

A single SNP in *NRT1.1B* has a major impact on nitrogen use efficiency in rice

DUAN DongDong & ZHANG HanMa*

Chongqing Key Laboratory of Molecular Adaptations of Plants, College of Life Science, Chongqing Normal University, Chongqing 401331, China

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Nitrogen (N) is one of the most important nutrients for plants. Its availability is a major limiting factor in crop productivity and yield. Since the “green revolution” in the middle of the 20th century, the amount of N fertilizer used in agriculture has risen dramatically worldwide, resulting in a significant reduction of nitrogen use efficiency (NUE) and serious economic, environmental, and public health concerns [1]. A key challenge for plant biologists and crop scientists is how to reduce N fertilizer consumption without negatively affecting crop yield. Developing crop varieties with improved NUEs is an important strategy for addressing this challenge, but little progress has been made because of the lack of understanding of the mechanisms controlling NUE in crop plants.

A recent study [2] by a multi-institutional research team led by Chengcai Chu of the Institute of Genetics and Development Biology at the Chinese Academy of Sciences opened a new avenue for the development of nitrogen-efficient varieties of rice (*Oryza sativa* L.), which is one of the most important food crops and is widely cultivated worldwide. The study demonstrated that a single nucleotide difference in a nitrate transporter gene, *NRT1.1B* (*OsNPF6.5*), has a significant impact on both NUE and yield, and that it is possible to improve NUE in rice by exploring natural variations using relatively simple conventional breeding methods.

In plants, N is primarily acquired from the soil in two in-

organic forms, nitrate (NO_3^-) and ammonium (NH_4^+), via specific transporters. Currently, three families of plant nitrate transporters have been identified: NRT1, NRT2, and CLC (Chloride channel) [3]. The NRT1 family primarily includes low affinity transporters, although some, such as AtNRT1.1 (CHL1) from *Arabidopsis*, can act as dual-affinity nitrate transporters. The NRT2 family is exclusively composed of high-affinity nitrate transporters. It has been known that two widely cultivated Asian rice subspecies, *indica* and *japonica*, exhibit different nitrogen uptake activities and NUEs [4], but the underlying molecular mechanisms remain unknown. Hu et al. [2] adopted a genetic approach to investigate these mechanisms. They explored the differences in nitrate uptake activities between the two subspecies, and used chlorate, a toxic analog of nitrate that can be transported to plant cells via nitrate transporters, to screen a population of 317 BC2F5 lines developed from crossing an *indica* variety IR24 (the donor) and a *japonica* variety Nipponbare (the recipient) for plants with elevated sensitivity to this chemical (hence, the higher nitrate uptake activity). They obtained seven such lines and selected the most sensitive one, which carried a single-segment substitution on chromosome 10 from the IR24 genome in the Nipponbare background for further mapping analyses. Eventually, they mapped the chlorate sensitive locus in this line to a 15-kb fragment containing a single gene (i.e., *NRT1.1B*) and found two single-nucleotide polymorphisms (SNPs) between the *indica* and *japonica* subgroups within the gene. Of these, only one SNP (c.980C >

*Corresponding author (email: hanmazhang@126.com)

T) resulted in an amino acid change, from threonine (Thr) in the *japonica* form to methionine (Met) in the *indica* form. Using a number of carefully designed analyses, they confirmed that NRT1.1B functioned as a nitrate transporter, and that the 980C>T nucleotide variations in the *NRT1.1B* gene could explain the differences between *indica* and *japonica* rice plants, with respect to both the nitrate uptake activity and NUE.

CHL1 (AtNRT1.1) is the closest homolog of NRT1.1B in *Arabidopsis*, and is known to act as both a dual-affinity nitrate transporter and a nitrate sensor [5]. Remarkably, NRT1.1B also possessed those functional properties, as NRT1.1B displayed nitrate-transport activity under both low and high nitrate conditions. In addition, *OsNIA1* and *OsNIA2*, two genes encoding nitrate reductase, were significantly upregulated in the NIL. Conversely, their induction by nitrate was greatly repressed in the *nrt1.1b* mutant.

Most significantly, NRT1.1B appeared to play an important role in NUE. In hydroponic culture with nitrate as the sole N source, the plants carrying the *NRT1.1B-indica* allele displayed a stronger performance in a range of agronomically important traits, such as chlorophyll content, photosynthetic rate, and biomass production, than plants carrying the *NRT1.1B-japonica* allele, especially under low nitrate conditions. This functional property in NUE regulation makes NRT1.1B an ideal target gene for modification during future efforts to develop nitrogen-efficient crops. Since every known plant genome contains a large number of *NRT1* genes, 53 and 80 in the *Arabidopsis* and rice genomes respectively, there are considerable natural variations among NRT1 proteins. The characterization of the functions of different NRT1 proteins in and the identification of variations with functional impacts on NUE may provide new strategies for developing nitrogen efficient cultivars of rice or other crops.

Interestingly, the amino acid affected by the variations identified in this study is not conserved. This raises a number of interesting questions. For example, how did the functional significance of the variations evolve, and is it appli-

cable to other members of the family in rice and other crops? Are there any other naturally occurring substitutions at this position that have an impact on NUE? Obviously, further work will be required to answer these questions and fully appreciate the implications of this study. Nevertheless, the findings of this study are significant for a number of reasons: 1) they offer an explanation for a well-known difference in NUEs between two widely cultivated rice subspecies, *indica* and *japonica*; 2) they provide an excellent example for the knowledge gained from a model plant (*Arabidopsis*) being successfully applied to an economically important crop. A commonly used justification for studying model plants is that knowledge gained from such studies can be applied to economically important plants. However, in reality this rarely happens. This study presents a perfect example of the functional conservation of two homologous proteins in two distant plant species, and the consequent knowledge transfer from a model species to a crop; and 3) they demonstrate that exploring natural variations can be an important and fruitful strategy for crop improvement. This strategy offers an ideal alternative to the use of genetic engineering in future plant breeding, with a considerably reduced risk of public hostility. Thus, this study begins a new chapter in our search for nitrogen efficient crops.

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