Combining Machine Learning, Traditional Breeding and Gene Editing to Improve Nitrogen Use Efficiency (NUE) in Rice

In this new proposal, each subproject has its own specialty to integrate machine learning, classical breeding, protein engineering as well as new and old transformation techniques to enhance Rice NUE.

Ⅰ *Sub-project 1* Enhancing Nitrate Remobilization in Rice and Identifying Causative Genes in the Rice NUE Mutants

First, to further improve remobilization, promoters of four putative nitrogen remobilization genes were chosen to drive the expression of hyperactive transporter NC4N to enhance NUE. 19 independent OsNPF2.4::NC4N transgenic lines are ready for homozygous screen. Second, gor the NUE mutants isolated from mutant pool of IR64, we currently focused on AZ1306, which showed higher nitrate content and U-shape improvement of grain yield. Mapping using AZ1306 backcross lines are underway. In the third approach, several N-related differences were found between *Japonica* and *indica*. Moreover, through RNAseq analysis of the difference between japonica and indica, OsNPF4.10 was identified and will be studied using oocyte assay and transgenic approach.

Ⅱ *Sub-project 2* Overexpressing GS2 to enhance N reassimilation in rice

Approximately 80% of the total N in the panicle comes from remobilized N (Mae and Ohira, 1981). Gln synthetase (GS) is the primary enzyme to assimilate the remobilized N. Overexpressing GS2 may enhance N assimilation and improve rice yield.

Summary of current progresses are:

- The team successfully obtained GS2 overexpressors by ectopically expressing AtGLN2 in rice.
- The rice GS2 CRISPR mutants are lethal, and characterizations of GS2 OE and mutant lines are underway

Ⅲ *Sub-project 3* This project conducts field fertilizer experiments and strain propagation for rice plants produced in sub-projects 1 and 2. In addition, we backcross breeding to introduce high NUE characteristics into the stem rice Tainan No. 11 and the indica rice Taichung Indica No. 10.

Ⅳ *Sub-project 4* The main goal of sub-project 4 is to create hyperactive nitrate transporters through single amino acid mutation of OsNPF proteins that will allow us to improve rice NUE using CRISPR. By engineering six rice NPF transporters, we have identified OsNPF2.4jp-L493H and OsNPF6.5id-L395P, which exhibit approximately 2 times higher nitrate transport activity compared to their wild type. Furthermore, the activity of OsNPF6.5id-L395P is 4.8 times higher compared to CHL1.

Ⅴ *Sub-project 5* A multi-perspective approach to uncover novel genetic features responsible for NUE

We have used three levels of genetic features—DNA, RNA, and post-transcriptional isoforms—to establish a multi-level feature fusion prediction model aimed at understanding the mechanisms responsible for the superior NUE of indica rice compared to japonica rice. Firstly, through transcriptomic analyses, we have identified that the N-response genes specific to indica are enriched in photosynthesis, while those specific to japonica are enriched in suberin biosynthesis. At the DNA level, we have identified cis-regulatory elements (CREs) specific to either indica or japonica, thus inferring the upstream transcription factor families involved in regulating NUE. At the post-transcriptional level, we have identified an RNA isoform of a nitrate transporter that show differences between the two subspecies.
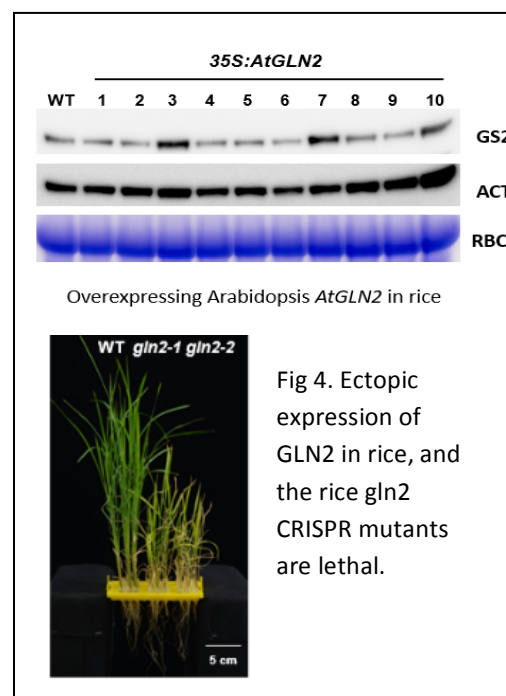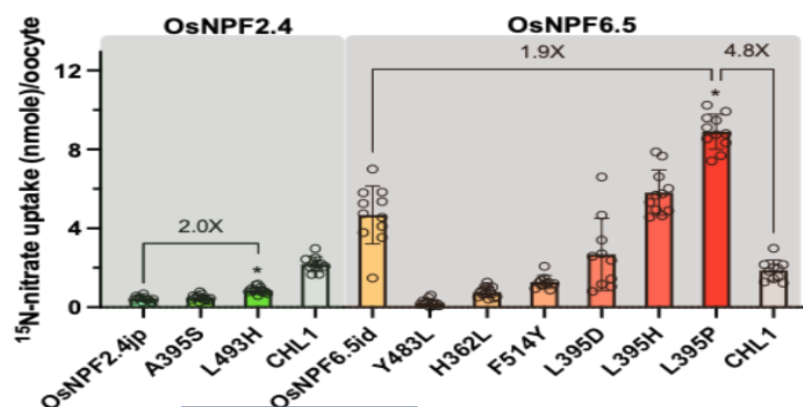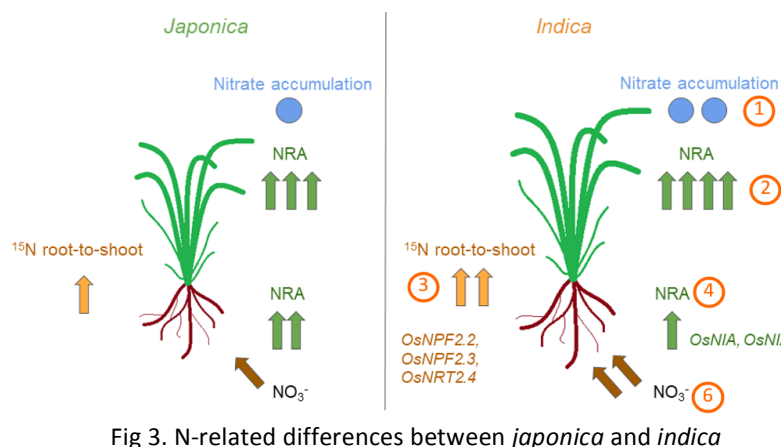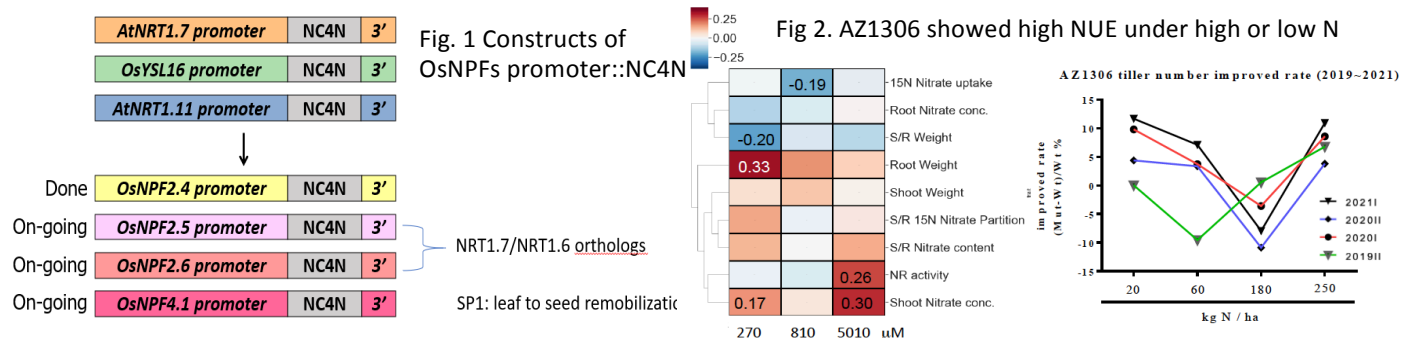
Fig. 1 Constructs of OsNPFs promoter::NC4N

Fig 2. AZ1306 showed high NUE under high or low N

Fig 3. N-related differences between *japonica* and *indica*

Overexpressing Arabidopsis *AtGLN2* in rice

Fig 4. Ectopic expression of GLN2 in rice, and the rice gln2 CRISPR mutants are lethal.

Figure 5. ^{15}N-nitrate uptake activity of engineering rice NPF transporters . OsNPF2.4jp-L493H and OsNPF6.5id-L395P (star-marked) shows 2X nitrate uptake activities compared to wild type.
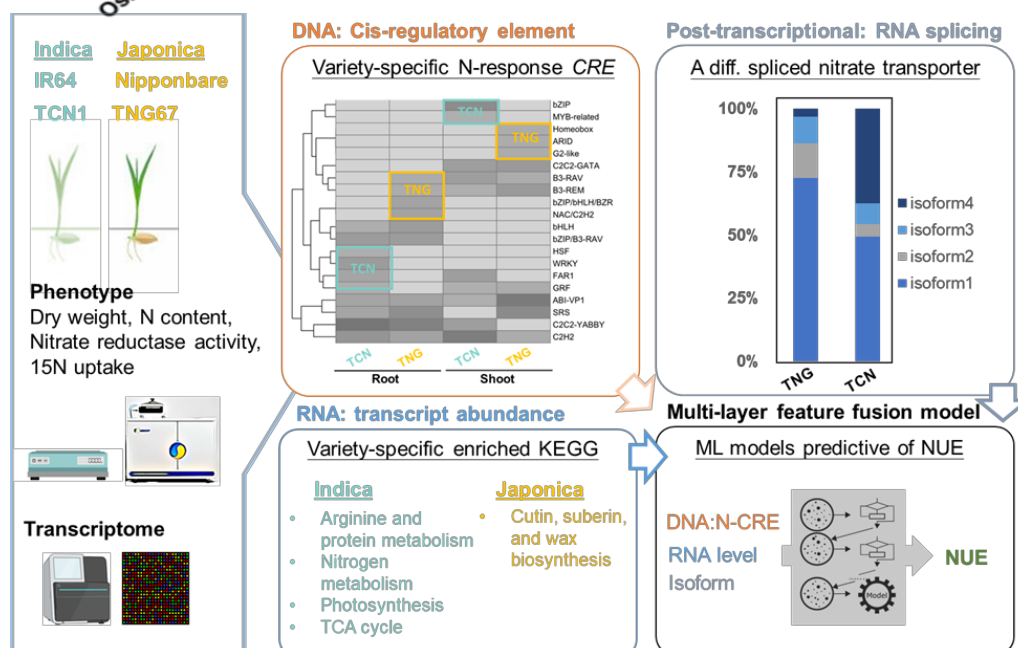

Figure 6. AI revealing novel genetic features linked to NUE