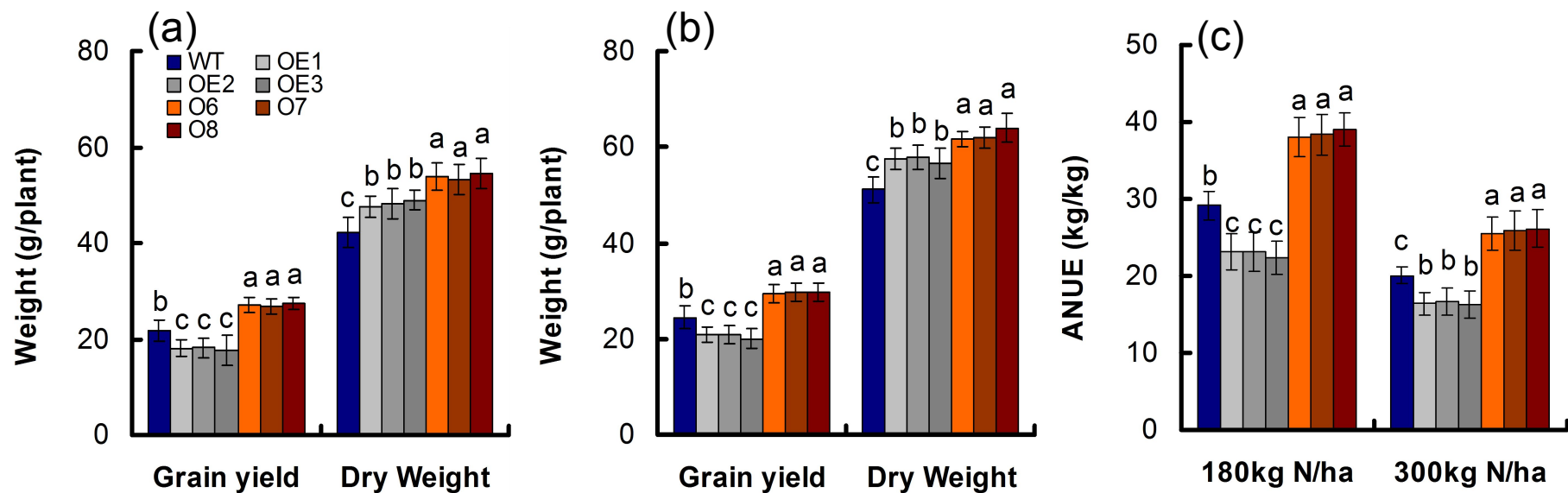


Figure S6. The diagramma of RNA sampling in T4 generation transgenic lines and WT plants.

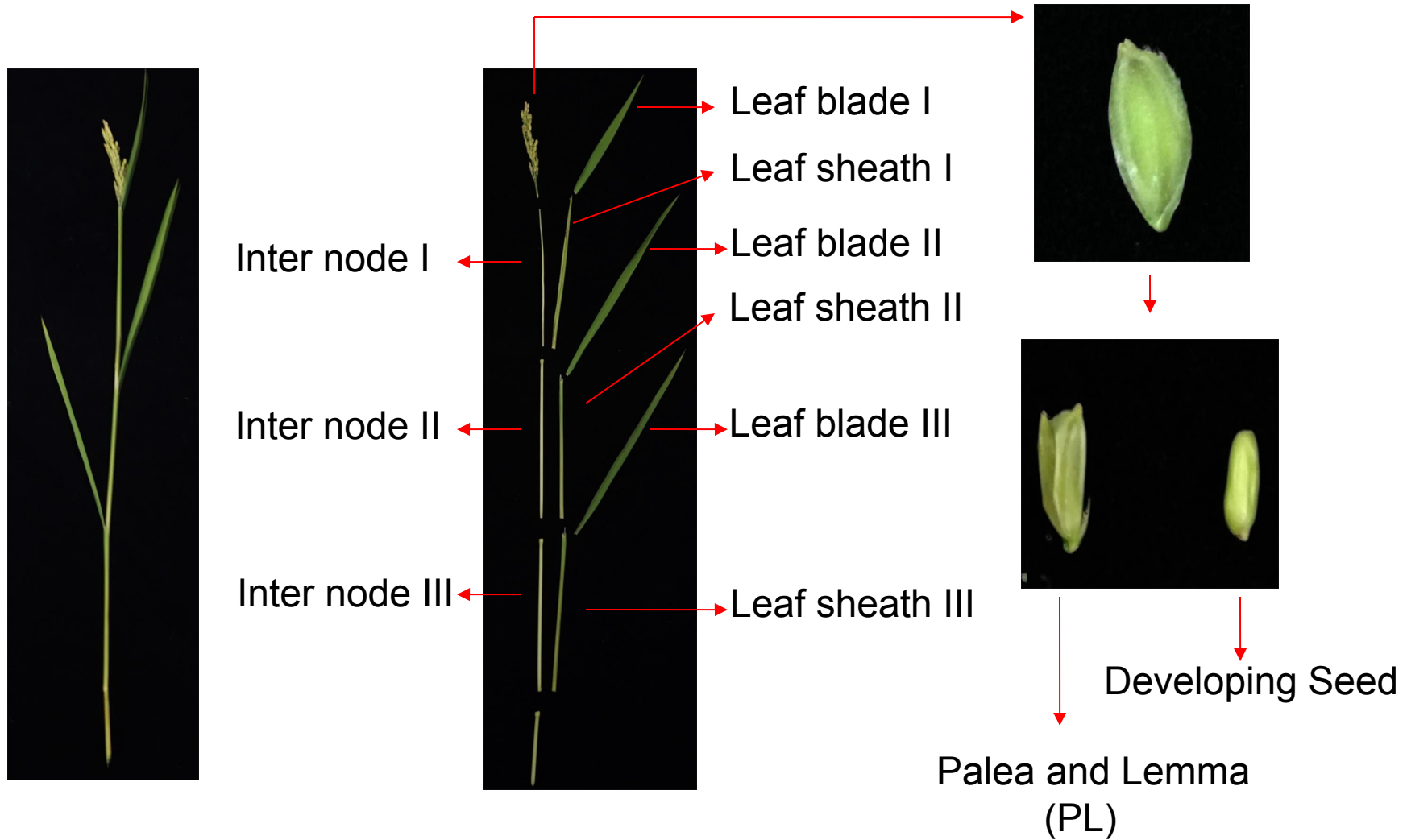


Figure S7. Ratios of *OsNRT2.1* to *OsNAR2.1* expression in different organs of WT and transgenic lines.

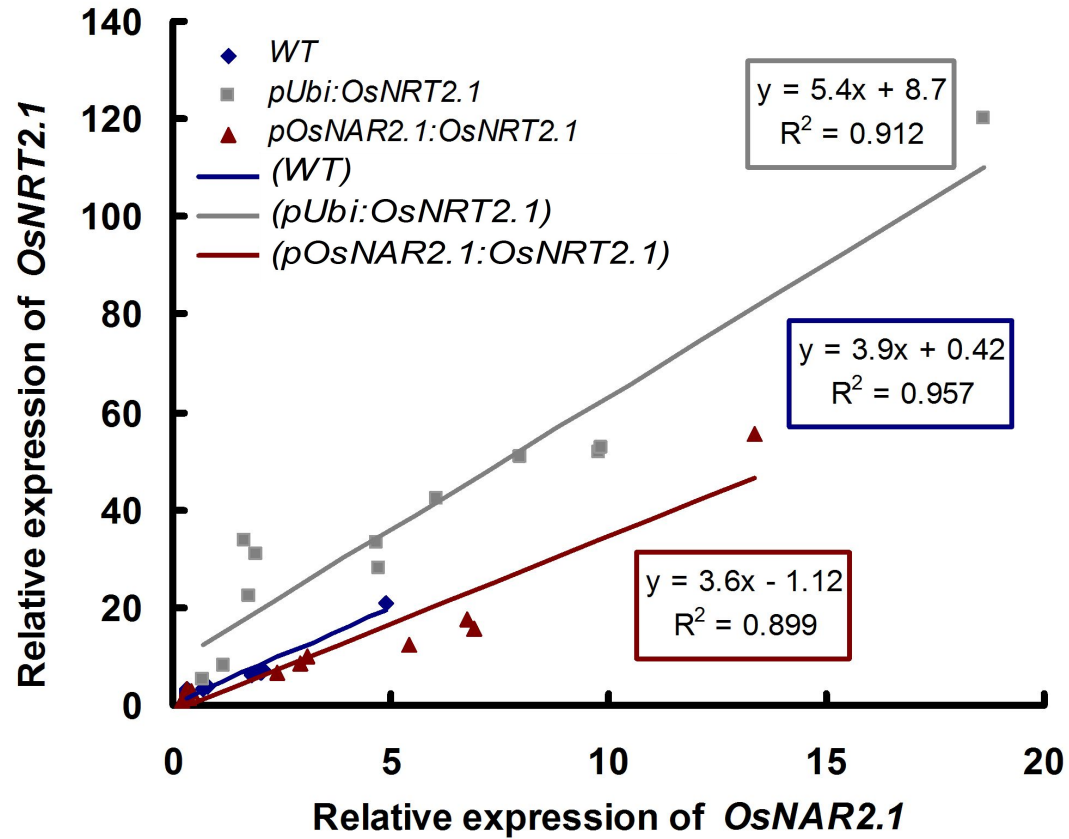


Figure S8. The diagramma of RNA sampling in T3 generation transgenic lines and WT plants.

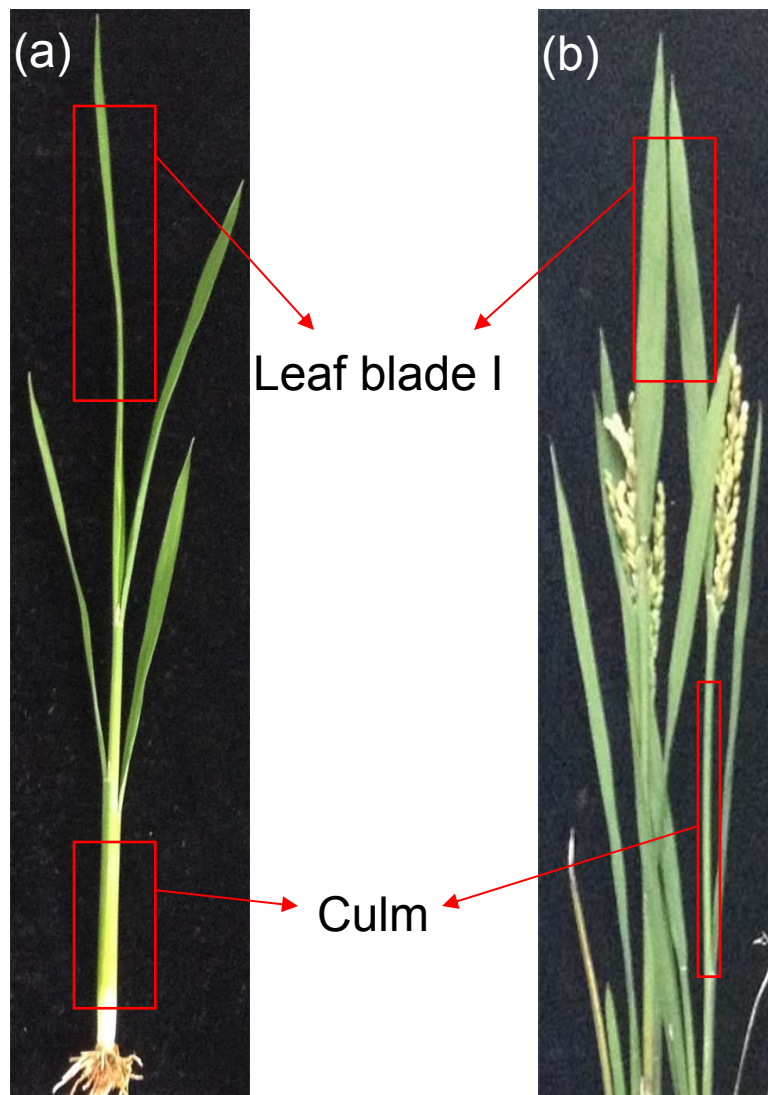


Figure S9. Changes in genes expression in leaf blade I throughout the experimental growth period.

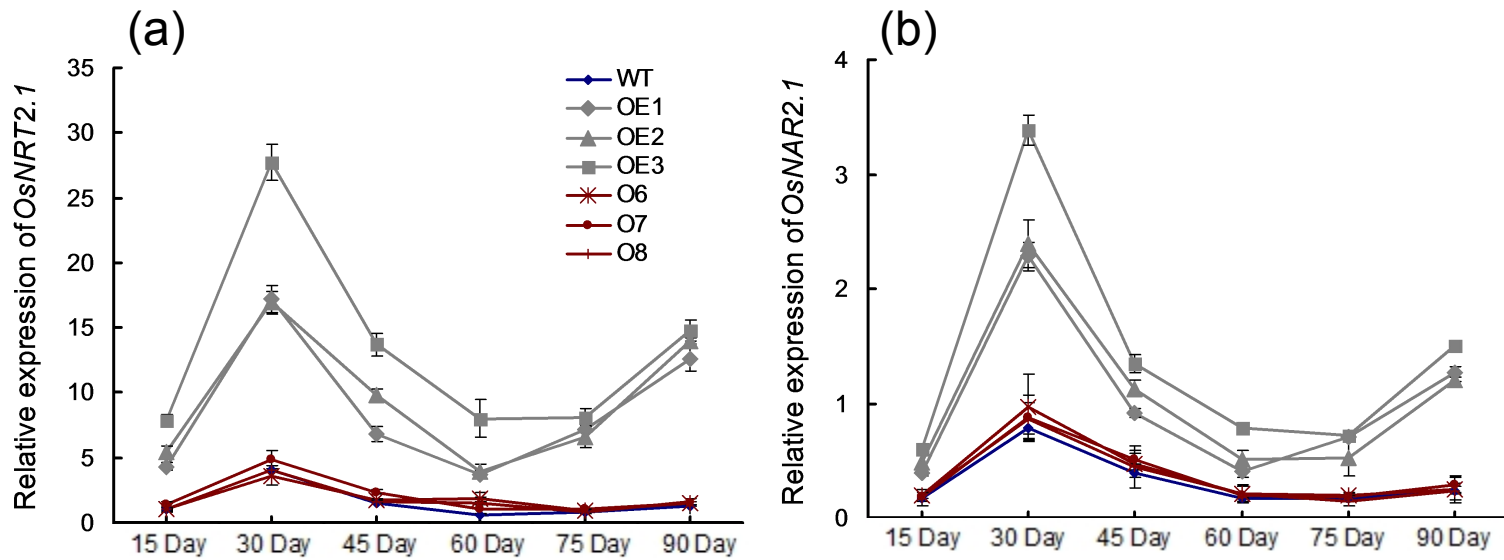


Figure S10. Ratios of *OsNRT2.1* and *OsNAR2.1* expression in the leaf blade I of WT and transgenic plants during different periods.

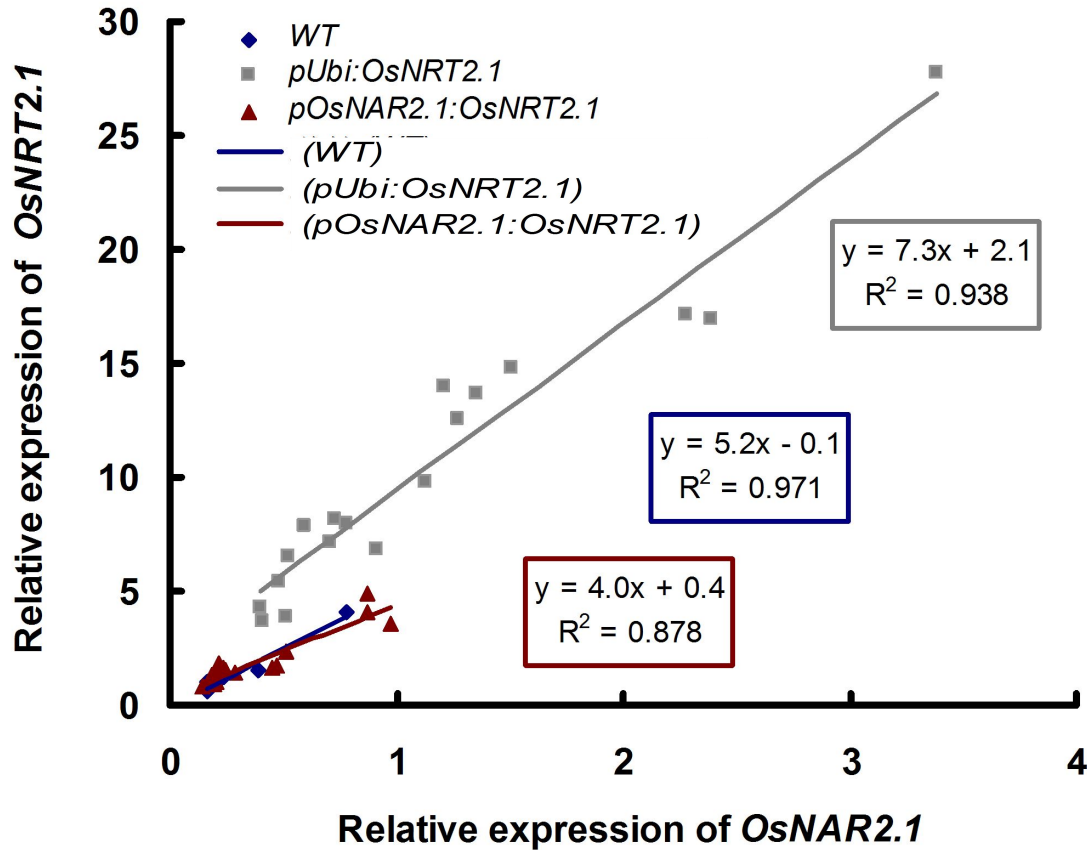


Figure S11. A field experiment picture of WT and T3 generation transgenic plants.

