Breeding Science 69: 429–438 (2019)
doi:10.1270/jsbbs.19014

Research Paper

Overexpression of an NADP(H)-dependent glutamate dehydrogenase gene, *TrGDH*, from *Trichurus* improves nitrogen assimilation, growth status and grain weight per plant in rice

Chang-Qing Du†, Jian-Zhong Lin†, La-Ai Dong†, Cong Liu, Dong-Ying Tang, Lu Yan, Ming-Dong Chen, Shan Liu and Xuan-Ming Liu*

Hunan Province Key Laboratory of Plant Functional Genomics and Developmental Regulation, College of Biology, Hunan University, Changsha 410082, Hunan, China

As glutamate dehydrogenases (GDHs) of microorganisms usually have higher affinity for NH_4^+ than do those of higher plants, it is expected that ectopic expression of these GDHs can improve nitrogen assimilation in higher plants. Here, a novel NADP(H)-GDH gene (*TrGDH*) was isolated from the fungus *Trichurus* and introduced into rice (*Oryza sativa* L.). Investigation of kinetic properties *in vitro* showed that, compared with the rice GDH (*Os*GDH4), *Tr*GDH exhibited higher affinity for NH_4^+ ($K_m = 1.48 \pm 0.11$ mM). Measurements of the NH_4^+ assimilation rate demonstrated that the NADP(H)-GDH activities of *TrGDH* transgenic lines were significantly higher than those of the controls. Hydroponic experiments revealed that the fresh weight, dry weight and nitrogen content significantly increased in the *TrGDH* transgenic lines. Field trials further demonstrated that the number of effective panicles, 1,000-grain weight and grain weight per plant of the transgenic lines were significantly higher than those of the controls, especially under low-nitrogen levels. Moreover, glutelin and prolamine were found to be markedly increased in seeds from the transgenic rice plants. These results sufficiently confirm that overexpression of *TrGDH* in rice can improve the growth status and grain weight per plant by enhancing nitrogen assimilation. Thus, *TrGDH* is a promising candidate gene for maintaining yields in crop plants via genetic engineering.

Key Words: glutamate dehydrogenase, *Trichurus*, ammonium metabolism, rice (*Oryza sativa* L.).

Introduction

Because of the deficiency and low utilization efficiency of nitrogen in most cultivated soils, nitrogen has become one of the limiting dominant factors in agricultural production (Frink *et al.* 1999, Stulen *et al.* 1998). However, a strong dependence on fertilizers is not economically sustainable, and even worse, more than half of the nitrogen in fertilizers applied to crops is not actually taken up by crop plants, giving rise to serious environmental problems (Socolow 1999). Thus, there is considerable interest in cultivating low-nitrogen-dependent crop varieties that present stable yields (Giles 2005). Nitrogen for plants mainly originates from inorganic nitrides such as ammonium (NH_4^+) and nitrate (NO_3^-). For crop species such as rice (*Oryza sativa* L.), the main form of nitrogen available in irrigated paddy fields is NH_4^+. Two pathways involved in the assimilation of NH_4^+ in higher plants have been identified: one is the glutamine synthetase (GS)/glutamate synthase (GOGAT) pathway (Lea and Miflin 1974), and the other is the glutamate dehydrogenase (GDH) pathway (Skopelitis *et al.* 2007). In the GS/GOGAT pathway, GS is the first enzyme responsible for ammonium assimilation to produce glutamine, and its activity requires energy in the form of adenosine triphosphate (ATP). GOGAT then transfers the amide nitrogen of glutamine to 2-oxoglutarate (2-OG) to form two glutamate molecules (Hodges 2002). In the GDH pathway, glutamate dehydrogenase (GDH, EC 1.4.1.2 and EC 1.4.1.4) catalyzes the reversible amination of 2-OG with NH_4^+ to form glutamate, which requires NAD(P)H as a cofactor but not ATP (Wootton 1983). Therefore, ammonia assimilation via the GDH pathway is purported to be more energy efficient than the GS/GOGAT pathway is (Helling 1998, Windass *et al.* 1980).

Although the GS/GOGAT pathways in plants were discovered in the 1970s (Lea and Miflin 1974) and are considered

Communicated by Toyoaki Anai
Received January 25, 2019. Accepted April 16, 2019.
First Published Online in J-STAGE on August 3, 2019.
*Corresponding author (e-mail: xml05@hnu.edu.cn)
† These authors contributed equally to this work

to constitute the main ammonium assimilation pathway, the role of GDH in ammonium assimilation remains controversial (Abiko *et al.* 2010, Helling 1998). There are at least two distinct GDH enzymes: NAD(H)-dependent glutamate dehydrogenase [NAD(H)-GDH; EC 1.4.1.2] and NADP(H)-dependent glutamate dehydrogenase [NADP(H)-GDH; EC 1.4.1.4]. In comparison with NAD(H)-GDH, NADP(H)-GDH has received very little attention in plants, and its role in plant metabolism remains obscure. Recently, NADP(H)-GDHs from microorganisms have been attracting widespread attention because they have been identified to play important roles in carbon and nitrogen metabolism in microorganisms, especially in fungi. For example, a mutant of *Aspergillus nidulans* deficient in NADP(H)-GDH (gdhA) showed poor growth when provided with ammonium as the sole nitrogen source (Kinghorn and Pateman 1973, Macheda *et al.* 1999). In particular, NADP(H)-GDHs from microorganisms such as bacteria and fungi exhibit a higher affinity for NH_4^+ (K_m = 0.2–4.5 mM; Du *et al.* 2014, Zhou *et al.* 2015b) than do those from higher plants (K_m = 10–80 mM; Oaks 1994), suggesting that NADP(H)-GDHs from microorganisms assimilate ammonium more efficiently. To date, several NADP(H)-GDHs from lower organisms have been introduced into crop plants to improve ammonium assimilation (Abiko *et al.* 2010, Ameziane *et al.* 2000, Du *et al.* 2014, Noor and Punekar. 2005, Tang *et al.* 2018, Zhou *et al.* 2014, 2015a, 2015b). For example, when an NADP(H)-GDH gene (*gdhA*) from *Aspergillus niger* was introduced into rice, the transgenic lines exhibited increased nitrogen utilization efficiency, and their dry weight, nitrogen content and grain weight per plant increased significantly compared with those of nontransformed plants (Abiko *et al.* 2010). Similarly, ectopic expression of another NADP(H)-GDH gene (*EcGDH*) from *Eurotium chevalieri* markedly improved the nitrogen assimilation and grain weight per plant in rice, especially under low-nitrogen conditions (Tang *et al.* 2018). To date, only *CeGDH* from *Cylindrocarpon ehrenbergii* and *EcGDH* from *Eurotium chevalieri* have been reported to be capable of improving grain yields in rice under low-nitrogen conditions (Tang *et al.* 2018, Zhou *et al.* 2015a).

GDHs are ubiquitous and present in both microorganisms and higher plants. It was reported that four GDH genes (*OsGDH1*, *OsGDH2*, *OsGDH3*, and *OsGDH4*) are present in the rice genome (Qiu *et al.* 2009). The NADP(H)-GDHs from fungi seem to be promising candidate genes for crop improvement and breeding due to their enhancement of nitrogen utilization efficiency in crops. However, except for the improvement of nitrogen assimilation, not all GDHs can always improve growth quality and grain yield, and even some GDHs severely inhibit seedling growth. For example, overexpression of *PcGDH* from *Pleurotus cystidiosus* showed improved nitrogen assimilation in rice, but no statistical enhancement in grain yields was detected between transgenic rice and wild-type plants (Zhou *et al.* 2014). Similarly, *MgGDH* from *Magnaporthe grisea* did not improve growth quality but did enhance tolerance to dehydration stress in transgenic rice (Zhou *et al.* 2015b). Moreover, overexpression of *SsGDH* from *Sclerotinia sclerotiorum* did not improve the growth quality or grain yield of rice, and even worse, it severely inhibited seedling growth (Du *et al.* 2014). Thus, to develop environmentally friendly crops with stable yields even under low-nitrogen fertility, we sought to isolate and identify new fungal NADP(H)-GDHs that can improve ammonium assimilation and production in crops.

The fungus *Trichurus* is a common soil saprophyte and usually causes some harm to plants and animals. When *Trichurus* infestation develops because of favorable environmental conditions, losses can be great, and control measures should be considered. However, the role of glutamate dehydrogenase from *Trichurus* in nitrogen metabolism remains unclear. In this study, an NADP(H)-GDH gene (*TrGDH*) was isolated from *Trichurus*, and the kinetic properties of *Tr*GDH were characterized. To further evaluate whether *Tr*GDH has a positive effect on ammonium assimilation efficiency as do other microorganism GDHs in rice, we tried to modify the ammonium assimilation pathway by overexpressing *Tr*GDH in rice as an additional enzyme for NH_4^+ assimilation and biochemical characterization. Changes in the nitrogen metabolism and growth status of rice following the introduction of fungal *TrGDH* were reported. Our results strongly demonstrated that ectopic expression of fungal *TrGDH* in rice alters the pathway for ammonium assimilation and, consequently, leads to a number of growth alterations in nitrogen metabolism, growth status and grain yield. These results will enrich the development of strategies to engineer enhanced nitrogen utilization efficiency via exogenous GDHs from fungal microorganisms in higher plant species such as rice.

Materials and Methods

Fungus and plant materials

The fungus *Trichurus* was kindly provided by Dr. Hong Liu (Fujian Agriculture and Forestry University, Fuzhou, China). Notably, the species name of this fungus has not been identified, so its generic name *Trichurus* is used to denote this fungus. The model rice (*Oryza sativa* sub. *japonica*) cultivar Kitaake was used to generate transgenic rice.

Phylogenetic analysis

To evaluate the evolutionary relationships between *Tr*GDH and previously characterized GDHs from various plants, fungi, and bacteria, a phylogenetic tree was constructed using MEGA 7.0 software and Clustal X based on their amino acid sequences (detailed information of all the GDHs is shown in **Supplemental Table 2**). Sequence information on the GDHs was obtained from the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/), TAIR (http://www.arabidopsis.org/) and GenBank (http://www.ncbi.nlm.nih.gov/).

Cloning of TrGDH from Trichurus and transformation of TrGDH in rice

Total RNA was extracted from *Trichurus* mycelia using Trizol reagent (Invitrogen, USA). The full-length cDNA of the glutamate dehydrogenase gene was amplified by PCR with degenerate primers (**Supplemental Table 1**) as described previously (Zhou *et al.* 2014). The sequence of the PCR product was verified and denoted as *TrGDH*. To assess the effects of *Tr*GDH in rice, *Tr*GDH fused to a FLAG tag was introduced into a pCAMBIA1301 binary vector (slightly modified) with the specific primers (**Supplemental Table 1**) as described previously (Zhou *et al.* 2014), and the resulting vector was designated as pCAMBIA1301-*Ubi::TrGDH-FLAG* (**Fig. 3A**). To investigate the effects of *TrGDH* on the nitrogen utilization efficiency in rice, the recombinant plasmid was introduced into *O. sativa* cv. Kitaake by the *Agrobacterium*-mediated (EHA105) transformation method (Zhou *et al.* 2018); hygromycin-resistant plants from calli were designated as composing the T_0 generation. The T_2 progeny that were from the transgenic rice plants and that expressed high levels of *TrGDH* were used in subsequent experiments. The *TrGDH* transcription level in transgenic plants was investigated by semi-RT-PCR using *TrGDH*-specific primers (**Supplemental Table 1**).

Expression and purification of His_6-TF-TrGDH

The full sequence of *TrGDH* was amplified by PCR with specific primers (**Supplemental Table 1**), and the PCR products were digested with *Bam*HI and *Hind*III and then inserted into a pCold-TF vector with the same sites to yield the recombinant prokaryotic expression vector pCold-TF-*TrGDH* with a trigger factor (TF) and a His-tag sequence at the 5′-end (Takara, Japan). In addition, the rice homogenous NADP(H)-GDH gene *OsGDH4* was also cloned and inserted into the same prokaryotic expression vector, which served as a control. The pCold-TF-*TrGDH* vector was then transformed into *E. coli* BL21 (DE3), and positive clones were isolated for His_6-TF-*Tr*GDH expression. The recombinant protein His_6-TF-*Tr*GDH was affinity purified with nickel-nitrilotriacetic acid (Ni-NTA) resin (Invitrogen, USA) according to previously described methods (Zhou *et al.* 2015a). To verify the His_6-TF-*Tr*GDH, a western blot was used to examine the purified fusion proteins using anti-His monoclonal antibodies (1:5,000; Abmart, China) as described previously (Zhou *et al.* 2015b).

NADP(H)-GDH enzyme activity assays

The NADP(H)-GDH enzyme activity was measured *in vitro* following the method described by Noor and Punekar (2005). The aminating activity of His_6-TF-*Tr*GDH was determined in a reaction mixture containing various NH_4Cl concentrations (1, 2.5, 5, and 7.5 mM) or various 2-oxoglutarate (2-OG) concentrations (1, 2.5, 5, and 10 mM) and 100 μM β-NADP(H) in Tris buffer (pH 8.0). The deaminating activity of His_6-TF-*Tr*GDH was determined in a reaction mixture containing various L-glutamate concentrations (25, 50, 75, and 100 mM) and 200 μM β-$NADP^+$ in Tris buffer (pH 9.3). The reactions were preincubated at 25°C before adding His_6-TF-*Tr*GDH protein and routinely started by the addition of a coenzyme [β-NADP(H) or β-$NADP^+$]. The enzyme activity assays were measured by monitoring the absorption change at 340 nm with a UV-1800 spectrophotometer (Shimadzu, Japan). One unit of enzyme activity is defined as the reduction or oxidation of 1 μM NADP(H)/second at 25°C. The kinetic constant (K_m) for different substrates was calculated by using the Lineweaver–Burk equation. The purified His_6-TF-*Os*GDH4 (as the control) was also used for the NADP(H)-GDH enzyme activity assay.

For the NADP(H)-GDH enzyme activity assay *in vivo*, the soluble proteins were extracted from the shoots and roots of *TrGDH* transgenic rice plants and nontransgenic plants (as controls) at the two-leaf stage according to the method described by Abiko *et al.* (2010), and their respective NADP(H)-GDH activities were tested in a reaction mixture containing NH_4Cl (5 mM), 2-oxoglutarate (2-OG; 5 mM), and β-NADP(H) (100 μM) in a Tris buffer (pH 8.0). The reactions were preincubated at 25°C before adding soluble proteins and were started by the addition of coenzyme β-NADP(H). The absorption change was monitored as described above. The data points represent the averages of three replicates and are consistent.

Reverse transcription-PCR (RT-PCR) analysis

For RNA expression level analysis, semiquantitative RT-PCR was used to investigate the expression of *TrGDH* in the T_2 progeny of the transgenic homozygous rice plants. RT-PCR was performed using a SYBR PremixEx Taq kit (Takara, Japan) according to the manufacturer's instructions with an Mx3000P instrument. Triplicate quantitative assays were performed on each cDNA sample. *OsActin1* was used as an internal control. The relative expression levels were measured as described by Livak and Schmittgen (2001). Each data point represents the average of three replicates. Three experiments were performed with consistent results. The primers used are shown in **Supplemental Table 1**.

Characterization of TrGDH transgenic rice plants at the seedling stage

Two T_2 progeny seeds of transgenic lines (*Ubi::TrGDH-4* and *Ubi::TrGDH-13*) and the controls were sterilized with 3% NaClO and then germinated, after which they were grown in an incubator at 28°C/26°C (day/night) and normally cultured in 1/2-strength MS liquid medium for 7 d. The seedlings were then cultured in IRRI nutrient solution (0.3 mM KH_2PO_4, 0.35 mM K_2SO_4, 1 mM $MgSO_4 \cdot 7H_2O$, 0.5 mM $Na_2SiO_3 \cdot 9H_2O$, 1 mM $CaCl_2 \cdot 2H_2O$, 9 μM $MnCl_2 \cdot 4H_2O$, 20 μM H_3BO_3, 0.77 μM $ZnSO_4 \cdot 7H_2O$, 0.32 μM $CuSO_4 \cdot 5H_2O$, 20 μM NaFeEDTA, and 0.39 μM $Na_2MoO_4 \cdot 2H_2O$, pH 5.5) with different concentrations of NH_4^+ (50 μM, 0.500 μM) as the only nitrogen source as described previously (Zhou *et al.* 2014) for 15 d to analyze the phenotype and physiology. The nutrient solution was

maintained at pH 5.6 and refreshed every 3 d. After treatment for 15 d, phenotypic images were taken, and the fresh weights, dry weights and total nitrogen contents of the transgenic and control plants were measured by Kjeldahl analysis (Zhou *et al.* 2015a). For the above parameters, each data point represents the average of three replicates, with consistent results.

Field trial

A field trial was performed as described previously (Zhou *et al.* 2014). *TrGDH* transgenic and control plants were planted in paddy fields with different nitrogen gradient levels (0, 37.5, 112.5, and 187.5 kg N/ha; in the form of urea) in Changsha, Hunan Province, China. The nitrogen level of the paddy field, which was planted with early rice and did not receive any nitrogen fertilizer, was defined as 0 kg/ha (the actual fertility was 0.65 g N/kg soil). Fertilizer was applied in three split doses: one dose was half that of the total nitrogen fertilizer of each gradient and was applied as the basal dose [50 kg phosphorus (P), 50 kg potash (K), 25 kg zinc sulfate ($ZnSO_4$) per hectare], whereas the other two doses were applied at the active tillering and panicle initiation stages. All rice plants in this study were grown as late rice in July. Two rows of control plants were planted as guard rows around the planted field. At harvest, agriculture traits including plant height, effective panicle number, filled grain rate and 1,000-seed weight were analyzed. The grain weight per plant represents the total weight of grain per plant, and after harvest, the date of grain weight per plant was measured from 20–30 rice plants in the middle two rows. The average value obtained through variance and statistical analyses of these data was evaluated using Student's *t*-test.

Glutelin and prolamine content analysis

Glutelin and prolamine contents were extracted according to the method described by Krishnan and Okita (1986) from the seeds of the transgenic and control plants. The protein concentrations of glutelin and prolamine were measured by the Cai *et al.* (2009). Protein assay using Coomassie Plus Protein Assay Reagent (Tiangen, China). Bovine serum albumin (BSA) was used as the standard protein.

Statistical analysis

All the data were subjected to analysis of variance (ANOVA) or Student's *t*-test using SPSS 13.0 (SPSS Co., USA).

Results

Phylogenetic analysis of TrGDH

Based on the conserved regions in GDHs among fungi and bacteria, a full-length cDNA encoding glutamate dehydrogenase was isolated from the fungus *Trichurus* and designated as *TrGDH*. The full-length *TrGDH* sequence consists of 1,359 nucleotides, which was predicted to encode 452 amino acids with a molecular mass of 49.0 kDa and a calculated pI of 5.7 (**Supplemental Fig. 1**). Notably, neither the nucleotide nor the amino acid sequence of *TrGDH* has been deposited in the NCBI database or GenBank, so *TrGDH* was considered a novel GDH gene. The phylogenetic tree shows that NADP(H)-GDH proteins form a separate cluster from the group containing plant NAD(H)-GDH proteins; moreover, *Tr*GDH belongs to the NADP(H)-GDH subgroup and exhibits high homology with GDHs in bacteria and fungi but low homology with GDHs in higher plants such as rice and *Arabidopsis thaliana* (**Fig. 1**). Thus, we speculate that the fungal *Tr*GDH also may play an important role in enhancing ammonium assimilation efficiency, similar to other microorganism GDHs, when introduced into rice (Abiko *et al.* 2010, Tang *et al.* 2018, Zhou *et al.* 2015a). It is notable that three NAD(H)-GDHs (*Os*GDH1, *Os*GDH2 and *Os*GDH3) and one NADP(H)-GDH (*Os*GDH4) are present in the rice (Qiu *et al.* 2009, **Supplemental Table 2**), whereas only *Os*GDH4 exhibits the highest homology with *Tr*GDH (**Fig. 1**). Moreover, we analyzed the protein sequence similarity between *Tr*GDH and other representative reported GDHs from fungi (*Magnaporthe oryzae*, *Chaetomium globosum* and *Neurospora crassa*), which shows a higher

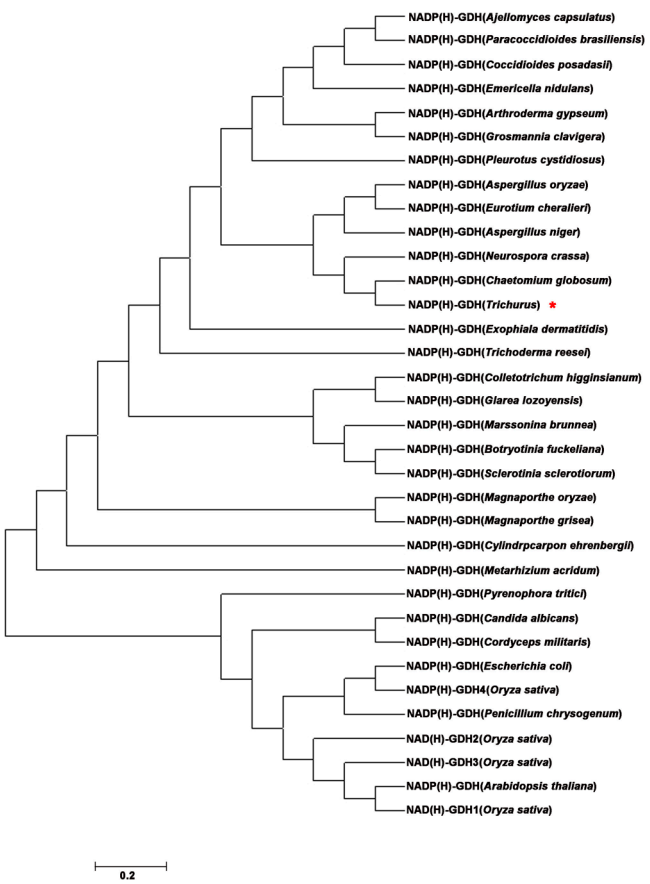


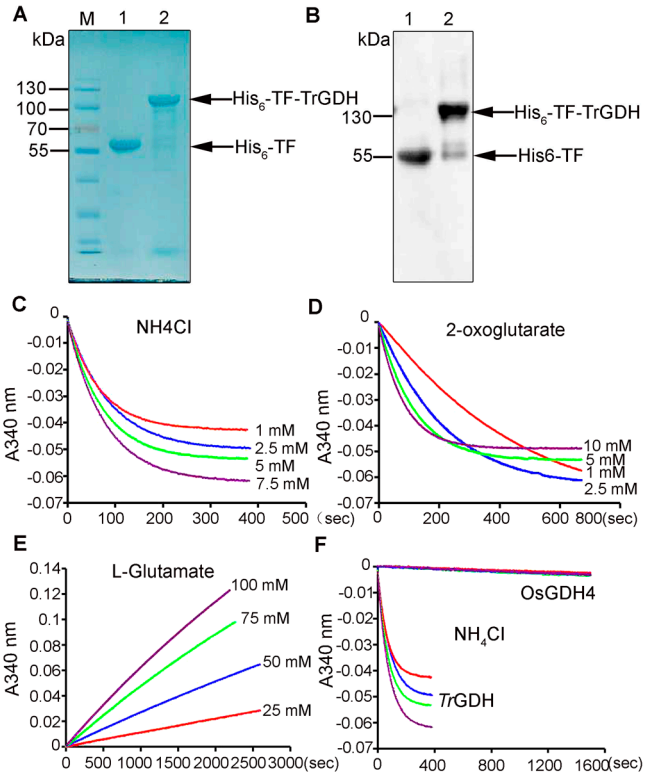
Fig. 1. Phylogenetic relationship of GDHs among bacterial, fungal, and higher plants. Phylogenetic tree was constructed by neighbor-joining method using Clustal X and MEGA 7.0. The information on the deduced amino acid sequences of the NAD(P)(H)-GDHs was obtained from NCBI or GeneBank. The length of the branch is proportional to the degree of divergence. *Tr*GDH was red asterisk marked.

homology with *Tr*GDH (**Fig. 1**), bacteria (*Escherichia coli*), and rice (*Oryza sativa*) according to MEGA 7.0 and GENEDOC (**Supplemental Fig. 2**). As expected, *Tr*GDH contains a Glu/2-OG binding site and an NADP(H) binding site, which are the characteristic conserved domains in NADP(H)-GDH proteins. Taken together, these results demonstrated that the NADP(H)-GDH gene *TrGDH* was successfully isolated from the fungus *Trichurus*, which allowed for its functional characterization and application in rice.

TrGDH exhibits higher affinity for ammonium than does OsGDH4 in vitro

To characterize the properties of *Tr*GDH *in vitro*, a pCold-TF-*TrGDH* recombinant plasmid was constructed and introduced into *E. coli* BL21 for His-tagged His_6-TF-*Tr*GDH recombinant protein expression. The recombinant protein was then affinity purified and subjected to western blotting and Coomassie Brilliant Blue staining. A single polypeptide band that corresponded to the deduced molecular size (104.0 kD) of His_6-TF-*Tr*GDH was detected, while the control showed only a 55.0 kDa band corresponding to that of the His_6-TF chaperone protein in the original vector (**Fig. 2A**). Western blotting analysis also confirmed the presence of His_6-TF-*Tr*GDH protein via anti-His monoclonal antibodies (**Fig. 2B**). The purified His_6-TF-*Tr*GDH recombinant protein was then used for glutamate dehydrogenase activity analysis.

To study the enzymatic properties of *Tr*GDH, the kinetic properties of the affinity of His_6-TF-*Tr*GDH for its substrates, including ammonium (NH_4^+), 2-oxoglutarate (2-OG), and L-glutamate, were determined *in vitro*. The absorption changes in the amination/deamination enzymatic reactions with different concentrations of NH_4Cl, 2-OG and L-glutamate were monitored at 340 nm (**Fig. 2C–2E**). Via the Lineweaver–Burk equation, the K_m values for NH_4^+, 2-OG and L-glutamate were then calculated. As shown in **Fig. 2G**, the affinity of the His_6-TF-*Tr*GDH protein for NH_4^+ ($K_m = 1.48 \pm 0.11$ mM) and 2-OG ($K_m = 1.57 \pm 0.19$ mM) was significantly higher than that for L-glutamate ($K_m = 410.09 \pm 44.84$ mM), indicating that *Tr*GDH prefers to utilize ammonium to produce glutamate (**Supplemental Fig. 3**). As described above, among four *Os*GDHs, *Os*GDH4 has the highest homology with *Tr*GDH (**Fig. 1**) and thus was selected for the comparison of enzyme activity with *Tr*GDH *in vitro*. As expected, the time course of the comparison between His_6-TF-*Tr*GDH and His_6-TF-*Os*GDH4 in absorbance at 340 nm in the amination reaction in conjunction with different NH_4Cl concentrations showed that the affinity of His_6-TF-*Tr*GDH for NH_4^+ was prominently higher than that of His_6-TF-*Os*GDH4 (**Fig. 2F**). Thus, these results indicate that, compared with the *Os*GDHs, *Tr*GDH has a markedly stronger affinity for ammonium, which makes it possible that *Tr*GDH can efficiently assimilate NH_4^+, even at very low ammonium concentrations.



G

Substrate	K_m(mM)
$NH4^+$	1.48±0.11
2-oxoglutarate	1.57±0.19
L-Glutamate	410.09±44.84

Fig. 2. Purification and enzymatic kinetic assay of recombinant protein His_6-TF-*Tr*GDH *in vitro*. (A) SDS-PAGE detection of purified His_6-TF-*Tr*GDH protein. M: protein marker; 1: His_6-TF-tag control; 2: His_6-TF-*Tr*GDH protein. The proteins were stained with Coomassie Blue dye after SDS-PAGE. (B) Western blot assay of purified His_6-TF-*Tr*GDH protein. 1: His_6-TF-tag control; 2: His_6-TF-*Tr*GDH protein. The proteins were detected using anti-His antibody (1:5,000). (C) Time course of changes in absorbance at 340 nm in amination reaction using different NH_4Cl concentrations (1, 2.5, 5, 7.5 mM). (D) Time course of changes in absorbance at 340 nm in amination reaction using different 2-OG concentrations (1, 2.5, 5, 10 mM). (E) Time course of changes in absorbance at 340 nm in deamination reaction using different glutamate concentrations (25, 50, 75, 100 mM). (F) Time course of changes comparison of His_6-TF-*Tr*GDH and His_6-TF-*Os*GDH4 in absorbance at 340 nm in amination reaction using different NH_4Cl concentrations (1, 2.5, 5, 7.5 mM). Three experiments were conducted with consistent results. The result from one set of experiments is presented here. (G) Kinetic property (*Km*) of purified His_6-TF-*Tr*GDH protein. One unit of enzyme activity is defined as the reduction or oxidation of 1 μM of NADP(H) per second at 25°C. The calculation was conducted by using the Lineweaver–Burk double-reciprocal protocol. Values represents the means of three independent experiments. Data represents the mean values ± SD (n = 3).

Identification of TrGDH transgenic rice plants

The results showed that a higher *TrGDH* transcription level was identified in two *TrGDH* transgenic lines,

Ubi::TrGDH-4 and *Ubi::TrGDH-13*, whereas no *TrGDH* was detected in the nontrangenic plants (negative controls) (**Fig. 3B**), indicating that *TrGDH* was successfully over-expressed in the transgenic rice lines. Furthermore, the *Tr*GDH-FLAG fusion proteins in the two transgenic lines were also confirmed by western blot analysis using anti-FLAG monoclonal antibodies. As shown in **Fig. 3C**, target bands of *Tr*GDH-FLAG were detected in the two transgenic lines but not in the control plants. This result further confirmed that transgenic lines constitutively expressing *TrGDH* were successfully obtained. Thus, these two transgenic lines, *Ubi::TrGDH-4* and *Ubi::TrGDH-13*, were used for subsequent physiological and phenotypic analysis.

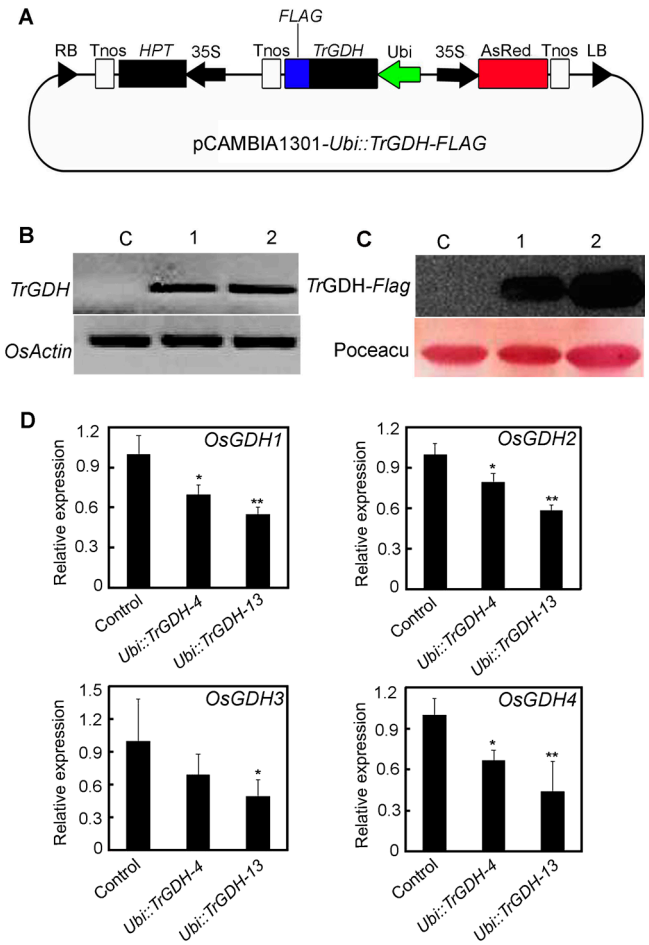


Fig. 3. Molecular confirmation of transgenic rice lines expressing *Tr*GDH-FLAG. (A) Schematics of plant expression vector pCAMBIA1301-*Ubi::TrGDH-FLAG*. *HPT* hygromycin resistance gene, Tnos terminator of nopaline synthase gene (nos), *FLAG* tag sequence, *AsRed* red fluorescence protein gene, 35S CaMV 35S promoter, Ubi ubiquitin promoter derived from *Zea mays*, LB left border, RB right border, and *TrGDH* NADP(H)-GDH gene derived from *Trichurus*. (B) Relative expression level analysis of *TrGDH* in the control and *TrGDH* transgenic rice lines by semi-quantitative RT-PCR. Two independent transgenic rice lines, *Ubi::TrGDH-4* and *Ubi::TrGDH-13*, were used for analysis. *OsActin1* was amplified as a control for mRNA levels. (C) Western blotting analysis of *Tr*GDH-FLAG in the control and *TrGDH* transgenic rice lines using anti-FLAG antibody (1:5000). Poceacu staining was used as a loading control. (D) Real-time quantitative PCR analysis of *OsGDH1*, *OsGDH2*, *OsGDH3*, *OsGDH4* in the control and *TrGDH* transgenic rice lines. *OsActin1* was amplified as a control for mRNA levels. Triplicate quantitative assays were performed on each cDNA sample. * indicates significant differences at $p < 0.05$. ** indicates significant differences at $p < 0.01$.

TrGDH improved the ammonium assimilation efficiency of rice seedlings

To evaluate the effects of *TrGDH* on rice homogenous

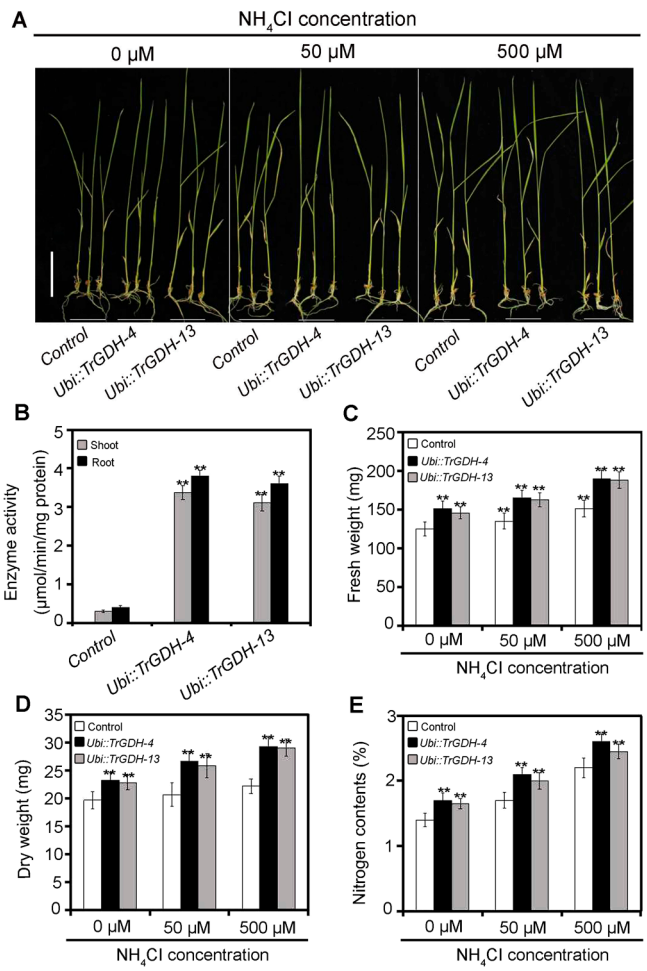


Fig. 4. Characteristics of transgenic rice lines expressing *Tr*GDH grow under different NH_4Cl concentrations (0, 50, and 500 μM). (A) Images of control and *TrGDH* transgenic seedlings grown under different NH_4Cl concentrations (0, 50, and 500 μM) for 15 d. Two independent transgenic rice lines, *Ubi::TrGDH-4* and *Ubi::TrGDH-13*, were used for analysis. Bar = 5 cm. (B) Amination activity assay of NADP(H)-GDH in the shoots and roots of control and *TrGDH* transgenic seedlings. Data are presented as average values ± SD (n = 3). (C, D) Fresh weight and dry weight of control and *TrGDH* transgenic seedlings grow under different NH_4Cl concentrations (0, 50, and 500 μM). Average values ± SD are shown (n = 20–30). From B–E, statistical differences were calculated by *t*-test. ** indicates significant differences at $p < 0.01$. (E) Nitrogen contents of control and *TrGDH* transgenic seedlings grow under different NH_4Cl concentrations (0, 50, and 500 μM). Data represent the mean values ± SD (n = 3).

Table 1. Agronomic traits of the control and transgenic rice plants grown in paddy filed

Nitrogen fertilizer concentration (kg N/ha)	Lines	Plant height (cm)	Number of effective panicles (per)	Filled grain rate (%)	Thousand grain weight (g)	Grain weight per plant (g)
0	Control	73.3 ± 2.0	16.2 ± 2.3	58.2 ± 2.1	21.4 ± 0.8	14.05 ± 1.5
	Ubi::TrGDH-4	70.7 ± 2.8	20.6 ± 2.1**	48.1 ± 1.3**	25.3 ± 0.7**	17.04 ± 0.9**
	Ubi::TrGDH-13	71.4 ± 3.2	21.5 ± 1.5**	50.2 ± 2.0**	25.7 ± 1.0**	16.64 ± 0.9**
37.5	Control	73.6 ± 3.2	20.6 ± 1.5	62.3 ± 1.1	24.8 ± 0.7	16.70 ± 0.7
	Ubi::TrGDH-4	73.0 ± 1.9	25.1 ± 1.2**	53.0 ± 1.4**	28.3 ± 0.4**	19.76 ± 1.2**
	Ubi::TrGDH-13	72.0 ± 3.4	26.6 ± 2.1**	54.2 ± 1.2**	28.9 ± 0.6**	21.95 ± 0.7**
112.5	Control	75.2 ± 3.1	25.7 ± 2.2	66.3 ± 1.3	26.1 ± 0.6	22.34 ± 0.9
	Ubi::TrGDH-4	73.8 ± 2.5	28.6 ± 1.3**	58.0 ± 1.5**	29.6 ± 0.5**	25.77 ± 1.0**
	Ubi::TrGDH-13	74.4 ± 3.2	29.7 ± 1.6**	59.1 ± 1.1**	30.0 ± 0.4**	26.64 ± 0.7**
187.5	Control	76.3 ± 2.1	29.8 ± 3.2	68.6 ± 1.6	28.3 ± 0.7	29.36 ± 1.2
	Ubi::TrGDH-4	74.1 ± 3.3	31.5 ± 2.2**	62.0 ± 1.7**	30.6 ± 0.4**	33.37 ± 1.2**
	Ubi::TrGDH-13	75.5 ± 2.3	32.7 ± 1.6**	61.6 ± 1.3**	30.8 ± 0.5**	32.57 ± 0.9**

A collection of quantitative data represent the mean values ± SE (n = 20–30). Statistical analysis of the data was performed by t-test. Asterisks indicate significantly differences at $p < 0.01$ (**).

*GDH*s, quantitative real-time PCR (qPCR) was performed to analyze the transcription levels of *OsGDH1*, *OsGDH2*, *OsGDH3* and *OsGDH4*. The results showed that the expression levels of *OsGDH1*, *OsGDH2*, *OsGDH3* and *OsGDH4* were downregulated (**Fig. 3D**), indicating that the heterologous *TrGDH* competitively disrupted the transcription of their endogenous *GDH*s in the transgenic rice plants.

To detect whether the heterologously expressed *Tr*GDH in the transgenic plants retained its function, the enzymatic activity (i.e., amination activity) of NADP(H)-GDH in the shoots and roots of the control and transgenic plants was measured, with NH_4^+ used as a substrate. The results showed that the NADP(H)-GDH activities of both the shoots and roots were significantly higher in the two transgenic lines than in the control plants (**Fig. 4B**), indicating that the heterologous *Tr*GDH was still functional and that it indeed enhanced the ammonium assimilation efficiency in rice. To further investigate the effects of *Tr*GDH on the ammonium assimilation efficiency of rice plants at the seedling stage, the phenotypic changes of seedlings of the controls and *TrGDH* transgenic lines were examined after treatment with different concentrations of ammonium (0, 50, and 500 μM NH_4Cl) (**Fig. 4A**), and their fresh weights, dry weights and total nitrogen contents were analyzed. The results showed that, under the three ammonium concentrations, both the fresh weight and dry weight of *TrGDH* transgenic seedlings were significantly greater than those of the control seedlings (**Fig. 4C**, **4D**), indicating that the heterologous *Tr*GDH enhanced biomass accumulation in rice. Moreover, the transgenic seedlings also accumulated more nitrogen than did the control seedlings (**Fig. 4E**), which was consistent with our hypothesis that *Tr*GDH can improve the ammonium assimilation efficiency in rice. Thus, we speculate that the marked increase in the absorption of nitrogen may give rise to improvement in rice growth status.

TrGDH improved the grain weight per plant and protein accumulation in seeds

To evaluate the effects of the *Tr*GDH introduction on rice productivity, transgenic lines (*Ubi::TrGDH-4* and *Ubi::TrGDH-13*) and control plants were planted and grown to maturity in paddy fields with different nitrogen concentrations (0, 37.5, 112.5 and 187.5 kg N/ha) in accordance with local standard practices for rice cultivation. Agronomic traits including plant height, effective panicle number, percentage of filled grains, 1,000-grain weight, and grain weight per plant were scored at harvest. Under different nitrogen concentrations, there was no difference in plant height between the control and transgenic plants (**Table 1**). However, the number of effective panicles, 1,000-grain weight and grain weight per plant of the transgenic lines were significantly higher than those of the control plants (**Table 1**). Surprisingly, an obvious decrease in the percentage of filled grains was observed in the transgenic rice lines compared with the control plants. In addition, the degree of

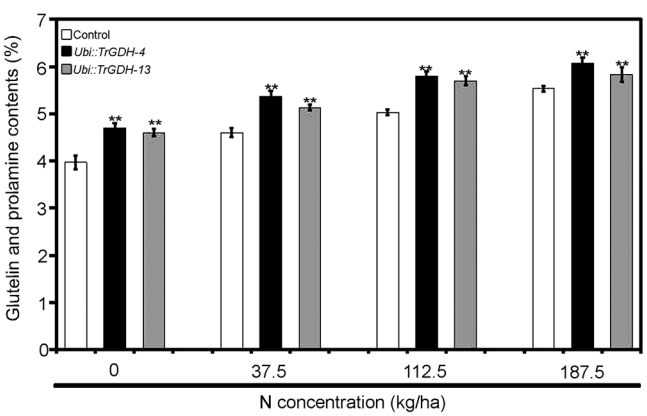


Fig. 5. Glutelin and prolamine contents of control and transgenic lines (*Ubi::TrGDH*-4 and *Ubi::TrGDH*-13) seeds. Average values ± SD are shown (n = 3). Statistical differences were calculated by t-test. ** indicates significant differences at $p < 0.01$.

increase in effective panicle number, 1,000-seed weight and grain weight per plant were reduced with the increase in the nitrogen gradient concentration, indicating that *Tr*GDH can effectively promote biomass accumulation in grain weight per plant, especially under low-nitrogen conditions (0 and 37.5 kg N/ha).

To investigate whether *TrGDH* introduction also affects protein accumulation in rice seeds, the glutelin and prolamine contents were further analyzed in the seeds of the control and transgenic plants. The results showed that the glutelin and prolamine contents of the seeds of transgenic plants were markedly higher than those of the control plants grown under the same field conditions (**Fig. 5**), indicating that *Tr*GDH can promote protein accumulation in rice seeds and improve their nutritional value. These results confirmed that ectopic expression of *Tr*GDH can improve nitrogen assimilation efficiency and grain weight per plant in rice.

Discussion

Using NAD^+ and $NADP^+$ as cofactors, glutamate dehydrogenase (GDH) catalyzes the reversible amination of 2-OG with NH_4^+ to form glutamate (Wootton 1983). In organisms, GDH contributes to important processes such as amino acid and carbohydrate metabolism, energy production and ammonia management through the Krebs cycle, interconnecting in parallel amino acid and carbohydrate metabolism (Hutson *et al.* 2011). Therefore, GDH is considered a key enzyme for metabolism and is essential for cell survival (Hudson and Daniel 1993). In plants, since the discovery of the glutamine synthetase (GS)/glutamate synthase (GOGAT) pathway in the 1970s, the role of GDH in ammonium assimilation has been a matter of controversy (Abiko *et al.* 2010). For example, a study involving feeding with both $^{15}NH_4^+$ and MSX in GDH1-null mutants of maize suggested that endogenous GDH does not catalyze the ammonium-assimilating reaction (Magalhães *et al.* 1990). By catalyzing the ATP-dependent condensation of ammonium with glutamate to produce glutamine, GS is therefore considered the key enzyme involved in the primary assimilation of ammonium in plants (Ireland and Lea 1999). Conversely, in microorganisms such as bacteria and fungi, both NADP(H)-dependent glutamate dehydrogenase (GDH) and GS play important roles in ammonium assimilation. In particular, in comparison with GDHs in plants, NADP(H)-GDHs in microorganisms assimilate ammonium more efficiently, as the affinity of the latter for ammonium is relatively stronger. Furthermore, Zhou *et al.* (2015b) reported that the K_m value of *Os*GDH4 for NH_4^+ is 12.43 ± 1.84 mM, which is markedly higher than that of *Tr*GDH (1.48 ± 0.11 mM). Therefore, the genes encoding fungal NADP(H)-GDHs have been proposed to be promising candidates for ectopic expression in crop plants for the production of an additional enzyme for ammonium assimilation (Abiko *et al.* 2010, Noor and Punekar 2005, Tang *et al.* 2018, Zhou *et al.* 2015a).

In this study, a novel NADP(H)-dependent glutamate dehydrogenase gene (*TrGDH*) was first isolated from the fungus *Trichurus*. Evolutionary relationship analysis demonstrated that *Tr*GDH shows high homology with the NADP(H)-GDHs of other microorganisms (**Fig. 1**). Glutamate dehydrogenase activity assay *in vitro* showed that the amination activity of His_6-TF-*Tr*GDH was significantly higher than the deamination activity (**Fig. 2C–2F**), and the K_m value for NH_4^+ (1.48 ± 0.11 mM) was markedly lower than that for L-glutamate (410.09 ± 44.84 mM) (**Fig. 2G**), which further demonstrated that *Tr*GDH tends to catalyze the amination of 2-OG with NH_4^+ to form glutamate. Additionally, Zhou *et al.* (2015b) reported that the K_m value of *Os*GDH4 from rice for NH_4^+ is 12.43 ± 1.84 mM, which is markedly higher than that of *Tr*GDH (1.48 ± 0.11 mM). Similarly, His_6-TF-*Os*GDH4 also exhibited a significantly lower affinity for NH_4^+ than did His_6-TF-*Tr*GDH (**Fig. 2F**). These results indicate that ectopic expression of *Tr*GDH for the production of as an additional enzyme for ammonium assimilation might improve nitrogen assimilation efficiency in rice. Hydroponic experiments subsequently revealed that overexpression of *TrGDH* actually improved the nitrogen assimilation efficiency and growth status and increased biomass production at the seedling stage in rice (**Fig. 4**). Surprisingly, overexpression of exogenous *TrGDH* resulted in the downregulation of the transcription of endogenous GDHs such as *OsGDH1*, *OsGDH2* and *OsGDH3* in rice (**Fig. 3D**). Although the endogenous GDHs of higher plants were reported not to catalyze the ammonium-assimilating reaction (Magalhães *et al.* 1990), their transcription was still competitively disrupted by the exogenous *TrGDH* in transgenic rice plants. Furthermore, the endogenous GDH transcript levels were downregulated, but the *TrGDH* transgenic rice plants still exhibited higher amination activity (**Fig. 4B**) and accumulated more nitrogen than did the control plants (**Fig. 4E**). These results further verified, at least in part, that the endogenous GDHs of higher plants are not involved in ammonium assimilation but that fungal GDHs such as *Tr*GDH play important roles in this physiological process.

As described above, GDHs from fungi are currently receiving increased amounts of attention for the improvement of ammonium assimilation in crops by genetic engineering. Except for their ability to enhance nitrogen assimilation, not all GDHs from fungi can always improve growth quality and grain yield (Abiko *et al.* 2010, Du *et al.* 2014, Zhou *et al.* 2014, 2015a, 2015b). Even worse, *SsGDH* from *Sclerotinia sclerotiorum* severely inhibited seedling growth in rice, but surprisingly, *SsGDH* transgenic rice plants exhibited a marked tolerance to Basta, which is an herbicide commonly used in agricultural production (Du *et al.* 2014). In this study, the field trial demonstrated that the number of effective panicles, 1,000-grain weight and grain weight per plant of the *TrGDH* transgenic lines were higher than those of the control plants at both low- and high-nitrogen levels, but the degrees of increase were more distinct at low-nitrogen levels (**Table 1**). Compared to that of the control

plants, the percentage of filled grains of the transgenic rice plants obviously declined, but their increase in the number of effective panicles and 1,000-grain weight compensated or even surpassed the yield loss by the decrease in the percentage of filled grains, ultimately leading to an obvious increase in grain yield. These results indicated that the ectopic expression of *Tr*GDH for the production of an additional enzyme for ammonium assimilation could indeed enhance the nitrogen utilization efficiency and grain weight per plant in rice. It is notable that *gdhA* from *Aspergillus niger* improved the grain yield of forage rice under low-nitrogen conditions (Zhang *et al.* 2016); however, it could increase the grain yield of a food rice cultivar only under high-nitrogen conditions and not low-nitrogen conditions (Abiko *et al.* 2010). Because *TrGDH* can enhance the grain weight per plant in rice under both low and high-nitrogen conditions, it is speculated that *TrGDH* might be a better fungal GDH gene than *CeGDH*, *EcGDH* and *gdhA* and has the potential for maintaining yields of crop species under various nitrogen fertility regimens. Interestingly, compared with the seeds of the control plants, the seeds of the *TrGDH* transgenic rice plants presented a greater accumulation of proteins such as glutelin and prolamine (**Fig. 5**), which means that *TrGDH* is a promising candidate gene for improving nutritional value as well as nitrogen utilization efficiency.

In conclusion, overexpression of *TrGDH* from *Trichurus* can improve nitrogen assimilation, growth status and grain weight per plant in rice, especially under low-nitrogen conditions. Thus, *TrGDH* is a promising candidate gene for maintaining yields of crop plants via genetic engineering.

Author Contribution Statement

JZL and XML conceived the project and designed the research. CQD, LAD, DYT and JZL conducted the experiments and analyzed the data. CL, LY, MDC and SL partly participated in the experiments and analyzed the data. JZL, CQD and CL wrote the manuscript. All authors read and approved the manuscript.

Acknowledgments

The authors thank Dr. Hong Liu for providing the fungus *Trichurus* and for the assistance with cloning *TrGDH* (Fujian Agriculture and Forestry University, Fuzhou, China). This research is financially supported by the National Science Foundation of China (Nos. 31571635, 31871595 and 31170172), Hunan Provincial Important Science and Technology Specific Projects (No. 2018NK1010), Hunan Provincial Natural Science Foundation of China (No. 2017JJ2042), Planned Science and Technology Project of Hunan Province (No. 2017WK2012), and Planned Science and Technology Project of Changsha City (Nos. kq1801001 and kq1701028).

Literature Cited

Abiko, T., M. Wakayama, A. Kawakami, M. Obara, H. Kisaka, T. Miwa, N. Aoki and R. Ohsugi (2010) Changes in nitrogen assimilation, metabolism, and growth in transgenic rice plants expressing a fungal NADP(H)-dependent glutamate dehydrogenase (gdhA). Planta 232: 299–311.

Ameziane, R., K. Bernhard and D. Lightfoot (2000) Expression of the bacterial *gdhA* gene encoding a NADPH glutamate dehydrogenase in tobacco affects plant growth and development. Plant Soil 221: 47–57.

Cai, H.M., Y. Zhou, X.H. Xiao, X.H. Li, Q.F. Zhang and X.M. Lian (2009) Overexpressed *glutamine synthetase* gene modifies nitrogen metabolism and abiotic stress responses in rice. Plant Cell Rep. 28: 527–537.

Du, C., J. Lin, Y. Yang, H. Liu, C. Li, Y. Zhou, Y. Li, D. Tang, X. Zhao, Y. Zhu *et al.* (2014) Molecular cloning, characterization and function analysis of a GDH gene from *Sclerotinia sclerotiorum* in rice. Mol. Biol. Rep. 41: 3683–3693.

Frink, C.R., P.E. Waggoner and J.H. Ausubel (1999) Nitrogen fertilizer: Retrospect and prospect. Proc. Natl. Acad. Sci. USA 96: 1175–1180.

Giles, J. (2005) Nitrogen study fertilizes fears of pollution. Nature 433: 791.

Helling, R.B. (1998) Pathway choice in glutamate synthesis in *Escherichia coli*. J. Bacteriol. 180: 4571–4575.

Hodges, M. (2002) Enzyme redundancy and the importance of 2-oxoglutarate in plant ammonium assimilation. J. Exp. Bot. 53: 905–916.

Hudson, R. and R. Daniel (1993) L-glutamate dehydrogenases: distribution, properties and mechanism. Comp. Biochem. Physiol. B. 106: 767–792.

Hutson, S.M., M.M. Islam and I. Zaganas (2011) Interaction between glutamate dehydrogenase (GDH) and L-leucine catabolic enzymes: intersecting metabolic pathways. Neurochem. Int. 59: 518–524.

Ireland, R.J. and P.J. Lea (1999) The enzymes of glutamine, glutamate, asparagine, and aspartate metabolism. *In*: Singh, B.K. (ed.) Plant amino acids: biochemistry and biotechnology. Marcel Dekker, New York, pp. 49–109.

Krishnan, H.B. and T.W. Okita (1986) Structural relationship among the rice glutelin polypeptides. Plant Physiol. 81: 748–753.

Lea, P.J. and B.J. Miflin (1974) Alternative route for nitrogen assimilation in higher plants. Nature 18: 614–616.

Livak, K.J. and T.D. Schmittgen (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2[^{-\Delta\ \Delta C}(_T)]$ method. Methods 25: 402–408.

Magalhães, J.R., G.C. Ju, P.J. Rich and D. Rhodes (1990) Kinetics of $^{15}NH_4^+$ assimilation in *Zea mays*. Plant Physiol. 94: 647–656.

Noor, S. and N.S. Punekar (2005) Allosteric NADP-glutamate dehydrogenase from *Aspergilli*: purification, characterization and implications for metabolic regulation at the carbon–nitrogen interface. Microbiology 151: 1409–1419.

Oaks, A. (1994) Primary nitrogen assimilation in higher plants and its regulation. Can. J. Bot. 72: 739–750.

Qiu, X., W. Xie, X. Lian and Q. Zhang (2009) Molecular analyses of the rice *glutamate dehydrogenase* gene family and their response to nitrogen and phosphorous deprivation. Plant Cell Rep. 28: 1115–1126.

Skopelitis, D.S., N.V. Paranychianakis, A. Kouvarakis, A. Spyros, E.G. Stephanou and K.A. Roubelakis-Angelakis (2007) The isoenzyme 7 of tobacco NAD (H)-dependent glutamate dehydrogenase

exhibits high deaminating and low aminating activities in vivo. Plant Physiol. 145: 1726–1734.

Socolow, R.H. (1999) Nitrogen management and the future of food: lessons from the management of energy and carbon. Proc. Natl. Acad. Sci. USA 96: 6001–6008.

Stulen, I., M. Perez-soba, L.J. Dekok and L. Van Der Eerden (1998) Impact of gaseous nitrogen deposition on plant functioning. New Phytol. 139: 61–70.

Tang, D., Y. Peng, J. Lin, C. Du, Y. Yang, D. Wang, C. Liu, L. Yan, X. Zhao, X. Li *et al.* (2018) Ectopic expression of fungal *EcGDH* improves nitrogen assimilation and grain yield in rice. J. Integr. Plant Biol. 60: 85–88.

Windass, J.D., M.J. Worsey, E.M. Pioli, D. Pioli, P.T. Barth, K.T. Atherton, E.C. Dart, D. Byrom, K. Powell and P.J. Senior (1980) Improved conversion of methanol to single-cell protein by *Methylophilus methylotrophus*. Nature 287: 396–401.

Wootton, J.C. (1983) Re-assessment of ammonium-ion affinities of NADP-specific glutamate dehydrogenases. Biochem. J. 209: 527–531.

Zhang, H., C. Liang, N. Aoki, K. Kawai, K.I. Takane and R. Ohsugi (2016) Introduction of a fungal NADP (H)-dependent glutamate dehydrogenase (gdhA) improves growth, grain weight and salt resistance by enhancing the nitrogen uptake efficiency in forage rice. Plant Prod. Sci. 19: 267–278.

Zhou, X., J. Lin, Y. Zhou, Y. Yang, H. Liu, C. Zhang, D. Tang, X. Zhao, Y. Zhu and X. Liu (2015a) Overexpressing a fungal gene improves nitrogen utilization and growth in rice. Crop Sci. 55: 811–820.

Zhou, Y., H. Liu, X. Zhou, Y. Yan, C. Du, Y. Li, D. Liu, C. Zhang, X. Deng, D. Tang *et al.* (2014) Over-expression of a fungal NADP(H)-dependent glutamate dehydrogenase *PcGDH* improves nitrogen assimilation and growth quality in rice. Mol. Breed. 34: 335–349.

Zhou, Y., C. Zhang, J. Lin, Y. Yang, Y. Peng, D. Tang, X. Zhao, Y. Zhu and X. Liu (2015b) Over-expression of a glutamate dehydrogenase gene, *MgGDH*, from *Magnaporthe grisea* confers tolerance to dehydration stress in transgenic rice. Planta 241: 727–740.

Zhou, Y., C. Liu, D. Tang, L. Yan, D. Wang, Y. Yang, J. Gui, X.Y. Zhao, L. Li, X.D. Tang *et al.* (2018) The receptor-like cytoplasmic kinase STRK1 phosphorylates and activates CatC, thereby regulating H_2O_2 homeostasis and improving salt tolerance in rice. Plant Cell 30: 1100–1118.