Rice OsLHT1 Functions in Leaf-to-Panicle Nitrogen Allocation for Grain Yield and Quality

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Proper allocation of nitrogen (N) from source leaves to grains is essential step for high crop grain yield and N use efficiency. In rice (Oryza sativa) grown in flooding paddy field, amino acids are the major N compounds for N distribution and re-allocation. We have recently identified that Lysine-Histidine-type Transporter 1 (OsLHT1) is the major transporter for root uptake and root-to-shoot allocation of amino acids in rice. In this study, we planted knockout mutant lines of OsLHT1 together wild-type (WT) in paddy field for evaluating OsLHT1 function in N redistribution and grain production. OsLHT1 is expressed in vascular bundles of leaves, rachis, and flowering organs. Oslht1 plants showed lower panicle length and seed setting rate, especially lower grain number per panicle and total grain weight. The concentrations of both total N and free amino acids in the flag leaf were similar at anthesis between Oslht1 lines and WT while significantly higher in the mutants than WT at maturation. The Oslht1 seeds contained higher proteins and most of the essential free amino acids, similar total starch but less amylose with lower paste viscosity than WT seeds. The mutant seeds showed lower germination rate than WT. Knockout of OsLHT1 decreased N uptake efficiency and physiological utilization efficiency (kg-grains/kg-N) by about 55% and 72%, respectively. Taken together, we conclude that OsLHT1 plays critical role in the translocation of amino acids from vegetative to reproductive organs for grain yield and quality of nutrition and functionality.

Keywords: rice, amino acids, transporter, OsLHT1, nitrogen allocation, grain yield, grain quality

INTRODUCTION

In natural soil ecosystem, nitrogen (N) is presented in diversity forms including both inorganic and organic N (Rasmussen et al., 2010; Robertson and Groffman, 2015). Rice (Oryza sativa) is commonly grown in flooding paddy field where ammonium is dominant form of inorganic N (Xu et al., 2012). Rice absorbs N rapidly at the vegetative stage, especially at the early stage of growth (Muhammad and Kumazawa, 1974; Masclaux-Daubresse et al., 2010). When the panicles emerge, the N is highly distributed to both the flag leaves and panicles (Muhammad and Kumazawa, 1974; Itoh et al., 2005). During grain-filling stage, the N supplied for panicles is mainly redistributed from leaves and about 70% of N can be transported into the panicle (Muhammad and Kumazawa, 1974).
The leaf blades are the main organ of photosynthesis and the N in the flag leaf contributes significantly to the yield (Ma et al., 1989). In flooding paddy soil, ammonium absorbed in rice roots is further assimilated in plastid into amino acids which are then transferred from roots to leaves via xylem. Amino acids in leaves are loaded into sieve elements and companion cell complexes and released to seeds (Tegeder, 2012; Tegeder, 2014; Tegeder and Masclaux-Daubresse, 2018). Import of amino acids into phloem and seeds is the key event in source to sink partitioning of N, and the sink development and grain yield rely on the amounts of amino acids translocation (Santiago and Tegeder, 2016; Tegeder and Hammes, 2018). Several membrane proteins for amino acid allocation have been characterized recently and manipulation of these transporters is able to regulate plant growth. In Arabidopsis, AtLHT1, with the strong expression in the surface of roots, young leaves, flowers, and siliques, is involved in amino acid uptake and import into the mesophyll cells (Chen and Bush, 1997; Hirner et al., 2006). Disruption of AtLHT1 exhibited decrease of shoot biomass and seed yield while AtLHT1 overexpression improved the N use efficiency under limiting N (Hirner et al., 2006). Furthermore, AtAAP2 is expressed in the phloem and functions in xylem-phloem transfer step. Increased amino acids allocation to leaves in aap2 mutants resulted in higher seed yields (Zhang et al., 2010; Perchlik and Tegeder, 2018). AtAAP8 is shown to be expressed in leaf phloem and siliques. It functions in amino acid loading into the phloem and import into seeds. Decreased amino acid loading into phloem and partitioning to sinks in aap8 mutants led to decreased siliques and seed numbers (Schmidt et al., 2007; Santiago and Tegeder, 2016). AtAAP1 is localized to Arabidopsis embryos and regulates amino acid distribution to the developing embryos. Amino acid loaded into embryo via AtAAP1 could also affect seed protein reserves and seed yield (Sanders et al., 2009). Similarly, in pea plants, overexpression of Amino Acid Permease1 (AAP1) in the phloem and embryos was able to improve seed yield and N use efficiency (Zhang et al., 2015; Perchlik and Tegeder, 2017).

In rice, 85 putative amino acid transporters were identified (Zhao et al., 2012). However, only a few of them have been associated with N translocation from source leaves to grains. OsAAP3 is localized to roots and leaves. Disruption of OsAAP3 decreased amino acid levels in various tissues, and promoted the tiller growth, and further improved the grain yield (Lu et al., 2018). OsAAP5 is expressed in leaves and panicles and regulates tiller growth by affecting cytokinin levels. Loss-of-function of OsAAP5 also decreased siliques and seed numbers (Schmidt et al., 2007; Santiago and Tegeder, 2016). AtAAP1 is involved in N translocation from source leaves to grains. The results show that OsLHT1 contributes to forming grain yield and quality, and high N use efficiency in rice.

**MATERIALS AND METHODS**

**Plant Materials, Growth Conditions, and Harvest**

Field experiments were conducted at the research base of Nanjing Agricultural University in Ledong county, Hainan Province, China. Seeds of Oslih1 mutant lines with Nipponbare background (Guo et al., 2020) were germinated and planted according to Chen et al. (2016) and Fan et al. (2016). Field experiments were managed according to local agricultural practices and 180-kg N/ha was applied during the growth season. For molecular and physiological analysis, rice plants were harvested at both anthesis and maturity. Panicles, leaves, and culms were collected, dried at 60°C for 3 days and weighed. Panicles were considered as reproductive tissues while leaves and culms were considered as vegetative tissues.

**RNA Extraction, and Gene Expression Analysis**

WT plants were planted in the paddy field (see above). At the anthesis stage, spikelet, rachis, leaf blades, leaf sheaths, nodes, and internodes were collected. Total RNA isolation and first-strand cDNA synthesis were performed according to Guo et al. (2020). RT-qPCR was performed with SYBR Premix Ex Taq™ II (Perfect Real Time) Kit (TaKaRa Biotechnology, Dalian, China) on the QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, MA, USA) using OsLHT1-specific primers (F: 5′-GGACTCCGCGAGATCATCA; R: 5′-CTGGTTTATCATGATGTCGCTA). Relative expression levels were normalized to the rice housekeeping gene OsActin1 using specific primer pairs (F: 5′-CAACACCCCTGTGTATGACG; R: 5′-CATCACAGATGCCAACCACAC) and presented as 2^(-ΔCT).

**Analysis of OsLHT1 Promoter-GUS Line**

Seeds of OsLHT1 promoter-GUS lines (Guo et al., 2020) were grown in the field (see above). GUS assays were done as described previously (Ai et al., 2009).

**Free Amino Acid Analysis**

Oslih1 mutant and WT plants were grown in the field (see above). At the anthesis or/maturity stage, flag leaves and dry
seeds were collected for amino acid analyses. Flag leaf free amino acid extraction was performed according to Peng B. et al. (2014). Amino acid contents in dry seeds were analyzed according to Zhou et al. (2009) and Wang et al. (2008) with some modifications. Rice grains were husked and ground to a flour consistency in a mill (JFS-13A, Hangzhou, China). Seed flour (0.100 g) was used for extraction with 5 ml of 6 M HCl in a sealed air-evacuated tube at 110°C for 22 hours. The hydrolysate was diluted to 100 ml. 1 ml dilution was dried in a Termovap Sample Concentrator MD200-1 (Allsheng, China) and re-dissolved in 1 ml water. Amino acid concentrations were determined using an L-8900 automatic amino acid analyzer (Hitachi, Tokyo, Japan). All steps were performed according to the manufacturer’s instructions. Most individual proteinous amino acid were analyzed, and results show the combined total amounts of Asn and Asp, and Gln and Glu, respectively (Peng B. et al., 2014; Guo et al., 2020).

Analysis of Total N, Protein, Starch, Amylose Concentrations and Pasting Properties

OsLht1 mutant and WT plants were grown in the field (see above). At the anthesis or/and maturity stage, panicles, leaves, culms, and dry seeds were collected for total elemental N, protein, starch, and amylose analyses. Rice tissues were husked and ground to a flour consistency in a mill (JFS-13A, Hangzhou, China). Total N content was analyzed according to the Kjeldahl method (Chen et al., 2016). Protein content was obtained by multiplying 5.95 as the protein conversion factor (GB5009.5-2016, National Food Safety Standards, China). Total starch was extracted using a starch assay kit (Megazyme, Wicklow, Ireland) according to the manufacturer’s instructions and determined with ultraviolet spectrophotometer U-1800 (Hitachi, Tokyo, Japan). Amylose content was analyzed according to Liu et al. (2009). Rapid Visco Analyser (RVA, Perton, Warriewood, NSW, Australia) were used for evaluating pasting properties of endosperm of both OsLht1 and WT seeds as described by Peng C. et al. (2014).

Analysis of N Uptake Efficiency (NUpE) and N Utilization Efficiency (NUtE)

NUpE and NUtE were calculated according to Moll et al. (1982) and Xu et al. (2012). NUpE is the percentage ratio of total accumulated N in the above-ground shoot to total supplied fertilizer N. NUtE is the ratio of total grain yield to total accumulated N in the above-ground shoot.

Seed Germination Analysis

A total of 100 respective fully filled seeds of OsLht1 mutant lines and Nipponbare WT were surface-sterilized for 30 min in 30% (v/v) NaClO solution, washed with sterilized water and then placed in water in Petri dishes (d=15 cm) with two sheets of filter paper. The seeds were incubated at 28°C in the dark for 2 days and then moved into a tissue culture chamber with 14-hour-days at a light intensity of 6000 lux, 40% humidity, and temperatures of 28°C. The water was exchanged every day. Seeds were photographed and plant root length, shoot length and germination rate were measured.

Statistical Analysis

Data are generally presented as means ± SD (standard deviation) of at least 5 biological repetitions. Significant differences were analyzed using IBM SPSS Statistics 20 program at one-way ANOVA followed by Tukey’s test.

Accession Numbers

Sequence data from this article can be obtained from The Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/) under the following accession numbers: *Oryza sativa japonica* LOC_Os08g03350 (OsLHT1), LOC_Os03g50885 (OsActin1).

RESULTS

OsLHT1 Is Highly Expressed in Vascular Bundles of Leaves and Rachis

We have previously shown that OsLHT1 transcripts were detected in roots and leaf blades at the seedling stage (Guo et al., 2020). Here we show OsLHT1 expression highly in leaf blades, leaf sheaths and rachis, and relatively weak in spikelet, node and internode at the anthesis stage (Figures 1A, B). Since the leaf 1 (the youngest or flag leaf) and developing panicles are growing organs and need large amount of nitrogenous compounds transported via stem from leaves (Muhammad and Kumazawa, 1974), the abundant expression of OsLHT1 in leaves and rachis hints that OsLHT1 may play a role in leaf-to-panicle amino acid allocation. GUS staining was observed throughout all cell types of root-shoot junction, with the strong expression levels in the vascular bundles (Figure 1C). GUS activity was present in leaf sheaths and leaf blades, specifically in the cells of the major and minor veins (Figures 1D–F). In addition, OsLHT1 is strongly expressed in flowering organs, hulls and germinated seeds (Figures 1G, H), demonstrating its potential role in rice reproductive growth.

Knockout of OsLHT1 Suppressed Development of Both Panicles and Grains

To detect the contribution of OsLHT1 in rice grain production, we compared the performance of WT and OsLht1 mutants in the paddy field. At the maturation, in comparison to WT, Osht1 plants showed shorter height, brown leaf and spikelet (Figure 2A), shorter panicle length (Figure 2B), lower grain number per panicle, seed setting rate, grain weight per panicle and total grain yield per plant (Figures 2C–F). The OsLHT1-mutation reduced 1000-grain weight about 10% (Figure 2G), which was relatively less affected in comparison to the other parameters.

Knockout of OsLHT1 Affected Distribution of Different N Components in Leaf and Grains

In cereal crops, there is a considerably higher distribution of N from flag leaf to the panicle after the emergence of panicles...
As expected, the contents of both total N and free amino acids in the flag leaf was dramatically decreased from anthesis to mature stage in both WT and Osllt1 mutant lines of rice (Figures 3A, D), confirming that large portion of grain N was allocated from the flag leaf in addition of direct contribution of root acquired N from soil. Remarkably, there was no significant difference of the content of total N (mg/g), total free amino acids and most of individual free amino acids (mg/kg FW) in the flag leaf between WT and Osllt1 plants at the anthesis stage (Figures 3A–C). However, their contents were significantly higher except similar level of aspartate, asparagine and serine in the mutant lines than in WT at the maturity stage (Figures 3D–F). In comparison to the increase of total N in Osllt1 lines by about 15-30%, total free amino acids accumulated in the mutants were increased by about 60-120% at the maturation (Figures 3D, E). Since amino acids represent the main form of N that was transported over long distances to the reproductive tissues and OsLHT1 is an amino acid transporter (Guo et al., 2020), the results demonstrated that OsLHT1 greatly contributes N re-allocation from source leaves to developing grains.

Since the mutation of OsLHT1 limited rice growth, as expected, total N per plant was significantly lower in the mutants than in WT at anthesis stage (Figure 4A), particularly at maturation stage (Figure 4C), while the difference of N in the leaves was relatively smaller than that in other organs (Figure 4C). At the anthesis stage, 67% of the total N was found in the flag leaf to be allocated to the reproductive tissues, indicating a high demand for N, as previously reported (Muhammad and Kumazawa, 1974; Zhang et al., 2007; Chen et al., 2016; Tegeder and Masclaux-Daubresse, 2018).
vegetative tissues and 33% in the reproductive tissues (Figure 4B). In contrast, in Oslht1 mutants, 77% of total N levels remained in the vegetative tissues while about 23% of the total plant N was detected in the reproductive tissues (Figure 4B). At the maturity stage, higher percentage of N was allocated to reproductive tissues compared to that at the anthesis stage. Oslht1 mutants only moved around 40% of total N to the reproductive tissues compared to 62% in WT plants (Figure 4D). These data indicated the great contribution of OsLHT1 to leaf-to-panicle allocation in the forms of amino acids.

Knockout of OsLHT1 Affected the Components of Grain Storage Compounds and Cooking Texture of Brown Rice

As reported, the dry seeds of both Oslht1 mutants and WT plants contained a high amount of total and individual free amino acids in addition to rich starch (Figure 5) (Fitzgerald et al., 2009; Li et al., 2018). Compared with WT, Oslht1 seeds accumulated higher total N and proteins (Figures 5A, D), total and individual free amino acids (Figures 5B, C) with the increase by up to 30-35%. In contrast, amylose content in Oslht1 seeds was reduced by

FIGURE 2 | Effect of OsLHT1 mutation on rice growth and grain yield components. Both wild-type (WT, cv. Nipponbare) and Oslht1 mutants were grown in paddy field and harvested at the maturity stage for determining yield components (see Materials and Methods). (A) Image of Oslht1 and WT plants in the field. (B) Mean panicle length. (C) Total grain number per panicle. (D) Total grain weight per panicle. (E) Total grain yield per plant. (F) Mean seed setting rate. (G) Mean 1000-seeds weight. Values are means ± SD (n = 7). Asterisks indicate significant differences between each Oslht1 mutant line and WT detected by one-way ANOVA followed by Tukey’s test. **p < 0.01.
about 31% (Figure 5F) even though total starch content in the mutant and WT seeds was kept at the same level (Figure 5E).

The altered storage compounds in Oslht1 grains affected cooked texture and pasting properties of the endosperm starch. Both WT and Oslht1 seed starches showed the similar patterns of pasting with peak viscosity and breakdown when the temperature was increased. However, compared with WT, the peak and cool paste viscosity of the Oslht1 starch were all decreased (Figure 5G).

**Knockout of OsLHT1 Affected Both N Uptake and Physiological Utilization Efficiency**

Increasing both the grain and N harvest indexes to drive N acquisition and utilization is an important approach for breeding future high N use efficient cultivars (Xu et al., 2012). In this study, we also analyzed both N uptake efficiency (NUpE) and N utilization efficiency (NUtE) (Xu et al., 2012) of Oslht1 plants and WT grown in the paddy field with the moderate application level of N fertilizer (180-kg/ha). Knockout of OsLHT1 resulted in decrease of NUtE by about 50% while it decreased NUpE by about 70% to 90% (Figure 6). The extreme low NUtE of Oslht1 was caused mainly by low grain harvest index (the grain to straw ratio) due to limited N re-allocation.

**Knockout of OsLHT1 Affected Seed Germination Speed and Rate**

To resolve if the change in Oslht1 grain storage compounds affects seed viability, we performed germination test of the seeds. The seeds of both WT and mutants began to germinate after soaking in water for 24 h and peaked at 72 h. After 72 h, the germination rate of WT seeds reached 95%, while Oslht1 seeds germinated only between 80-86% and no further increase after that (Figure 7A). Furthermore, the significant differences of seedling growth were also observed between Oslht1 and WT (Figure 7B). After 12-day growth in nutrient-free water, in comparison to WT, Oslht1 root and shoot length showed incredibly significant decrease (Figures 7C, D). The results clearly showed an inhibitory effect on the germination efficacy of the seeds in the loss-of-function of OsLHT1.
DISCUSSION

OsLHT1 Functions in Amino Acid Allocation to Panicles and Grains

Amino acids in mature leaves are transported via phloem of minor veins to sink tissues (Noctor et al., 2002; Tegeder, 2014). In our previous study, the promoter-GUS assay in rice indicated that OsLHT1 is localized in the leaf blade, especially in the cells of the major and minor veins (Guo et al., 2020). Here we further present high expression of OsLHT1 in multiple tissues including leaf blades, sheaths, nodes, rachis and spikelets at the anthesis stage, especially in the major and minor veins of leaf blade (Figure 1), supporting the role of OsLHT1 in the phloem loading of leaf or root-synthesized amino acids.

Next, OsLHT1 functions in N allocation most probably in the broad spectrum of amino acids as shown in the seedling stage (Guo et al., 2020). In this study, N translocation from source leaves to panicles was clearly established with analysis of four independent Oslht1 mutant lines. Inactivation of OsLHT1 resulted in increased accumulation of total N and amino acids in the flag leaves of Oslht1 mutants from anthesis to maturity stage (Figure 3). Considering that OsLHT1 is involved in root-to-shoot allocation and loss function of OsLHT1 reduced root-synthesized amino acid levels in leaves (Guo et al., 2020), the large amounts of amino acids accumulated in the mutant leaves suggested that the process of amino acids synthesized in source leaves might be stimulated, or less amino acid amounts were allocated into phloem. Since lower percentage of N was contained in the reproductive tissues of Oslht1 mutants (Figure 4), it provides strong support that lack of OsLHT1 function reduced the amino acids allocation from source leaves to panicles. Since OsLHT1 is localized in the major and minor veins of leaf blade and serves as an amino acid importer (Figure 1F; Guo et al., 2020), this decrease might result from the reduced amino acid import into phloem. In Arabidopsis, many members of AAP family are expressed in the phloem of major and minor veins and contribute to source to sink allocation at the reproductive stage (Sanders et al., 2009; Zhang et al., 2010; Santiago and Tegeder, 2016; Perchlík and Tegeder, 2016).
Although AAP and LHT transporters are two distinct groups, they usually have overlapping functions in amino acid translocation (Tegeder and Ward, 2012; Perchlik et al., 2014). Therefore, OsLHT1, a member of LHT family, is one of the major transporters responsible for the transfer process of amino acids in rice.

Amino acids move from the location of the synthesis to the sites of usage to satisfy the demand of plant growth (Tegeder, 2014). At the seedling stage, amino acids are synthesized in the root and exported to supply the developing leaves. In our previous studies, compared with WT, total amounts of free amino acids were reduced in leave blades of Oslht1 mutants (Guo et al., 2020). At the reproductive stage, amino acids could move from roots to flag leaves and, at the same time, flag leaf could also serve as the source organs to supply amino acids to panicles (Muhammad and Kumazawa, 1974). Since no difference of the free amino acid content in the flag leaf between WT and Oslht1 lines was observed at the anthesis stage, amino acids imported into flag leaves and exported from flag leaves might be dynamically balanced before anthesis (Figures 3A, B). At the maturity stage, much higher percentage of amino acids needs to be allocated to the panicles for meeting the demand of seed production. The higher free leaf amino acids and total N in Oslht1 mutants than in WT (Figures 3D–F) indirectly supports the conclusion that OsLHT1 functions in amino acid allocation to panicles and grains.

**OsLHT1 Contributes to Grain Production and Nutrition Quality and Functionality of Brown Rice**

A number of studies indicated that rice sink production is positively related to root N uptake and the amounts of N supplied by vegetative tissues (Fan et al., 2016; Wang et al., 2018). In Arabidopsis, LHT1 is crucial for organic N uptake from soil (Ganeteg et al., 2017). Atlh1 T-DNA insertion mutants displayed dramatic growth inhibition when grown in soil (Hirner et al., 2018).
In Oslht1 mutants, less N distributed into shoot at early stage and panicle sinks at late stage decreased shoot biomass and grain yield production (Figure 2; Wang X. et al., 2019; Guo et al., 2020). We have discussed the mechanism of OsLHT1 function in dramatically alteration of rice growth (Guo et al., 2020). We proposed that OsLHT1 mutation could decrease both xylem loading and leaf amino acid import, resulting in N deficiency and growth impairment as observed in Oslht1 mutant (Hermans et al., 2006; Grechi et al., 2007; Guo et al., 2020). The altered leaf structure and size of Oslht1 mutants can affect transpiration, water transport and photosynthesis which further limits root N uptake, allocation and re-distribution, as well as downstream carbon-N interaction (Oliveira and Coruzzi, 1999; Lejay et al., 2003; Palenchar et al., 2004; Gutiérrez et al., 2007). In addition, the reduced import of amino acids into mesophyll cells by Oslht1 mutation could decrease C-, N-regulatory and C/N interaction pathways play crucial roles in seed germination (Osuna et al., 2015). OsLHT1-mutation resulted in significant increase of total N and most of individual amino acids, and decrease of amylose concentration in rice grains (Figure 5). Internal C/N status could affect seed germination by moderating gibberellin acid and abscisic acid metabolism (Koornneef et al., 2002; Yamaguchi and Kamiya, 2002; Osuna et al., 2015). In addition, seed size is commonly showing positive correlation with seed germination (Ambika et al., 2014). Therefore, the relatively smaller seed size, lower amylose and higher amino acids of Oslht1 mutants could contribute to the delay of seed germination (Figure 2G). Furthermore, decreased grain weight was partially resulted from less seed storage amylose content (Figure 5F) while few seed number was caused by decreased amino acid allocation (Figures 3 and 4) (Tan et al., 2010; Santiago and Tegeder, 2016). Both root-to-shoot and leaf-to-panicle amino acid allocation were reduced, and eventually led to low grain number (Figure 2) (Guo et al., 2020). However, total N and protein levels per grains were still higher in Oslht1 mutants (Figures 5A, D). The results may hint that the trade-off occurs due to the extremely low seed setting as well as reduction in grain weight (Figures 2F, G).
between producing more seed number and keeping high N per seed during the grain filling phase (Seiffert et al., 2004; Drechsler et al., 2015). Overexpressing AAP1 in pea plants could allocate more N to seeds and produce high seed number (Perchl and Tegeder, 2017). However, in Arabidopsis, decreased amino acid partitioning to sinks led to decreased seed numbers while seed protein levels were unchanged (Schmidt et al., 2007; Santiago and Tegeder, 2016). In rice, amino acid transporter OsAAP6 functions as an important regulator of grain protein content and nutritional quality. OsAAP6 showed no effect on grain yield. However, transgenic plants with higher OsAAP6 expression levels produced much more grain storage protein (Peng B. et al., 2010; Ranathunge et al., 2014; Bao et al., 2015; Hu et al., 2015; Fan et al., 2016). Rice plants mainly acquire ammonium and amino acids from root to shoot (Guo et al., 2020) and redistribution of N to above ground parts in the forms of amino acids (Xu et al., 2012). Many genes involved in N uptake, assimilation, translocation and regulation have been identified to make great contributions to the NUE, such as amino acid transporter AtAAP2 in Arabidopsis, nitrate transporter OsNRT1.1b, OsNRT2.3b and ammonium transporter in rice (Zhang et al., 2010; Ranathunge et al., 2014; Bao et al., 2015; Hu et al., 2015; Fan et al., 2016). Rice plants mainly acquire ammonium and transport of N to above ground parts in the forms of amino acids (Xu et al., 2012). Since OsLHT1 plays the key role in the allocation of amino acids from root to shoot (Guo et al., 2020) and N redistribution from source leaves to developing grains (Figures 3–5), the inhibitory effect of OsLHT1 mutation on shoot growth acted the feedback role in preventing root N acquisition, which resulted in low N Ute (Figure 6). The confined panicle development in OsLHT1 plants decreased grain to straw ratio, which in turn reduced N Ute for root acquired N to produce grains (Figure 6).

In summary, as shown in Figure 8, OsLHT1 is not only for root acquisition and long-distance transport of amino acids, it also plays the major role in N allocation from source leaves to sinks.
developing panicle and grains for reaching the potential grain yield and quality of nutrition and functionality.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

NG and HQ conceived the research and analyzed the data. NG, JH, and MG performed the experiments. NG, HQ, and GX wrote the article. All authors contributed to the article and approved the submitted version.

REFERENCES

Ai, P., Sun, S., Zhao, J., Fan, X., Xin, W., Guo, Q., et al. (2009). Two rice phosphate transporters, OsPht1; 2 and OsPht1; 6, have different functions and kinetic properties in uptake and translocation. Plant J. 57 (5), 798–809. doi: 10.1111/j.1365-313X.2008.03726.x

Ambika, S., Manonmani, V., and Somasundar, G. (2014). Review on effect of seed size on seedling vigour and seed yield. Res. J. Seed Sci. 7 (2), 31–38. doi: 10.3923/rjss.2014.31.38

Balsubramanian, V., Alves, B., Aulakh, M., Bekunda, M., Cai, Z., Drinkwater, L., et al. (2004). Crop, environmental, and management factors affecting nitrogen use efficiency. Agriculture and the Nitrogen Cycle: Assessing the impacts of fertilizer use on food production and the environment. SCOPE (65), 19–33.

Bao, A., Liang, Z., Zhao, Z., and Cai, H. (2015). Overexpressing of OsAMT1-3, a high affinity ammonium transporter gene, modifies rice growth and carbon-nitrogen metabolic status. Int. J. Mol. Sci. 16 (5), 9037–9063. doi: 10.3390/ijms16050937

Chen, L., and Bush, D. R. (1997). LHT1, A Lysine- and Histidine-Specific amino acid transporter in Arabidopsis. Plant Physiol. 115 (3):1127. doi: 10.1104/pp.115.3.1127

Chen, Y., Wang, M., and Ouwerkerk, P. B. (2012). Molecular and environmental factors determining grain quality in rice. Food Energy Secur. 1 (2), 111–132. doi: 10.1002/fees.111

Chen, J., Zhang, Y., Tan, Y., Zhang, M., Zhu, L., Xu, G., et al. (2016). Agronomic nitrogen-use efficiency of rice can be increased by driving OsNAR2.1 expression with the OsNAR2.1 promoter. Plant Biotechnol. J. 14 (8), 1705–1715. doi: 10.1111/pbi.12531

Drechsler, N., Zheng, Y., Bohner, A., Nobmann, B., von Wirén, N., Kunze, R., et al. (2015). Nitrate-dependent control of shoot K homeostasis by the nitrate transporter/peptide transporter family member NPF7.3/NRT1.5 and the nitrate ammonium transporter gene, modified by SKOR in Arabidopsis. Plant J. 80 (3), 413–423. doi: 10.1111/pce.12881

Fageria, N., and Baligar, V. (2005). Enhancing nitrogen use efficiency in crop plants. Adv. Agron. 88, 97–185. doi: 10.1016/S0065-2113(05)80004-6

Fan, X., Tang, Z., Tan, Y., Zhang, Y., Luo, B., Yang, M., et al. (2016). Overexpression of a pH-sensitive nitrate transporter in rice increases crop yields. Proc. Natl. Acad. Sci. U.S.A. 113 (26), 7118–7123. doi: 10.1073/pnas.1525184113

Fitzgerald, M. A., McCouch, S. R., and Hall, R. D. (2009). Not just a grain of rice: the quest for quality. Trends Plant Sci. 14 (3), 133–139. doi: 10.1016/j.tplants.2008.12.004

Ganeteg, U., Ahmad, I., Jamtgard, S., Aguetoni-Cambui, C., Inselsbacher, E., Svennerstam, H., et al. (2017). Amino acid transporter mutants of Arabidopsis provides evidence that a non-mycorrhizal plant acquires organic nitrogen from agricultural soil. Plant Cell Environ. 40 (3), 413–423. doi: 10.1111/pce.12881

Grechi, I., Vivin, P., Hilbert, G., Milin, S., Robert, T., and Gaudillère, J.-P. (2007). Effect of light and nitrogen supply on internal C:N balance and control of root-to-shoot biomass allocation in grapevine. Environ. Exp. Bot. 59 (2), 139–149. doi: 10.1016/j.envexpbot.2005.11.002

Guo, N., Hu, J., Yan, M., Luo, L., Qu, H., Tegeder, M., et al. (2020). Oryza sativa Lysine-Histidine-type Transporter 1 functions in root uptake and root-to-shoot allocation of amino acids in rice. Plant J. 103 (1), 395–411. doi: 10.1111/tpj.14742

Gutiérrez, R. A., Lejay, L. V., Dean, A., Chiaromonte, F., Shasha, D. E., and Coruzzi, G. M. (2007). Qualitative network models and genome-wide expression data define carbon/nitrogen-responsive molecular machines in Arabidopsis. Genome Biol. 8 (1), R7. doi: 10.1186/gb-2007-8-1-17

Hermans, C., Hammond, J. P., White, P. J., and Verbruggen, N. (2006). How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci. 11 (12), 610–617. doi: 10.1016/j.tplants.2006.10.007

Hired, B., Le Gous, J., Ney, B., and Gallais, A. (2007). The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J. Exp. Bot. 58 (9), 2369–2387. doi: 10.1093/jxb/erm097

Hirner, A., Ladwig, F., Stransky, H., Okumoto, S., Keinath, M., Harms, A., et al. (2006). Arabidopsis LHT1 is a high-affinity transporter for cellular amino acid uptake in both root epidermis and leaf mesophyll. Plant Cell 18 (8), 1931–1946. doi: 10.1105/tpc.106.041012

Hu, B., Wang, W., Ou, S., Tang, J., Li, H., Che, R., et al. (2015). Variation in NRT1.18 contributes to nitrate-use divergence between rice subspecies. Nat. Genet. 47 (7), 834–838. doi: 10.1038/ng.3337

Itoh, I.-I., Nonomura, K.-I., Ikeda, K., Yamaki, S., Inukai, Y., Yamagishi, H., et al. (2005). Rice plant development: from zygote to spikelet. Plant Cell Physiol. 46 (1), 23–47. doi: 10.1093/pcp/pci501

Koornneef, M., Bentsink, L., and Hilhorst, H. (2002). Seed dormancy and germination. Curr. Opin. Plant Biol. 5 (1), 33–36. doi: 10.1016/S1369-5266(01)00219-9

Lejay, L., Gansel, X., Cerezo, M., Tillard, P., Müller, C., Krapp, A., et al. (2003). Regulation of root ion transporters by photosynthesis: functional importance and relation with hexokinase. Plant Cell 15 (9), 2218–2232. doi: 10.1105/tpc.013516

Li, Y., Xiao, J., Chen, L., Huang, X., Cheng, Z., Han, B., et al. (2018). Rice functional genomics research: past decade and future. Mol. Plant 11 (3), 359–380. doi: 10.1016/j.molp.2018.01.007

Liu, L., Ma, X., Liu, S., Zhu, C., Jiang, L., Wang, Y., et al. (2009). Identiﬁcation and characterization of a novel Waxy allele from a Yunnan rice landrace. Plant Mol. Biol. 71 (6), 609–626. doi: 10.1007/s11103-009-9544-4

Liu, G., Ji, Y., Bhuuyan, N. H., Pilot, G., Selvaraj, G., Zou, J., et al. (2010). Amino acid homeostasis modulates salicylic acid-associated redox status and defense responses in Arabidopsis. Plant Cell 22 (11), 3845–3863. doi: 10.1105/tpc.10709392

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Muhammad, S., and Kumazawa, K. (1974). The absorption, distribution, and maintenance. Front. Plant Sci. 52 (3), 1602–1609. doi: 10.1016/00380768.1989.104347E8

Guo et al. Leaf-to-Particale Nitrogen Allocation for Grain Yield
overexpression of the basic amino acid transporter AtCAT1. *Plant Cell Environ.* 37 (6), 1404–1414. doi: 10.1111/pce.12244

Zhang, W., Zhou, Y., Dibley, K. E., Tyerman, S. D., Furbank, R. T., and Patrick, J. W. (2007). Review: Nutrient loading of developing seeds. *Funct. Plant Biol.* 34 (4), 314–331. doi: 10.1071/FP06271

Zhang, L., Tan, Q., Lee, R., Trethewy, A., Lee, Y. H., and Tegeder, M. (2010). Altered xylem-phloem transfer of amino acids affects metabolism and leads to increased seed yield and oil content in Arabidopsis. *Plant Cell* 22 (11), 3603–3620. doi: 10.1105/tpc.110.073833

Zhang, L., Garneau, M. G., Majumdar, R., Grant, J., and Tegeder, M. (2015). Improvement of pea biomass and seed productivity by simultaneous increase of phloem and embryo loading with amino acids. *Plant J.* 81 (1), 134–146. doi: 10.1111/tpj.12716

Zhao, H., Ma, H., Yu, L., Wang, X., and Zhao, J. (2012). Genome-wide survey and expression analysis of amino acid transporter gene family in rice (*Oryza sativa* L.). *PloS One* 7 (11), e49210. doi: 10.1371/journal.pone.0049210

Zhao, H., Cai, H., Xiao, J., Li, X., Zhang, Q., and Lian, X. (2009). Over-expression of aspartate aminotransferase genes in rice resulted in altered nitrogen metabolism and increased amino acid content in seeds. *Theor. Appl. Genet.* 118 (7), 1381–1390. doi: 10.1007/s00122-009-0988-3

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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