

Supplementary information

MicroRNA393 is involved in nitrogen-promoted rice tillering through regulation of auxin signal transduction in axillary buds

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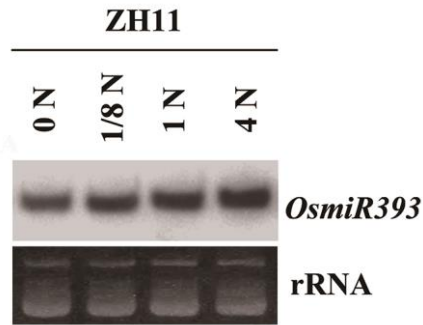


Figure S1. Supply of NH_4NO_3 triggered *OsmiR393* accumulation. Small RNA gel blot analysis of *OsmiR393* expression level in ZH11 under elevated NH_4NO_3 levels.

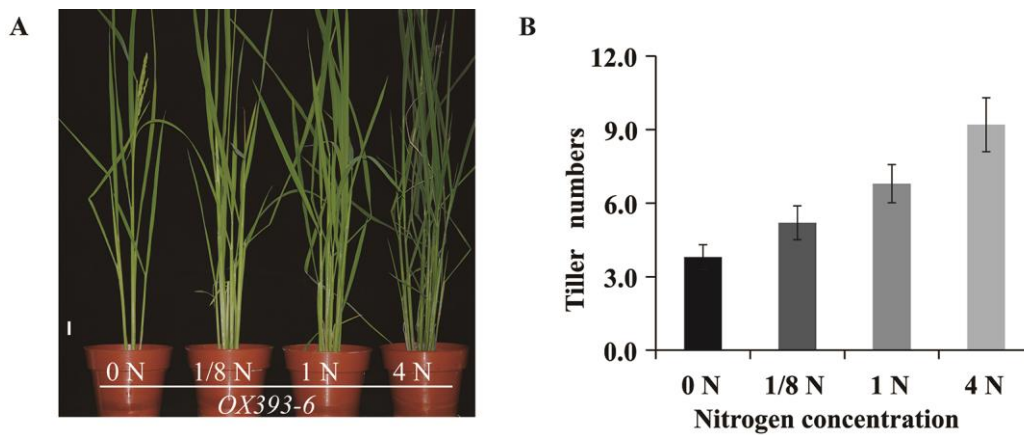


Figure S2. *OsmiR393* overexpressing line (*Ox393-6*) treated with NH_4NO_3 , exhibit increased tiller number, which is not to the extent of ZH11 in Fig. 1. B-C, Tillers of *Ox393-6* treated with gradient NH_4NO_3 concentrations. Bar = 3 cm. B, Statistical analysis of (A) by *t*-test, Vertical bars indicate standard error deviation. Three individual repeats were performed.

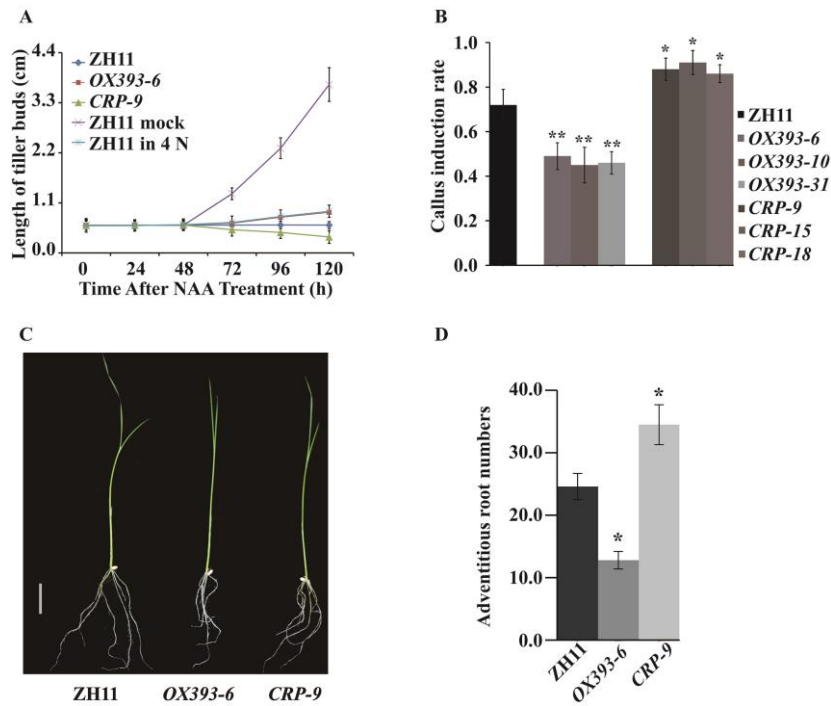


Figure S3. OsmiR393 involved in auxin signaling transduction.

A, Effect of NAA treatment on the outgrowth of rice tiller buds at the third leaf axils. Error bars represent mean \pm standard error ($n = 20$). The NAA spray concentration is 5 mg L^{-1} . **B**, Callus induction rate from seeds of ZH11, OsmiR393-overexpressing lines (OX393), and *OsMIR393* knock-out mutants (CRP) with 2 mg L^{-1} NAA. **C**, Root architecture of ZH11, OX393-6, and CRP-9 under 1 N level. Bar = 3 cm. **D**, Statistical analysis of adventitious root numbers in ZH11, OX393-6, and CRP-9. Eighteen 7-day-old seedlings of each line were used for adventitious roots number counting. The error bar represents mean \pm SE. The asterisk indicates significant differences ($* P \leq 0.05$ and $** P \leq 0.01$) compared with ZH11 by *t*-test.

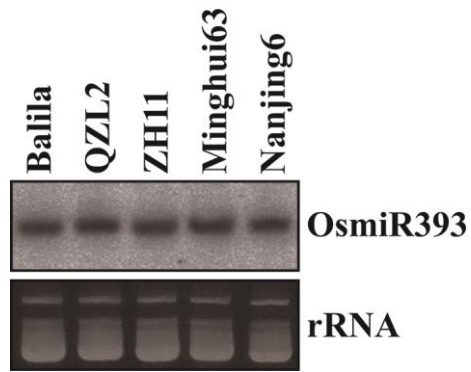


Figure S4. OsmiR393 expression level in N less-responsive and highly sensitive rice varieties. A small RNA gel blot assay was performed to detect OsmiR393 expression in N less-responsive varieties (ZH11, QZL2, and Balila), and in highly sensitive varieties (Minghui 63 and Nanjing 6). The total rRNA was used as a loading control.

Supplementary Table S1 Primer sequences used in this study

Name	Sequence (5' - 3')
393-q-F	GACTGTCCAAAGGGATCGCATT
393-q-R	GTGCAGGGTCCGAGGTATTC
Actin-q-F	CGGTGTCATGGTCGGAAT
Actin-q-R	GCTCGTTGTAGAAGGTGT
U6 q-F	CGATAAAATTGGAACGATACAGA
U6 q-R	ATTTGGACCATTCTCGATTTGT
U6-RT	ATTTGGACCATTCTCGATTTGT
OsAFB2- q-F	GAGCGGGATGGTAGCAATGAAATG
OsAFB2- q-R	CCGAGATAAGGGAGGCACACCAAC
OsTIR1-q-F	TCCAGGTGCTCCGCCTCGTCTCCT
OsTIR1-q-R	CCGGGAAGAGGCTGAGCCAATGAA
393-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACGACGAT
36080-q-F	TCAAGGAGGACCAGCAGTTCACCA
36080-q-R	GAGGACACCCGTGCTGACCAAAGT
41010-q-F	GCGGAGGTGGAGGCGGGAAGG
41010-q-R	CGCGAGGGAGACGAGGTGGTTG
39790-q-F	TATGGTATGCCCGGTGGAAGAAGT
39790-q-R	ATACTGCTGCCTCAGGATGATTGG
52320-q-F	TGCTTCCCCTACCACCGTCACCAC
52320-q-R	GCGCACTCTTCCACCTTCCCATTG
58734-q-F	TTGCTTCCGCCTCCTCATCTTCCT
58734-q-R	GCCGTGCGAACCATATTCCTTTGA
393 probe	GATCAATGCGATCCCTTTGGA
CRISPR-F	GGCAAAGGATCAATGCGATCCCTT
CRISPR-R	AAACAAGGGATCGCATTGATCCTT
CRISPR-g-F	CCTCACCAAATAACGAAAGAG
CRISPR-g-R	CAGTCACGACGTTGTAAAAC
cMYC-TIR1-F	GGATCC ATGGGGCGCGGCGGCTCGCG
MYC-TIR1-R	GGTACC CTAGAGTCCATGCATTTCCA
cMYC-OsIAA6-F	GGATCC ATGGAAGAAGGGTCCAACAA
cMYC-OsIAA6-R	GCGGCCGC GACCCTAGCAGTAGCTCCAA

Supplementary Table S2 Statistical analysis data of axillary meristem between ZH11 and *OX393-6*

	0 N (NAM/TP)	1 N (NAM/TP)	4 N (NAM/TP)	8 N (NAM/TP)
ZH11	2/20	9/20	17/20	20/20
<i>OX393-6</i>	/	13/20	/	/

ZH11 and *OX393-6* were sown and cultivated in hydroponic solution according to IRRI nutrient solution³⁸ under various levels of NH₄NO₃ fertilizer treatment whereas *OX393-6* was under normal (1 N) NH₄NO₃ fertilizer concentration. After 14 days, 20 samples were sectioned to observe axillary meristems both in ZH11 and in *OX393-6*. NAM: observed numbers of axillary meristem, TP: total plants sampled.