Involvement of a truncated MADS-box transcription factor ZmTMM1 in root nitrate foraging

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Supplementary Data

Fig. S1: The exon-intron structure of AGL17-like genes in Arabidopsis and rice.

Fig. S2: Root preferential expression of ZmTMM1 in maize.

Fig. S3: Expression of a N-responsive marker gene ZmGS1.1 and total N concentration in maize roots in responses to local N supply in split-root system.

Fig. S4: Localization of AGL21 and ANR1 promoter activities in Arabidopsis roots.

Fig. S5: LR growth phenotype of Arabidopsis dko mutant.

Fig. S6: Ectopic expression of ZmTMM1 in the dko mutant.

Fig. S7: Construction and analysis of ZmTMM1-, ANR1S- and ANR1-GR fusion transgenic lines.

Fig. S8: Phenotypic analysis of ZmTMM1-RNAi transgenic maize under local nitrate supply.

Fig. S9: Expression of Arabidopsis AGL17-like genes in response to local nitrate supply.

Table S1: Gene structure of truncated AGL17-like genes and orthologs in monocots identified from comparative genome analysis.

Table S2: Primers used in this study.

Dataset S1: Comparative genome analysis of AGL17-like genes in monocots.
Supplementary Fig. S1. The exon-intron structure of AGL17-like genes in Arabidopsis and rice

Gene structure of AGL17-like genes in Arabidopsis (A) and rice (B). Gene structure annotation was conducted by yrGATE algorithm. Blue blocks stand for exons and blue lines for introns. Magenta-colored lines highlight the intron inserted into the gap between I- and K- domain of MIKC-type MADS-box genes. Green and red arrow heads indicate the site of start and stop codons, respectively.
Supplementary Fig. S2. Root preferential expression of *ZmTMM1* in maize

Expression level of *ZmTMM1* transcript was analyzed by quantitative real-time PCR and normalized by maize GAPDH (*gi22302*). Superscript a denotes samples were collected from field-grown maize plants at seedling stage (28 days after germination), superscript b at silking stage, and superscript c at stage of 15 days after pollination. Data represent means ± SD (n = 3 replicates; each replicate represents a single seedling).
Supplementary Fig. S3. Expression of a N-responsive marker gene ZmGS1.1 and total N concentration in maize roots in responses to local N supply in split-root system

After a N starvation for 3 days, maize seedlings with four crown roots were transferred to a split-root system containing either 1 mM KNO₃ or 0.5 mM (NH₄)₂SO₄ in the +N compartment, and 0.5 mM K₂SO₄ in the -N compartment, respectively. Roots were sampled for gene expression and total nitrogen concentration analysis. (A) Relative expression level of ZmGS1.1 determined by qPCR and normalized by maize Tubulin 4 (AJ420856). (B) Total N concentration in the roots. Data represent means ± SD (n = 3 replicates; each replicate represents a single seedling). Asterisks indicate significant differences between the values detected in root samples from the +N and -N compartments at *, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, not significant (Student’s t-test).
Supplementary Fig. S4. Localization of AGL21 and ANR1 promoter activities in Arabidopsis roots

The transgenic Arabidopsis plants expressing AGL21 promoter-GFP-NLS and ANR1 promoter-GFP-NLS were grown on agar plates supplied with nitrate for observation of GFP (green) using a confocal microscope. (A) PR tips; (B) LR tips; (C) LR primordia. Roots were counter-stained by propidium iodide (red in A and B). Ep: epidermis; C: cortex; En: endodermis; LRC: lateral roots cap; QC: quiescent center; CRC: columella cell of root caps.
Supplementary Fig. S5. LR growth phenotype of Arabidopsis dko mutant.

(A) Schematic diagram of dSpm transposon insertions in anr1 and agl21 single mutants used for generating anr1 agl21 (dko) mutant.

(B) Root growth of Col-0 and dko plants under homogenous nitrate supply. Plants were grown on N-free half-strength MS agar plates supplemented with 1 mM KNO₃ for 12 days. Bars represent means ± SE (n = 16 replicates; each replicate represents a single seedling). Asterisk indicates significant differences between dko and Col-0 at *, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, not significant (Student’s t-test).

(C) Root response of Col-0 and dko mutant to localized nitrate supply in vertically split segmented agar plates. Arabidopsis seedlings pruned to have only two first-order LRs were transferred to split agar plates containing 1 mM KNO₃ in the +N patch and 0.5 mM K₂SO₄ in the -N patch, respectively. Root phenotypes were measured 6 days after transfer. Bars represent means ± SE (n = 12 replicates;
each replicate represents a single seedling). Different letters represent significant differences among means at $P < 0.05$ (Tukey's test).
Supplementary Fig. S6. Ectopic expression of ZmTMM1 in dko mutant

(A) Ectopic expression of ZmTMM1 in dko mutant. Driven by CaMV 35S promoter, ZmTMM1 was overexpressed in Arabidopsis dko mutant, and three transgenic lines were selected, named 2-5; 26-2; 14-1, respectively. Transcripts of ZmTMM1 in transgenic lines were detected by RT-PCR. ACTIN (AT3G18780) was used as the internal control.

(B) Root growth of Col-0, dko and ZmTMM1-overexpressing lines under homogenous nitrate supply. Plants were grown on N-free half-strength MS agar plate supplemented with 1 mM KNO₃ for 12 days. Bars = 1 cm.
Supplementary Fig. S7. Construction and analysis of ZmTMM1-, ANR1S- and ANR1-GR fusion transgenic lines

(A) Sequence alignment of ZmTMM1, intact ANR1 and truncated ANR1S proteins.

(B) Organization of ZmTMM1-, ANR1S- and ANR1-GR fusion gene constructs.

(C) Expression of ZmTMM1-GR, ANR1-GR, ANR1S-GR transcripts in transgenic Arabidopsis plants
determined by RT-PCR.

(D) ZmTMM1-GR, ANR1-GR and ANR1s-GR fusion lines grown on local DEX supplied plates. Vertically splitted agar plates contained N-free half-strength MS medium supplemented with 1 mM nitrate or 0.5 mM Gln as N the sources. Arabidopsis seedlings harboring two 1st-order LRs were transferred to split root plates containing 1 µM DEX in the +DEX side and no DEX in the -DEX side. Root images were taken 8 days after transfer.
Supplementary Fig. S8. Phenotypic analysis of ZmTMM1-RNAi transgenic maize under local nitrate supply

Three independent ZmTMM1-RNAi transgenic maize lines and the corresponding wild-types (WT)
with four crown roots were cultivated in a split-root system containing 1 mM KNO₃ in the +N compartment and 0.5 mM K₂SO₄ in the -N compartment, respectively.

(A) Phenotypes of ZmTMM1-RNAi transgenic lines under local nitrate supply in split-root systems. Photographs were taken at 5 days after transfer. (B) ZmTMM1, ZmMADS2 and GRMZM2G055782 transcript levels in response to local nitrate supply in WT and ZmTMM1-RNAi lines. Gene expression was detected 12h after the transfer of seedlings to local nitrate treatment using split-root systems. Relative transcript levels of indicated genes were determined by qPCR and normalized by maize Tubulin 4 (AJ420856). Data represent means ± SD (n = 4 replicates; each replicate represents a single seedling). Different letters indicate significant differences between the four bars in each group at P < 0.05 (Tukey’s test). (C) LR development of WT and ZmTMM1-RNAi lines in response to local nitrate supply. Root growth were measured at 5 days after the transfer to the split-root system. Data represent means ± SD (n = 4 replicates; each replicate represents two seedlings). Different letters indicate significant differences between the four bars in each group at P < 0.05 (Tukey’s test).
Supplementary Fig. S9. Expression of Arabidopsis *AGL17-like* genes in response to local nitrate supply.

Arabidopsis seedlings were cultivated in a split-root system which contained 1 mM KNO$_3$ in the +N patch and 0.5 mM K$_2$SO$_4$ in the -N patch. After 12 h of local nitrate treatment in the split-root system, transcript levels of *AGL16, AGL17, AGL21* and *ANR1* in roots were determined by qPCR and normalized by *AtUBQ10*. Data represent means ± SD (n = 3 replicates; each replicate represents a single seedling). Asterisks indicate significant differences between the gene expression in roots on +N and -N patches at: *, p < 0.05; **, p < 0.01; ns, not significant (Student’s t-test).
**Supplementary Table S1. Gene structure of truncated AGL17-like genes and orthologs in monocots identified from comparative genome analysis**

| Species                  | Gene name          | Protein structure | Protein length | First half on genome | Second half on genome | Length of the intron or gap |
|--------------------------|--------------------|-------------------|----------------|----------------------|------------------------|-----------------------------|
| *Zea mays*               | ZmTMM1             | Truncated protein | 89 aa          | GRMZM2G044408, Chr 5 | -                      | -                           |
| *Zea mays*               | ZmMADS2            | Complete protein  | 240 aa         | GRMZM2G316366, Chr 5 | GRMZM2G492156, Chr 5  | 5477 bp                     |
| *Zea mays*               | GRMZM2G055782      | Truncated protein | 91 aa          | GRMZM2G055782, Chr 4 | GRMZM2G133568, Chr 4  | 21253 bp                    |
| *Zea mays*               | GRMZM2G032905      | Truncated protein | 89 aa          | GRMZM2G032905, Chr 2 | GRMZM2G033093, Chr 2  | 20902 bp                    |
| *Oryza sativa*           | OsMADS61           | Complete protein  | 246 aa         | LOC_Os04g38770, Chr 4 | LOC_Os04g38780, Chr 4 | 4169 bp                     |
| *Brachypodium distachyon*| BRADI5G12440       | Truncated protein | 91 aa          | BRADI5G12440, Chr 5 | BRADI5G12450, Chr 5   | 14428 bp                    |
| *Sorghum bicolor*        | Sb04g024010        | Truncated protein | 91 aa          | Sb04g024010, Chr 4  | -                      | -                           |
| *Sorghum bicolor*        | Sb06g019040        | Truncated protein | 95 aa          | Sb06g019040, Chr 6  | -                      | -                           |
| *Sorghum bicolor*        | Sb07g021110        | Truncated protein | 73 aa          | Sb07g021110, Chr 7  | Sb07g021100, Chr 7    | 9402 bp                     |

Data source: Sequence information of AGL17-like genes in *Oryza sativa*, *Brachypodium distachyon*, *Sorghum bicolor*, and *Zea mays* were obtained from PlantGDB.
Supplementary Table S2. Primers used in this study

| Primer name          | Primer sequence                          | Purpose                  |
|----------------------|------------------------------------------|--------------------------|
| qZmTM1M1-F           | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'            | qRT-PCR                 |
| qZmTM1M1-R           | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'            | qRT-PCR                 |
| qZmMADS2-F           | 5'-GCAACCTGACTGCAACTGAG-3'              | qRT-PCR                 |
| qZmMADS2-R           | 5'-CCCTCGACTGCAACTGAG-3'                | qRT-PCR                 |
| qGRM2M2G05572-F      | 5'-GGCTCTGATAAGATTAATCTGGTCCGTTG-3'     | qRT-PCR                 |
| qGRM2M2G05572-R      | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qZmGS1.1-F           | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qZmGS1.1-R           | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qZmGAPDH-F           | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qZmGAPDH-R           | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qZmTUB4-F            | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qZmTUB4-R            | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qOsMADS57-F          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qOsMADS57-R          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qOsMADS23-F          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qOsMADS23-R          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qOsMADS27-F          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qOsMADS27-R          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qOsMADS61-F          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qOsMADS61-R          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qOsMADS25-F          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qOsMADS25-R          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qOsACTIN-F           | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qOsACTIN-R           | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qANR1-F              | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qANR1-R              | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qAGL16-F             | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qAGL16-R             | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qAGL17-F             | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qAGL17-R             | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qAGL21-F             | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qAGL21-R             | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qAtU1BQ10-F          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qAtU1BQ10-R          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qAtACTIN2-F          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| qAtACTIN2-R          | 5'-GGCAAGAAGCTGCAACTTCAGAG-3'           | qRT-PCR                 |
| ZmTM1M1-in situ antisense | AAGAAGAAGCTGCAACTTCAGAG-3'        | In situ hybridization    |
| ZmTM1M1-in situ sense | AAGAAGAAGCTGCAACTTCAGAG-3'            | In situ hybridization    |
ZmTMM1-OX-F 5'-GGAGCCATGGGGAGGGGAAGATAGT-3'
ZmTMM1-OX-R 5'-ACACACGTGTCACCCTACATGAGTTCT-3'
ZmTMM1-GFP-F 5'-AGAGACTAGTATGGGGGAAGATAGT-3'
ZmTMM1-GFP-R 5'-TTGTGGTACCCGTCATGAGTTCT-3'
ZmTMM1-RNAi-F1 5'-CGGAGTCTTTTCTCCAACACACATG-3'
ZmTMM1-RNAi-R1 5'-CCGGTGTCACCGTGACTACACCAACATG-3'
ZmTMM1-RNAi-F2 5'-TGGAACATGCCTGTCGTACTACGACCA-3'
ZmTMM1-RNAi-R2 5'-AGGGCCATGGGGAGGGGAAGATAGT-3'
ZmTMM1-GR-F 5'-TCGTACGCTACCAGTTCTGAAGTTGCA-3'
ZmTMM1-GR-R 5'-TCGTACGCTACCAGTTCTGAAGTTGCA-3'
ANR1-GR-F 5'-AGGGCCATGGGGAGGGGAAGATAGT-3'
ANR1-GR-R 5'-TCGTACGCTACCAGTTCTGAAGTTGCA-3'
ANR1S-GR-F 5'-AGGGCCATGGGGAGGGGAAGATAGT-3'
ANR1S-GR-R 5'-TCGTACGCTACCAGTTCTGAAGTTGCA-3'
GR-F 5'-CAGACGTAGTACCCAGTTAGCTACAGGACC-3'
GR-R 5'-CTGGGTGACCTTTCTAGAAGCGGCTC-3'
AGL21_2021TOPO 5'-CACCCACAGCAGAATAAACACACAATTAC-3'
AGL21_1R 5'-CAGGTGGGTCTTTCTCATGAGTACCAAGC-3'
ANR1_2032TOPO 5'-CAGGTGGGTCTTTCTCATGAGTACCAAGC-3'
ANR1_1R 5'-CTGTGTCTCCCAAAAGACTAC-3'
SG2 5'-AAGCAACGTATGACATGGAAGATGC-3'
SG10 5'-GACATGAGTGTGGGGTTTG-3'
dSpm1 5'-CTTATTTACAGTAAAGTGTGGGGTTTG-3'

p35S::ZmTMM1 construct
p35S::ZmTMM1 construct
p35S::ZmTMM1-GFP construct
p35S::ZmTMM1-GFP construct
pUBQ::ZmTMM1-RNAi construct
pUBQ::ZmTMM1-RNAi construct
pUBQ::ZmTMM1-RNAi construct
pUBQ::ZmTMM1-RNAi construct
p35S::ZmTMM1-GR construct
p35S::ANR1-GR construct
p35S::ANR1-GR construct
p35S::ANR1S-GR construct
p35S::ANR1S-GR construct
GR amplification
GR amplification
AGL21 promoter-GFP-NLS
AGL21 promoter-GFP-NLS
ANR1 promoter-GFP-NLS
ANR1 promoter-GFP-NLS
Gene specific primer of ANR1
Gene specific primer of ANR1
dSpm specific primer
|   | 5'-sequence                  | Type                      |
|---|------------------------------|---------------------------|
| SG3 | 5'-GAACCCCGCATCAGAAGTCAAG-3' | Gene specific primer of AGL21 |
| SG4 | 5'-GTGTTGTGTGTTACAGTTTTGGCAG-3' | Gene specific primer of AGL21 |
| SG6 | 5'-GAATCCGGAAAAGCAATCGTTC-3' | Gene specific primer of AGL21 |
| dSpm8 | 5'-GTTTTGGCGACACTCTACC-3' | dSpm specific primer |