Identification of a gene that promotes ammonium nitrogen (NH_4^+-N) uptake in rice

Nitrogen is the most essential nutrient to plant growth and grain yield. There are two forms of inorganic nitrogen available to plants: one is NO_3^--form and the other is NH_4^+-form. NH_4^+-form is the major form in paddy fields, thus rice plants grown in the fields uptake mainly NH_4^+-form nitrogen as nitrogen source in roots. Therefore, promoting NH_4^+-form uptake is one of the major targets to increase grain yield in rice. It is known that NH_4^+ influx into roots by a high-affinity transport system (HAT) is down-modulated in response to elevated NH_4^+ concentration around soil surface. We hypothesized that canceling the down-modulation of NH_4^+ influx by HAT would be beneficial on the uptake of NH_4^+-form nitrogen in rice. However, no gene that modulates NH_4^+ influx by HAT has been identified in rice. In this study, we aimed to identify the gene that modulates HAT of rice roots with gene expression analyses. Furthermore, we tried to isolate a gain-of-function gene to promote uptake of NH_4^+-form nitrogen with rice *Tos17* insertion mutants.

To isolate candidate genes concerned in down-modulation of HAT in roots of rice, we performed four-biological-repeat transcriptome analyses. A total of 28,381 out of 36,444 filtered probes were selected as differentially expressed genes based on a false discovery rate of ≤ 0.05. A strong candidate gene for down-modulation of HAT, the coding protein kinase gene *OsACTPK1*, showed 1,071 times higher expression in roots under NH_4^+-rich condition as compared with NH_4^+-deficient condition (Table 1). We then analyzed the detail of *Tos17*-inserted mutant of *OsACTPK1* (*actpk1* mutant) to elucidate *OsACTPK1* as down-modulator of HAT. Kinetic analyses of NH_4^+ influx by HAT revealed that *V*max value of *actpk1* mutant was 2 times higher than that of wild type, whereas there was no significant difference of *K*m value between these two lines (Fig. 1). These results indicated that down-modulation of HAT was canceled in *actpk1* mutant due to the loss-of-function *OsACTPK1*. Total nitrogen content of the *actpk1* mutant was significantly higher (+32%) than that of wild type in 1,000 μM NH_4Cl condition (NH_4^+-rich condition), while the significant difference was not observed in 5 μM NH_4Cl condition (NH_4^+-deficient condition) (Fig. 2A). Furthermore, the longest-root length of the *actpk1* mutant was significantly lower (-22%) than that of wild type in 1,000 μM NH_4Cl condition, while the significant difference was observed in 5 μM NH_4Cl condition (Fig. 2B).

We concluded that *OsACTPK1* was a down-modulator of HAT and that loss-of-function *ACTPK1* (*actpk1* gene) could maintain activity of NH_4^+ influx even in elevated NH_4^+ concentration. The *actpk1* gene could be effective in improving nitrogen use efficiency in a rice molecular breeding program. Also, reduction of root elongation in *actpk1* mutant would be used as phenotypic marker in the program. Further analyses to characterize nitrogen use and grain yield of *actpk1* are required for the program since *OsACTPK1* would function to avoid NH_4^+ toxicity.

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Table 1. Details for the *OsACTPK1* gene, a strong candidate for down-modulation of HAT

Item	Description
Increase of gene expression in response to elevated external NH_4^+ concentration	1,071-fold
RAP ID	Os02g0120100
Protein function	protein kinase

Rice Oligo DNA Microarray (4X44K RAP-DB) was used in this research. Total RNA was extracted from roots of rice plants grown for 10 days in 5 and 1,000 μM NH_4Cl.

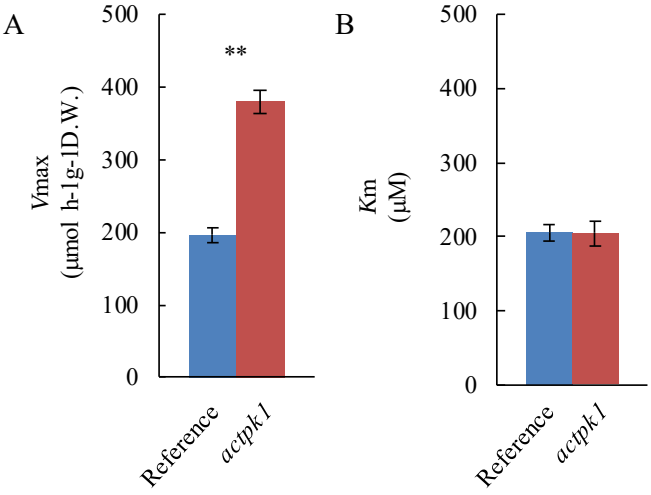

Fig. 1. Kinetic properties of HAT in *actpk1* mutant.
*V*max value (A) and *K*m (B) to NH_4^+ were expressed in blue column for reference, Nipponbare and red column for *actpk1*. Plants were hydroponically grown for 10 days in 1,000 μM NH_4Cl. Mean value with standard error (n=3-6) was plotted. Asterisks indicate probability of less than 0.01 between reference and *actpk1* (ANOVA).

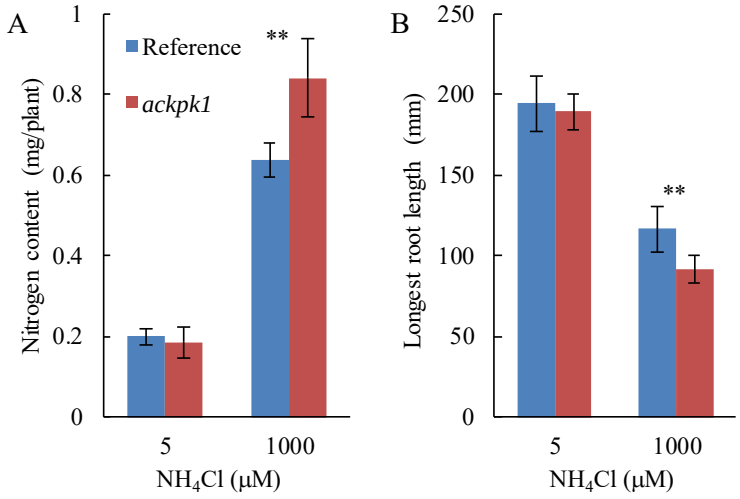

Fig. 2. Total nitrogen content (A) and longest root length (B).
Plants were hydroponically grown for 10 days in two NH_4^+ conditions, 5 μM as deficient and 1,000 μM as rich condition of NH_4^+. Mean value with standard deviation (n=6 for total nitrogen content, n=14 for longest root length) was expressed in blue column for Nipponbare as reference and red column for *actpk1*, respectively. Asterisks indicate probability of less than 0.01 between reference and *actpk1* (ANOVA).