**Table S1. Primers used to amplify the *OsNRT2.3b* open reading frame and identification primers of hygromycin.**

| Gene name | Primer | Sequence |
|---|---|---|
| OsNRT2.3b | 5' (F) | GAGATGGAGGCTAAGCCGGTGGCG |
| | 5' (R) | TTCTTCACAC CCCGGCCGGC GACG |
| Hygromycin | 5'(F) | CTTCTGCGGGCGATTTGT |
| | 5"(R) | CAGCGTCTCCGACCTGAT |

**Table S2. Primers used for quantitative real-time polymerase chain reaction.**

| Gene name | Forward primer (5'to 3') | Reverse primer (5'to3') |
|---|---|---|
| HvActin | AGCAACTGGGATGACATGGAG | GGGTCATCTTCTCTCTGTTGGC |
| OsNRT2.3b | GCCATCCACAAGATCGGTAG | TGTGGAGCTTCCCGTAGTTG |
| HvNRT2.1 | TTGTGCACTTCCCACAATGG | GACCCTTGGCTTTCTCCTCT |
| HvNRT2.2 | ACCCAGCTCATTATGCCACT | CCATCACCACGTGCATCATT |
| HvNRT2.3 | GTGCCCTTTGGAACATCTGG | AGGGTGCGACACCAAAGATA |
| HvNAR2.3 | AAGAAGGACAAGACCTGCCA | GTGATGCTGACGACGTTGAA |

**Table S3. The effect of 0.2 mM $NH_4^+$ treatment on the distribution of total N, P and Fe in shoots and roots.**

0.2mM $NH_4^+$

| Distribution ration of shoot (% ) | WT | Ubi-1 | Ubi-2 | Ubi-3 | RSs1-1 | RSs1-2 | RSs1-3 |
|---|---|---|---|---|---|---|---|
| Total N | 69.79a | 72.48a | 73.31a | 78.63a | 75.85a | 71.30a | 71.52a |
| Total P | 56.07a | 59.80a | 62.80a | 73.94a | 70.27a | 63.67a | 60.61a |
| Total Fe | 14.05b | 9.73b | 11.84b | 26.25a | 16.98b | 17.11b | 15.29b |

| Distribution ration of root (% ) | WT | Ubi-1 | Ubi-2 | Ubi-3 | RSs1-1 | RSs1-2 | RSs1-3 |
|---|---|---|---|---|---|---|---|
| Total N | 30.21a | 27.52a | 26.69a | 21.37a | 24.15a | 28.70a | 28.48a |
| Total P | 43.93a | 40.20a | 37.20a | 26.06a | 29.73a | 36.33a | 39.39a |
| Total Fe | 85.95a | 90.27a | 88.16a | 73.75b | 83.02a | 82.89a | 84.71a |

**Table S3. The effect of 0.2 mM $NH_4^+$ treatment on the distribution of total N, P and Fe in shoots and roots.** Significant differences between transgenic and WT lines are indicated by different letters ($P < 0.05$, one-way ANOVA).

**Table S4. Comparison of the expression of *OsNRT2.3b* in transgenic barley driven by different promoters under 0.2mM $NH_4^+$/$NO_3^-$ treatment.**

| Leaves | Ubi-1 | Ubi-2 | Ubi-3 | RSs1-1 | RSs1-2 | RSs1-3 |
|---|---|---|---|---|---|---|
| 0.2mM $NH_4^+$ | 0.000958e | 0.001218e | 0.001239e | 0.000788e | 0.000822e | 0.000499e |
| 0.2mM $NO_3^-$ | 0.018984c | 0.035601b | 0.079687a | 0.009516d | 0.009085d | 0.013484d |
| Sheath | Ubi-1 | Ubi-2 | Ubi-3 | RSs1-1 | RSs1-2 | RSs1-3 |
| 0.2mM $NH_4^+$ | 0.0000259d | 0.000203d | 0.000387d | 0.000195d | 0.000123d | 0.000278d |
| 0.2mM $NO_3^-$ | 0.0217b | 0.0439a | 0.0123c | 0.0149c | 0.0149c | 0.0148c |
| Root | Ubi-1 | Ubi-2 | Ubi-3 | RSs1-1 | RSs1-2 | RSs1-3 |
| 0.2mM $NH_4^+$ | 0.0000477c | 0.000253c | 0.000271c | 0.000126c | 0.000126c | 0.0000588c |
| 0.2mM $NO_3^-$ | 0.00404ab | 0.004614a | 0.001561bc | 0.0000219c | 0.0000851c | 0.000447c |

**Table S4. Comparison of the expression of *OsNRT2.3b* in transgenic barley driven by different promoters under 0.2mM $NH_4^+$/$NO_3^-$ treatment.** Significant differences between $NO_3^-$ and $NH_4^+$ condition in transgenic lines are indicated by different letters ($P < 0.05$, two-way ANOVA).

**Figure S1. Identification of transgenic barley lines by southern blot.**

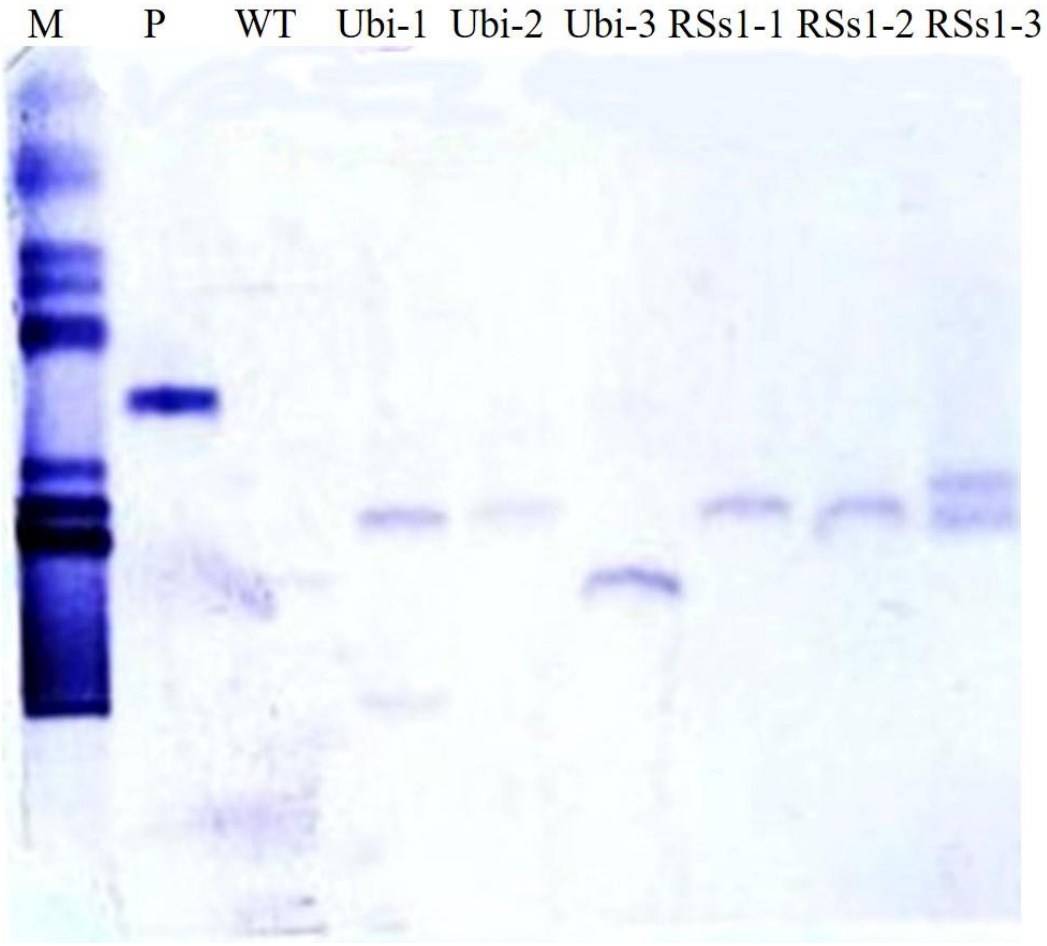


**Fig. S1. Identification of transgenic barley lines by southern blot.** Genomic DNA was isolated from WT and transgenic barley lines. Hybridization was performed using a hygromycin gene probe. M: maker, P: positive control.

## Figure S2. The seed concentrations of Mn and Mg in different barley lines.

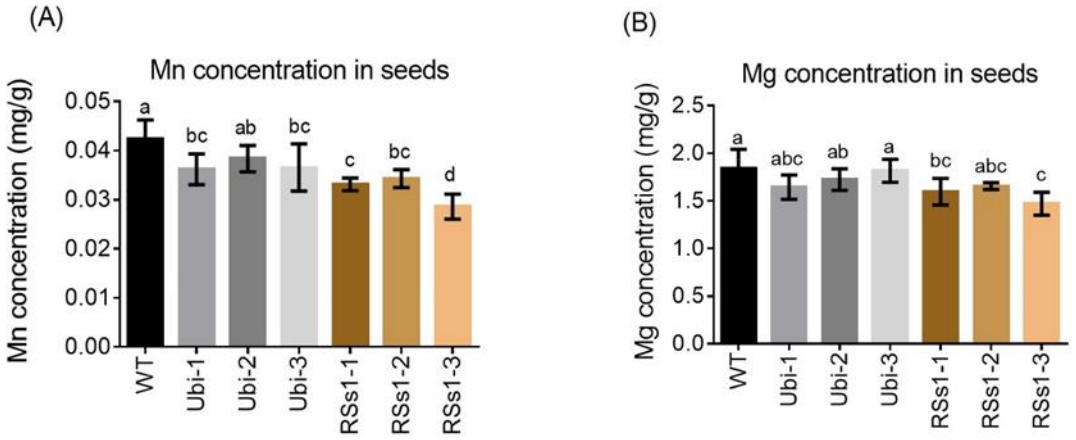


**Fig. S2. The seed concentrations of Mn and Mg in different barley lines.** (A) The concentration of Mn and (B) Mg in seeds. Significant differences between transgenic and WT lines are indicated by different letters ($P < 0.05$, one-way ANOVA). Error bars: standard error (n = 4 plants).

## Figure S3. Homology analysis of *NRT2s* genes.

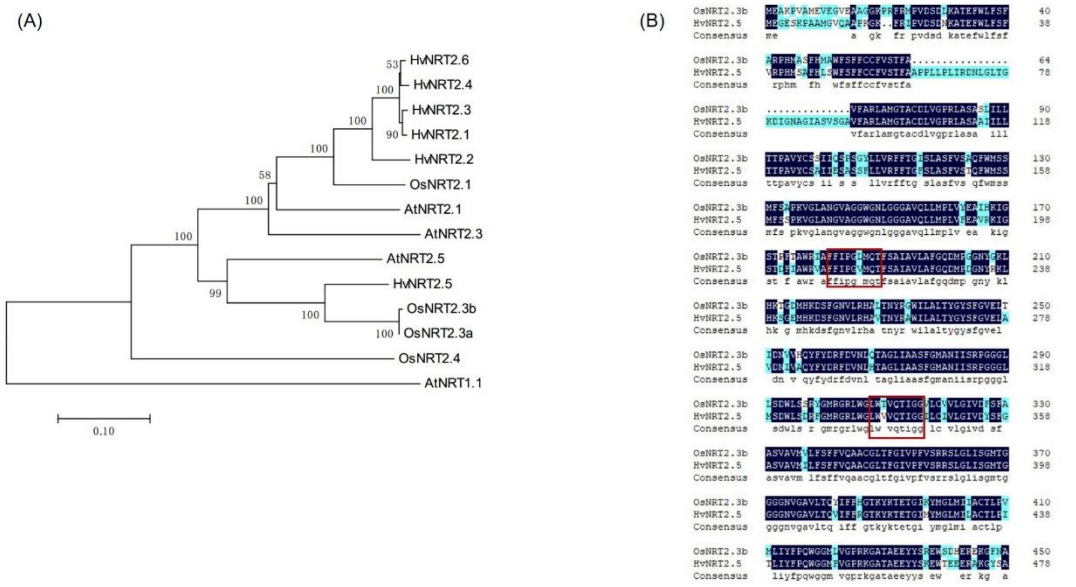


**Fig. S3. Homology analysis of *NRT2s* genes.** (A)The phylogenetic tree of the *NRT2* gene family in Arabidopsis, rice and barley. (B) HvNRT2.5 and OsNRT2.3b protein sequence alignment. The red box represented the pH sensing motif that was identified by OsNRT2.3b.

**Figure S4. The relative expression of *HvNRT2s* in different plants parts under 0.2mM $NH_4^+$ and 0.2mM $NO_3^-$ treatments.**

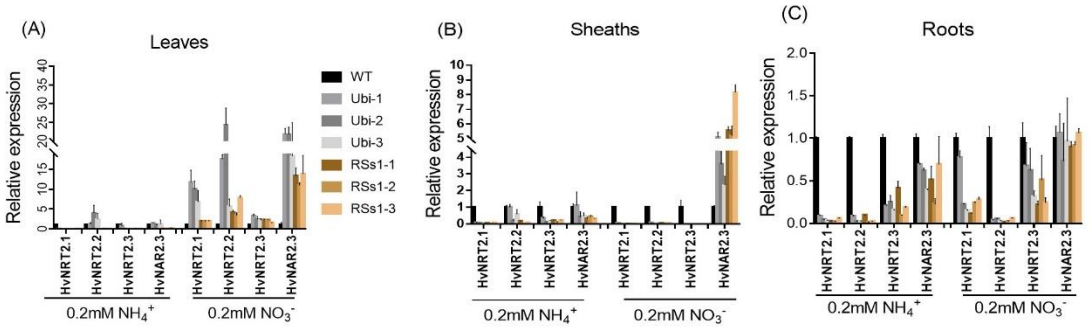


**Fig. S4. The relative expression of *HvNRT2s* in different plants parts under 0.2mM $NH_4^+$ and 0.2mM $NO_3^-$ treatments.** The relative expression of *HvNRT2.1/2.2/2.3* and *HvNAR2.3* in (A) leaves, (B) sheaths and (C) roots of all barley lines under 0.2mM $NH_4^+$ and 0.2mM $NO_3^-$ treatments. Significant differences between transgenic and WT lines are indicated by different letters ($P < 0.05$, one-way ANOVA). Error bars: standard error (n = 4 plants).

**Figure S5. Phosphorus (P) and iron (Fe) concentrations in different plant parts under 0.2 mM $NH_4^+$ and 0.2mM $NO_3^-$ treatments.**

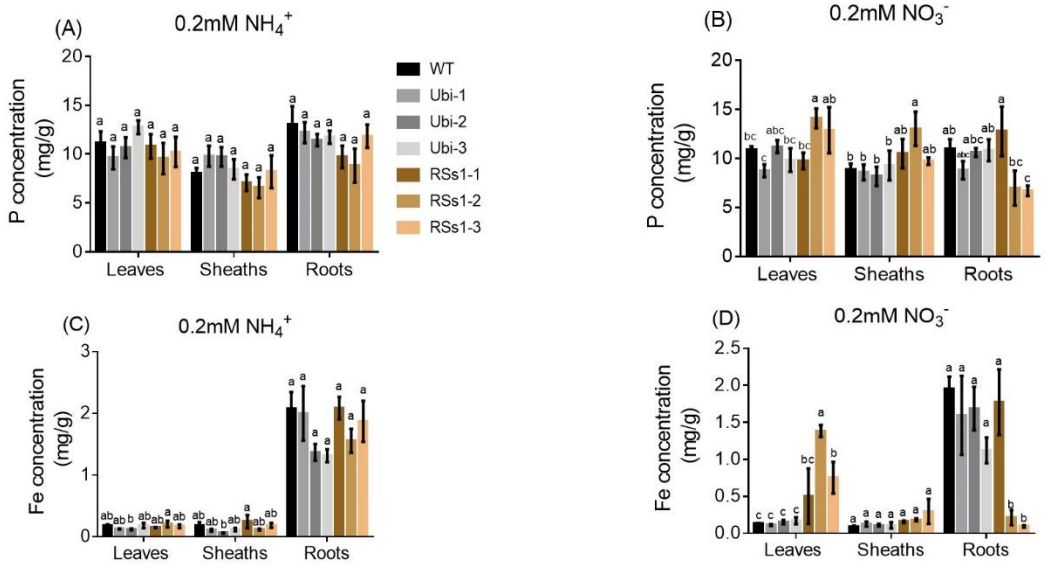


**Fig. S5. Phosphorus (P) and iron (Fe) concentrations in different plant parts under 0.2 mM $NH_4^+$ and 0.2mM $NO_3^-$ treatments.** (A) The concentration of P and (C) Fe in leaves, sheaths and roots of all barley lines under 0.2 mM $NH_4^+$ condition. (B) The concentration of P and (D) Fe in leaves, sheaths and roots of all barley lines under 0.2mM $NO_3^-$ condition. Significant differences between transgenic and WT lines are indicated by different letters ($P < 0.05$, one-way ANOVA). Error bars: standard error (n = 4 plants).

**Figure S6. The characterization of all barley lines. Under 10 mM $NH_4^+$ condition after 10 mM $NH_4^+$/$NO_3^-$ condition.**

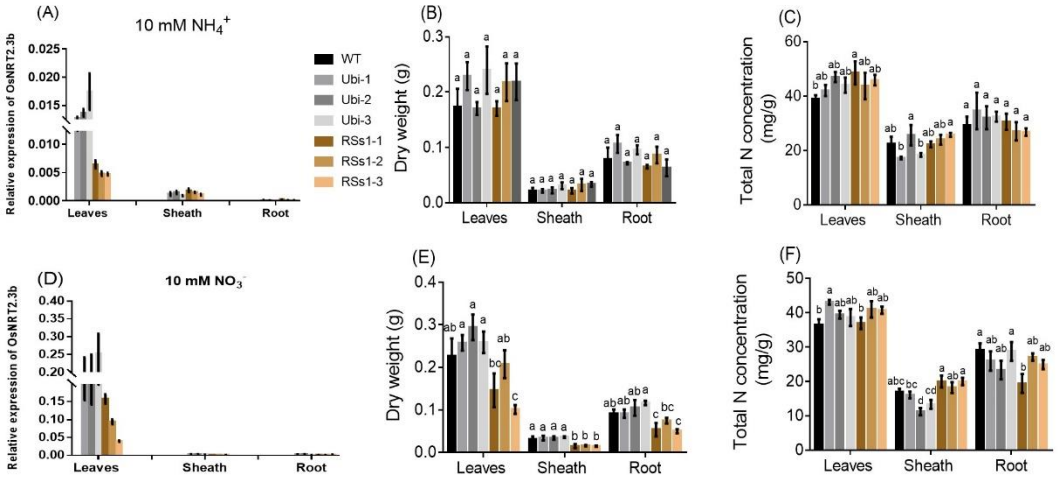


**Fig. S6. The characterization of all barley lines. Under 10 mM $NH_4^+$ condition after 10 mM $NH_4^+$/$NO_3^-$ condition.** (A−C) 10 mM $NH_4^+$ (A) the relative expression of *OsNRT2.3b*, (B) the dry weights and (C) total N concentration of different plant parts. (D-F) 10 mM $NO_3^-$, (D) the relative expression of *OsNRT2.3b*, (E) the dry weight and (F) total N concentration of different plant parts. Significant differences between transgenic and WT lines are indicated by different letters ($P < 0.05$, one-way ANOVA). Error bars: standard error (n = 4 plants).