THE BETTER GROWTH PHENOTYPE OF DVGS1-TRANSGENIC ARABIDOPSIS TALIANA IS ATTRIBUTED TO THE IMPROVED EFFICIENCY OF NITROGEN ASSIMILATION

Chenguang Zhu*, Guimin Zhang, Shilin Chen, Wei Wang, Yuanping Tang and Rentao Song

Shanghai Key Laboratory of Bio-Energy Crops, School of Life Sciences, Shanghai University, Shanghai, China

*Corresponding author: cgzhu@shu.edu.cn

Abstract: The overexpression of the algal glutamine synthetase (GS) gene DVGS1 in Arabidopsis thaliana resulted in higher plant biomass and better growth phenotype. The purpose of this study was to recognize the biological mechanism for the growth improvement of DVGS1-transgenic Arabidopsis. A series of molecular and biochemical investigations related to nitrogen and carbon metabolism in the DVGS1-transgenic line was conducted. Analysis of nitrogen use efficiency (NUE)-related gene transcription and enzymatic activity revealed that the transcriptional level and enzymatic activity of the genes encoding GS, glutamate synthase, glutamate dehydrogenase, alanine aminotransferase and aspartate aminotransferase, were significantly upregulated, especially from leaf tissues of the DVGS1-transgenic line under two nitrate conditions. The DVGS1-transgenic line showed increased total nitrogen content and decreased carbon:nitrogen ratio compared to wild-type plants. Significant reduced concentrations of free nitrate, ammonium, sucrose, glucose and starch, together with higher concentrations of total amino acids, individual amino acids (glutamate, aspartate, asparagine, methionine), soluble proteins and fructose in leaf tissues confirmed that the DVGS1-transgenic line demonstrated a higher efficiency of nitrogen assimilation, which subsequently affected carbon metabolism. These improved metabolisms of nitrogen and carbon conferred the DVGS1-transgenic Arabidopsis higher NUE, more biomass and better growth phenotype compared with the wild-type plants.

Key words: glutamine synthetase; Arabidopsis thaliana; nitrogen use efficiency; transgenic; biological mechanism

Received: April 14, 2015; Revised: May 5, 2015; Accepted: May 5, 2015

INTRODUCTION

Nitrogen is the limiting nutrient during the growth of plants. It is absorbed from soil mainly in the form of nitrate (NO\textsubscript{3}⁻), ammonia/ammonium (NH\textsubscript{3}/NH\textsubscript{4}⁺) or urea (CO(NH\textsubscript{2})\textsubscript{2}). Insufficient nitrogen severely affects the yield of crops, whereas oversupply has no effect on yield and contributes significantly to nitrogen pollution (Amiour et al., 2012; Liao et al., 2012).

Nitrogen use by plants involves two steps: uptake and utilization. The utilization can be further compartmentalized into assimilation and translocation/remobilization (Masclaux-Daubresse et al., 2010). The nitrate and ammonium are usually major forms of nitrogen resources taken up by plants (Good et al., 2004; Miller et al., 2007). Nitrate is absorbed from the environment by two families of transporters (NRT1 and NRT2) (Miller et al., 2007), and is converted to ammonium by the reduction of nitrate reductase (NR) and nitrite reductase (NiR) (Masclaux-Daubresse et al., 2010). Assimilation of ammonium into glutamine and glutamate is catalyzed through the glutamine synthetase/glutamate synthase (GS/GOGAT) system and glutamate dehydrogenase (GDH) (Suzuki and Knaff, 2005; Lehmann and Ratajczak, 2008). Once nitrogen has been taken up and assimilated, it is transported throughout the plant predominantly as glutamine, asparagine, glutamate and aspartate for utilization and storage (Okumoto and Pilot, 2011). The conversion of glutamine to asparagine and glutamate to aspartate requires two aminotransferases: asparagine synthetase (ASN) and
aspartate aminotransferase (AspAT), respectively (Hodges, 2002). Another aminotransferase, the alanine aminotransferase (AlaAT) can serve as a marker of nitrogen use efficiency (NUE) (Cañas et al., 2010). AlaAT-overexpressed rice plants grown in nitrogen-limiting conditions show increased biomass and yield, as well as increases in total nitrogen and key metabolites (Gln, Glu and Asn) (Shrawat et al., 2008).

Current attempts at improving plant NUE via transgenic approaches focus on altering nitrogen uptake, assimilation, remobilization and regulation. They include the transgenic expression of genes encoding AlaAT (Shrawat et al., 2008), GS (Brauer et al., 2011), NADH-GOGAT (Chickova et al., 2001), NADP(H)-GDH (Abiko et al., 2010), AspAT (Zhou et al., 2009), ASN (Lam et al., 2003), etc. Expression of additional factors, including those involved in nitrogen assimilation, carbon-nitrogen balance and signaling, may also have roles in improving NUE (McAllister et al., 2012). However, transgene targeted metabolic engineering may also be limited by the complexity and correlation of many metabolic processes. Currently, the regulation of genes that are involved in nitrogen metabolism and starvation response is not well-understood (Fischer et al., 2013).

It is thought that changes in the expression of GS (either GS1 or GS2) as well as changes in the activity of GS have an effect on nitrogen metabolism in plants, potentially affecting NUE (Eckes et al., 1989; Fei et al., 2003). Our previous work also reports that overexpression of the algal GS gene DvGS1 in Arabidopsis thaliana results in higher biomass and better growth phenotype. The fresh and dry weights, leaf GS activity and soluble protein concentration of the DvGS1-transgenic Arabidopsis showed various degrees of increase compared with the wild-type plants (Zhu et al., 2014). Therefore, a series of molecular and biochemical investigations related to nitrogen and carbon metabolism in the DvGS1-transgenic Arabidopsis were respectively conducted in this study, in order to understand the molecular mechanism for the improvement of growth phenotype, which would be useful in engineering transgenic crops with a high efficiency of nitrogen utilization.

**MATERIALS AND METHODS**

**Plant growth conditions**

The seeds of DvGS1-transgenic Arabidopsis T3 homozygotes were germinated on 1/2 MS agar plates after vernalization, and grew vertically until the root length was about 1-1.5 cm. Then the seedlings were transferred to plant nutrient solution (PNS) as previously described (Zhu et al., 2014) for hydroponic culture. During the growth, two concentrations of nitrate (9 mM and 2 mM KNO₃) were used. A regular day/night cycle was set to a day length (illumination of 4000 lux at 22°C) of 16 h and a dark period (at 20°C) of 8 h. The air humidity was maintained at the level of 60-70%. After 30 days of PNS culture, the plants were harvested, including leaves, roots and stems. The plant materials were frozen in liquid nitrogen and stored at -80°C until use.

**Determination of total nitrogen and carbon contents**

After drying and weighing, the plant materials were ground to a homogenous powder. A sample of 10-20 mg was carefully weighed in tin capsules to determine total nitrogen and carbon contents using a Vario MICRO cube organic elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

**Amino acid and saccharide analysis**

Total amino acid content was determined after extraction from fresh leaf tissues with 2% (w/v) solution of 5-sulfosalicylic acid by the Rosen colorimetric method (Rosen, 1957) using glycine as a reference. The concentrations of free amino acids were monitored by ion exchange chromatography on an L-8900 amino acid analyzer according to instructions of the manufacturer (Hitachi Ltd, Tokyo, Japan). The content of hexoses, sucrose and starch in the plant tissues was analyzed using kits for the detection of saccharide content according to the protocol (colorimetric assay) provided by the manufacturer (Suzhou Comin Biotechnology Co., Ltd, Suzhou, China).
Real-time quantitative PCR

The total RNA from DvGS1-transgenic Arabidopsis plants was extracted using Trizol reagent (Invitrogen), followed by treatment with DNase I to remove the contaminating genomic DNA. Reverse transcription was performed using the reverse transcriptase ReverTra Ace (TOYOBO) to obtain a cDNA template. The quantitative PCR (qPCR) was performed using SYBR Green Real-time PCR Master Mix (TOYOBO) according to the manufacturer’s recommendations. Primers for genes in A. thaliana were designed according to the corresponding sequences in the genome database of Arabidopsis. All the primers are listed in Supplementary Table 1. Real-time qPCR was carried out with a Realplex system (Eppendorf, Hamburg, Germany) using SYBR Green to monitor the dsDNA synthesis. The UBQ2 (At2G36170) and EF1α (At5G60390) genes were used as the internal standards for normalization of all the treatments. The relative expression levels of the target genes are presented after normalization to the internal standards from the technical repeats of three independent biological repeats.

Enzymatic assays

Enzymes were extracted from frozen plant materials that were previously stored at -80°C. All the extractions were performed at 4°C. The AspAT and AlaAT activities were measured in NADH-coupled colorimetric assays according to the protocol described in Cazetta et al. (1999). The GS activity was assayed by the previously described approach (Zhu et al., 2014). The GDH activity was assayed for the amination reaction according to the method of Turano et al. (1996). The NR, NiR and Fd-GOGAT activities were determined according to the method described in Debouba et al. (2007). The activity of NADH-GOGAT was measured in NADH-coupled colorimetric assay according to the protocol reported by Ertan (1992).

Nitrate and ammonium determination

Free ammonium was extracted from fresh leaves with 60% (v:v) methanol and was determined by the salicylate dichloroisocyanurate assay (Berthelot reaction) (Husted et al., 2000). Free nitrate was extracted from fresh leaves with hot deionized water (80°C) and the supernatant was determined by salicylic acid-H2SO4 method (Cataldo et al., 1975) using KNO3 as the standard.

Statistical analyses

Statistical analysis for all the biochemical detection was carried out using two-way analysis of variance (ANOVA). Data followed a normal distribution.
Means and standard errors were calculated by Excel software. Significances were determined using the two-tailed *t*-test on the mean of the technical repeats of biological repeats.

**RESULTS**

**DvGS1-transgenic Arabidopsis displays higher nitrogen content and lower C:N ratio**

In this study, with the purpose of understanding the biological mechanism for the improvement of growth phenotype in *DvGS1*-transgenic *Arabidopsis* plants, a transgenic line DvGS1-1 was selected to investigate the comprehensive effects of various changes in nitrogen and carbon assimilations in this plant. Under both nitrate conditions (9 mM and 2 mM), the fresh weights of transgenic line DvGS1-1 were 14-23% increased compared with the wild type, and the dry weights of DvGS1-1 were 8-15% higher than the wild type (Fig. 1A, B). As a result of higher nitrogen concentrations (N%; Fig 1G) and lower or similar carbon concentrations (C%; Fig 1H), the DvGS1-1 line showed a significantly lower C:N ratio than the wild type under both nitrate conditions (Fig. 1I).
In order to determine the nature of C:N changes in the transgenic line DvGS1-1, the concentrations of soluble proteins, total amino acids, ammonium and nitrate in the leaves of the transgenic line DvGS1-1 were detected. The soluble protein concentrations and total amino acid concentrations from leaf tissue of the DvGS1-1 line were increased by 16-18% and 36-53% compared to those of the wild type under both nitrate conditions (Fig. 1C, D). The nitrate and ammonium contents from the leaf tissue of the DvGS1-1 line were 15-19% and 8-15%, respectively, lower than those in the wild type under both nitrate supplies (Fig. 1E, F). The concentrations of soluble and insoluble saccharides from different tissues of the transgenic line DvGS1-1 were also investigated. Results showed that relative to the wild type, higher fructose concentrations were found in the transgenic line DvGS1-1 under both normal (9 mM) and low nitrate (2 mM) supplies (Fig. 2B, D). However, reduced concentrations of sucrose and glucose were observed in DvGS1-1 under both nitrate conditions (Fig. 2A, C, E, G). The starch concentrations of the transgenic line DvGS1-1 were reduced in stem and root tissues, and unchanged in leaf tissue under normal nitrate supply (Fig. 2F). Under low nitrate supply, the starch concentration of transgenic line DvGS1-1 was reduced in the stem, increased in leaf and unchanged in the root tissue (Fig. 2H).

Steady-state transcriptional levels of NUE-related genes

The mRNA accumulation of a number of genes that are involved in nitrogen assimilation and amino acid (Asn, Asp, Ala, Glu, Gln) biosynthesis was quantified in root, stem and leaf tissues of Arabidopsis DvGS1-1 line and wild type under both nitrate conditions. Under normal nitrate supply, the most significant differences in transcript abundance were observed in the leaf tissue. The transcriptional levels of GDH2, AlaAT1, AspAAT and ASN1 in the leaf of the DvGS1-1 line were increased from 3- to 6-fold as compared to the transcriptional levels in the wild type; the levels of GDH1, GDH3, Gln1-2, Gln1-3, AspAT2 and Fd-
GOGAT1 in the DvGS1-1 line were also increased by 100%, the levels of ASN2, ASN3, NRI and NiR were significantly downregulated by 1- to 4-fold (Fig. 3E). In the stem, the transcriptional levels of Gln1-4, AspAAT, AlaAT1, AlaAT2, Fd-GOGAT1 and NADH-GOGAT were increased by 41-69% in the DvGS1-1 line, but the levels of NR1, Gln2, GDH3 and Gln1-1 were downregulated by 44-68% (Fig. 3C). In the root tissue, the most significant increase in transcriptional level (39-66%) appeared in the ASN1, NADH-GOGAT, Gln1-1, Gln1-3, AspAT2 and AspAT4, the levels of GDH3 and NR1 were downregulated by more than 80%, and the levels of other genes including Gln1-2, AspAT1, NR2 and NiR were also reduced compared with the wild type (Fig. 3A). Under low nitrate supply, the most significant differences in transcript abundance were observed in leaf and stem tissues. The transcriptional levels of AlaAT1, ASN1 and GDH2 in leaf tissue of the DvGS1-1 line were 3- to 5-fold higher than in the wild type (Fig. 3F). The levels of AspAT1, AspAT3, AspAT4, Fd-GOGAT1, Gln1-1 and Gln1-3 in the DvGS1-1 line were also increased by 45-128%. The levels of NR1, NR2, NiR, ASN2, ASN3, GDH3 and AspAT5 were significantly downregulated by 61-86% (Fig. 3F). In the stem tissue, the transcriptional levels of ASN1, AspAT3, AlaAT2, AspAT4, GDH2 and Gln1-4 in the DvGS1-1 line were 2- to 7-fold higher as compared to those in the wild type, but the levels of NR1, NR2, NiR, Fd-GOGAT1, AspAT1 and Gln2 were downregulated by 37-78% (Fig.
3D). In the root tissue, the most significant increase in transcriptional levels (62-170%) appeared in the GDH1, AspAT1, ASN1 and Gln1-3 (Fig. 3B). The levels of NR1, NiR, GDH3, NADH-GDH, Gln1-1, Gln2 and AspAT2 were downregulated by more than 50%, the levels of other genes including Gln1-4, AspAT5, ASN2, NR2, Fd-GOGAT2 and NADH-GOGAT were also reduced compared with the wild type (Fig. 3B).

The enzymatic activities of NUE-related genes

According to the mRNA levels, seven types of genes (GS, GDH, AlaAT, AspAT, ASN, NADH-GOGAT, Fd-GOGAT) in the DvGS1-1 line showed significantly increased transcriptional levels compared with the wild type under either normal or low nitrate conditions. Consequently, the activities of these enzymes (except ASN, as there is no reference describing an approach for ASN assay) together with the NR and NiR, were assayed to test the concordance between mRNA level and enzymatic activity. In leaf tissue, it was observed that the activities of GS, GDH, Fd-GOGAT, AspAT, and AlaAT in the DvGS1-1 line were generally increased by 13-21% under both nitrate supplies (Fig. 4A-E, G, I-L). The NR and NiR activities were reduced by 10-18% (Fig. 4M-P), and the NADH-GOGAT activity had no significant change compared with the wild type under both nitrate supplies (Fig. 4F, H). In stem tissue, the activities of GS and GDH were increased by 12-16% under low nitrate supply but showed no significant changes under normal nitrate condition (Fig. 4A-D). The AspAT activity was increased by 14-20%, the activities of Fd-GOGAT and NADH-GOGAT had no significant changes compared with wild type under both nitrate supplies (Fig. 4E-H, J, L). The AlaAT activity was increased by 13% under normal nitrate conditions but increased by 27% under low nitrate conditions (Fig. 4I, K). The NR and NiR activities were reduced by 7-10% under both nitrate supplies (Fig. 4M-P). The activities of AspAT and Fd-GOGAT were not significantly changed under either nitrate supply (Fig. 4E, G, J, L).

Free amino acid profiles

The free amino acid profiles in fresh leaves of the Arabidopsis DvGS1-1 line and wild type under both nitrate conditions are presented in Table 1. Under normal nitrate conditions, the concentrations of Asp, Asn, Glu and Met showed a significant increment in the DvGS1-1 line. The Asn and Glu showed the most increased levels; their concentrations in the DvGS1-1 line were increased by 97% and 92%, respectively, compared with those in wild type (Table 1). The concentrations of Asp and Met were increased by 51-60%, and the other amino acids showed no significantly different concentrations compared with those in the wild type (Table 1). Under low nitrate conditions, the concentrations of Asp, Asn, Glu and Met in DvGS1-1 line were significantly increased. The highest concentration of Glu, more than 3-fold higher than in the wild type value, was detected in the DvGS1-1 line (Table 1). The concentration of Asp was increased by 34%, while the concentrations of Asn and Met were increased by 95-153%. The other amino acids showed similar concentrations and no significant differences compared to the wild type.

Discussion

Although previous work reported that the DvGS1-transgenic line demonstrated an improvement in growth phenotype under both normal and low nitrate conditions, it was not clear what the molecular mechanism for the growth improvement of DvGS1-transgenic lines was (Zhu et al., 2014). In this study, for the first time we showed that in leaves of the transgenic line Arabidopsis, DvGS1-1 was responsible for a reduction in concentrations of nitrate and ammonium, as well as increased concentrations of total amino acids and soluble proteins. It can be concluded
that more inorganic nitrogen was assimilated into amino acids, which resulted in the synthesis of more amounts of soluble proteins. The total nitrogen content of the DvGS1-1 line was higher than in the wild-type plants. This indicated that expression of DvGS1 actually affected the host’s nitrogen metabolism. An increased nitrogen content and at the same time decreased or almost identical concentration of carbon resulted in the decreased C:N ratio of the DvGS1-1 line compared with the wild type under both nitrate supplies. The reduction in the C:N ratio probably effected the better growth phenotype of the DvGS1-1 transgenic line. It is known that a high C:N ratio in the cell usually results in reduced proliferation of lateral roots and smaller shoots of plants (Malamy and Ryan, 2001; Kant et al., 2008). In trying to improve the NUE, it has been widely recognized that the link between carbon and nitrogen is crucial (Makino et
al., 1997; Reich et al., 2006). The lower concentrations of glucose, sucrose and starch, together with the higher accumulation of fructose in different tissues of the DvGS1-1 line confirmed that the increased total nitrogen content caused by the overexpression of DvGS1 also affected the metabolism of total saccharides directly or indirectly. Sucrose occupied the maximum ratio of concentration of the four saccharides and the reduction in sucrose concentration in leaf and stem tissues under both nitrate conditions was in correlation with the reduction of total carbon content in the DvGS1-1 line. Generally, we showed that the lower C:N ratio of the whole plant and synthesis of increased amounts of soluble proteins in leaf tissue resulted in the production of a higher plant biomass (higher fresh weight and dry weight in DvGS1-1 line) in the DvGS1-1 line as compared to the wild-type plaTo further elucidate the molecular mechanism for the NUE improvement of DvGS1 transgenic Arabidopsis, the transcriptional levels and enzymatic activities of NUE-related genes in various tissues of the DvGS1-1 line together with the concentrations of individual free amino acids from leaf tissue of the DvGS1-1 line under both nitrate conditions, were subsequently investigated. It was concluded that overexpression of DvGS1 directly resulted in up- or downregulated transcriptional levels of several NUE-related genes that play important roles in nitrogen assimilation and amino acid biosynthesis. As for the leaf tissues, the higher enzymatic activities of Fd-GOGAT, GDH, AlaAT, AspAT and GS in the DvGS1-1 line under both nitrate supplies revealed that these increased activities were attributable to the higher levels of gene expression, because the transcriptional levels of AlaAT1, GDH2, Fd-GOGAT1, AspAT1/AspAAT and Gln1-3 were significantly upregulated under both nitrate supplies (Fig. 3E, F). Previous investigations showed that GS/GOGAT systems play key roles in ammonium assimilation in plants (Masclaux-Daubresse et al., 2010; Foyer et al., 2011). It was also shown that GDH is involved in ammonium assimilation to form glutamate, and is confirmed to fuel the tricarboxylic acid (TCA) cycle (Miyashita and Good, 2008). In plants, the primary anabolism of alanine, the nontoxic storage form of nitrogen, appears to be catalyzed by AlaAT (Good and Beatty, 2011). As well as participating in plant nitrogen storage, AlaAT is also involved in the photosynthesis of C4 plants (Hatch and Mau, 1977). The AspAT catalyzes a reversible reaction transferring an amino group from glutamate to oxaloacetate to form aspartate and 2-oxoglutarate, and this enzyme is a link between amino acid and carbohydrate metabolism (McAllister et al., 2012). Therefore, it can be concluded that GDH AlaAT and AspAT played important roles not only in nitrogen assimilation but also in photosynthesis and carbon metabolism (TCA cycle) of the DvGS1-1 line. The higher accumulation of fructose and lower concentrations of sucrose, glucose and starch in the DvGS1-1 line also showed that the higher activities of GDH, AlaAT and AspAT could affect the carbon metabolism. The transcriptional level of ASN1 was significantly upregulated in root, stem and leaf tissues of the DvGS1-1 line under a low nitrate supply and was upregulated in root and leaf under normal nitrate supply. It was postulated that the ASN1 gene was associated with the increased NUE of the DvGS1-1 transgenic line. Previous reports showed that ASN synthesized nontoxic asparagine, which influenced the stored nitrogen to be remobilized and transported throughout the plants (Cañas et al., 2010). The significantly reduced concentration of ammonium in leaf tissues of the DvGS1-1 line (Fig. 1F) confirmed that higher efficiency of ammonium assimilation appeared in its leaf cells. This was possibly because the overexpression of DvGS1 resulted in higher GS activity and subsequently induced the enhancement of Fd-GOGAT and GDH activity under both nitrate conditions. The activities of NR and NiR were reduced in leaf and root tissues of the DvGS1-1 line under both nitrate supplies because of the downregulated transcriptional levels of these genes in the DvGS1-1 line. Since the transgene DvGS1 pushed the upregulated transcription of some important genes for nitrogen assimilation, which resulted in the reduction of free nitrate and ammonium concentrations in leaf tissues, and the normal/low nitrate conditions showed that there was no excess nitrogen to be utilized from the environment, it was probable that the DvGS1 transgenic plant reduced the transcriptional levels and en-
zymatic activities of NR and NiR to maintain more efficient and economical metabolism of nitrogen inside the leaf and root tissues of the DvGS1-1 line.

The higher concentrations of total amino acids and individual amino acids in the leaves of the Arabidopsis DvGS1-1 line were also closely related to the improved NUE. The concentrations of Asp, Glu and Asn in the DvGS1-1 line were significantly increased under both nitrate conditions. These amino acids represent nontoxic storage forms of nitrogen in plants and also play important roles in nitrogen transport and remobilization (Miyashita et al., 2007; McAllister et al., 2012). The Met also showed significantly increased levels (153% and 60%, respectively, higher than wild-type leaves under low and normal nitrate conditions). This sulfur-containing amino acid is an essential amino acid for protein biosynthesis and plays an important role in seed germination, plant growth and the quality of crops (Müntz, 1998). As the total amino acid concentration of the DvGS1-1 line was increased by 36-53% compared to the wild type under both nitrate conditions, the four types of amino acids (Asp, Asn, Glu, Met) should account for the major proportion of the total amino acids in the leaves. Therefore, it was concluded that the significantly increased concentrations of Glu, Asp, Asn and Met resulted in a higher concentration of total amino acids, and the higher concentrations of amino acids were associated with the rise of soluble protein concentration. The increased efficiency of nitrogen assimilation also affected the biosynthesis of carbohydrates, which resulted in the reduction of the C:N ratio in the whole plant.

CONCLUSIONS

Our study confirmed that the overexpression of DvGS1 in A. thaliana directly enhanced the expression of GS, GOGAT, GDH, ASN, AlaAT and AspAT genes, which were important to nitrogen assimilation and remobilization. The enhanced enzymatic activities of the proteins encoded by these genes resulted in higher concentrations of total amino acids and soluble proteins. The higher activities of AlaAT, AspAT and GDH simultaneously affected carbon metabolism in the Arabidopsis DvGS1-1 transgenic line, which resulted in increased total nitrogen content and decreased C:N ratio in the whole plant. Finally, these improved metabolisms of nitrogen and carbon conferred the DvGS1-transgenic Arabidopsis higher NUE, more global biomass and better growth phenotype compared to wild-type plants.

Acknowledgments: This work was supported by grants from Shanghai Municipal Natural Science Foundation (14ZR1414500) and Ministry of Agriculture of China (2014ZX08003-005).

Authors’ contributions: CZ is the main author who contributed to the design of the research and conception of the project, organization and analysis of the data and writing of the manuscript. GZ, SC, WW and YT contributed to the design of the research and data analyses. CZ and RS were supervisors of the research project and reviewed several drafts of the manuscript. All authors read and approved the final manuscript.

Conflict of interest disclosure: The authors declare that there are no conflicts of interest.

REFERENCES


Shrawat, A., Carroll, R., DePauw, M., Taylor, G. and A. Good (2008). Genetic engineering of improved nitrogen use effi-


