

RESEARCH

Open Access



# Mapping of Candidate Genes in Response to Low Nitrogen in Rice Seedlings

Jia Li<sup>†</sup>, Wei Xin<sup>†</sup>, Weiping Wang, Shijiao Zhao, Lu Xu, Xingdong Jiang, Yuxuan Duan, Hongliang Zheng, Luomiao Yang, Hualong Liu, Yan Jia, Detang Zou\* and Jingguo Wang\*

## Abstract

Nitrogen is not only a macronutrient essential for crop growth and development, but also one of the most critical nutrients in farmland ecosystem. Insufficient nitrogen supply will lead to crop yield reduction, while excessive application of nitrogen fertilizer will cause agricultural and eco-environment damage. Therefore, mining low-nitrogen tolerant rice genes and improving nitrogen use efficiency are of great significance to the sustainable development of agriculture. This study was conducted by Genome-wide association study on a basis of two root morphological traits (root length and root diameter) and 788,396 SNPs of a natural population of 295 rice varieties. The transcriptome of low-nitrogen tolerant variety (Longjing 31) and low-nitrogen sensitive variety (Songjing 10) were sequenced between low and high nitrogen treatments. A total of 35 QTLs containing 493 genes were mapped. 3085 differential expressed genes were identified. Among these 493 genes, 174 genes showed different haplotype patterns. There were significant phenotype differences among different haplotypes of 58 genes with haplotype differences. These 58 genes were hypothesized as candidate genes for low nitrogen tolerance related to root morphology. Finally, six genes (*Os07g0471300*, *Os11g0230400*, *Os11g0229300*, *Os11g0229400*, *Os11g0618300* and *Os11g0229333*) which expressed differentially in Longjing 31 were defined as more valuable candidate genes for low-nitrogen tolerance. The results revealed the response characteristics of rice to low-nitrogen, and provided insights into regulatory mechanisms of rice to nitrogen deficiency.

**Keywords:** Rice, Genome-wide association study, RNA-seq, Low-nitrogen tolerance

## Background

Rice (*Oryza sativa* L.) is one of the three major food crops in the world. More than 50% of the world's population rely on rice as their main food source (Zibae 2013). At present, the high yield of crops in many countries and regions mainly depends on the application of chemical fertilizers, especially nitrogen fertilizer. Nitrogen is an essential nutrient element affecting plant growth, development and final yield formation (Lawlor 2002).

Although the application of nitrogen fertilizer has greatly increased the yield of rice, it has also led to problems such as the reduction of nitrogen use efficiency (Purwani et al. 2021), the aggravation of water eutrophication (Yin et al. 2007) and soil hardening (Meng et al. 2005), which have brought enormous pressure to the ecological environment. How to ensure high yield on the premise of reducing the amount of nitrogen fertilizer has become one of the main challenges faced by rice breeding researchers.

Root system has the functions of fixing plants, absorbing nutrients and water, and synthesizing some physiologically active substances, and is necessary for plant growth and development (Karthika et al. 2018). In recent years, more and more researches have focused on studying the effect of nitrogen on root system. The nitrogen supply level significantly affects the root architecture and

<sup>†</sup>Jia Li and Wei Xin contributed equally to this work

\*Correspondence: zoudtneau@126.com; wangjg@neau.edu.cn

College of Agriculture, Northeast Agricultural University/Key Laboratory of Germplasm Enhancement and Physiology & Ecology of Food Crop in Cold Region, Ministry of Education, Harbin 150030, Heilongjiang Province, People's Republic of China

physiological characteristics of rice (Kant et al. 2011). Nitrogen absorption and nitrogen utilization are two main factors affecting nitrogen use efficiency (Latshaw et al. 2016). Root length and root diameter were the most significant positive and negative correlation factors affecting nitrogen absorption efficiency (Ren et al. 2013). Root activity was closely related to nitrogen absorption, and a reasonable nitrogen ratio was conducive to the improvement of runoff intensity (Xiong et al. 2016). An effective solution to improve yield yet free of pollution is the reduction of nitrogen fertilizer and the improvement of nitrogen use efficiency.

In order to adapt to the nitrogen-deficient environment, plants activate a "foraging response" of their own roots, and change root structure to further affect the perception and absorption of nitrogen in the rhizosphere micro-environment (Lawlor 2005). Low-nitrogen tolerant variety can not only efficiently absorb nitrogen in soil, but also effectively utilize the absorbed nitrogen to form yield. At present, researchers have found that increased yield was largely given to the improvement of crop tolerance to nitrogen stress, and the heritability of crop nitrogen use efficiency under low-nitrogen condition was significantly greater than that under high-nitrogen condition (Tollenaar and Wu 1999; Zou et al. 2008; Liu et al. 2013). In addition, scientists have identified and cloned some genes that are expected to significantly improve rice yield and nitrogen use efficiency, such as *DEP1* (Huang et al. 2009), *GRF4* (Duan et al. 2015), *NRT1.1B* (Fan et al. 2016), *NR2* (Gao et al. 2019) and *NAC42-NPF6.1* (Tang et al. 2019). Therefore, it is effective to alleviate crop nitrogen deficiency by fully exploiting the abundant plant germplasm resources, screening low-nitrogen tolerant crop genotypes, and cultivating low-nitrogen tolerant varieties through genetic manipulation.

With the advent of next-generation sequencing, genome-wide association study (GWAS) that aims to identify direct associations between genotype and phenotype in a natural population has rapidly become a powerful gene/QTL mapping tool (Zhang et al. 2012; Korte and Farlow 2013; Kim and Reinke 2019; Han et al. 2018; Zhou et al. 2019). GWAS has been successfully used to identify QTLs associated with disease resistance (Wang et al. 2015), abiotic stress (Challa and Nee-lapu, 2018), yield (Liu et al. 2018) and other traits in rice. Transcriptome has also been applied to various aspects of plant biology, such as the clarifying signal transduction mechanism of drought stress response in Arabidopsis (Harb et al. 2010) and excavating genes involved in adult resistance of wheat (El et al. 2021). Genes related to nitrogen metabolism in crop roots such as wheat (Guo et al. 2014), common beans (Nanjareddy et al. 2017), pears (Chen et al. 2018) and potatoes (Zhang et al. 2020)

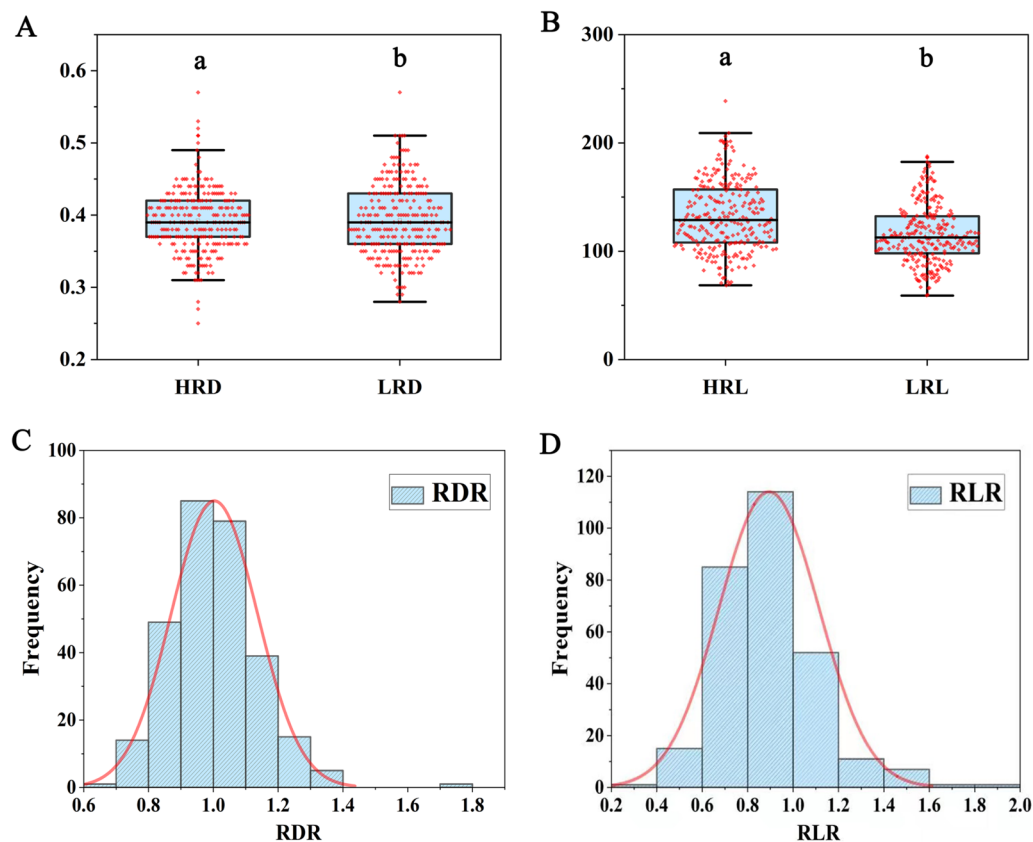
under nitrogen treatment had been identified through transcriptome analysis. The genetic mechanisms of many complex traits have been analyzed by integrating GWAS and RNA-seq. For example, 39 salt-responding differential expressed genes (DEGs) related to signal regulation of salt tolerance, including *CYPs*, *LRR-KISS* and *CML* were verified in barley (Tu et al. 2021); sixteen candidate genes of seed fatty acid metabolism and protein synthesis of cultivated peanut were screened (Zhang et al. 2021b); five DEGs were identified as promising candidate genes between long and short maize roots at seedling stage (Wang et al. 2019).

In this study, GWAS and RNA-seq were combined to identify candidate genes of low-nitrogen tolerance in major QTLs interval. Root length (RL) and root diameter (RD) of 295 rice varieties were measured under low and high nitrogen treatments at seedling stage. GWAS was performed based on phenotype (RL and RD) and 788,396 single-nucleotide polymorphisms (SNPs) developed by re-sequencing 295 rice varieties. Two rice varieties showing significant phenotypic differences of root under low-nitrogen treatment were chosen to conduct RNA-seq. Furthermore, six low-nitrogen tolerance-related candidate genes were obtained by haplotype and expression analysis. Results of this study have provided theoretical basis for rapid exploration of low-nitrogen response genes and the improvement of low-nitrogen tolerance in rice.

## Results

### Phenotypic Data Analysis

In order to evaluate phenotypic variation of nitrogen uptake in 295 rice accessions, the root length (RL) and root diameter (RD) at seedling stage were measured under low and high nitrogen treatments. The mean value, interval, standard deviation and coefficient of variation of the root morphology traits (RL and RD) are shown in Additional file 2: Table S1. Under low and high nitrogen treatments, the coefficients of variation ranged from 12.25 to 23.53 and 11.07 to 24.15, respectively. Among the trait relative values, the relative value of root diameter (RDR) under low and high nitrogen treatments showed the smallest coefficient of variation. RL of high-nitrogen treatment was higher than that of low-nitrogen treatment. Abundant genetic variations were displayed for each trait in association panel and all phenotypic values were approximately normally distributed (Fig. 1, Additional file 1: Fig. S1). Significant correlations were observed between RL and RD whether under low or high nitrogen treatment, but the correlation between RDR and the relative value of root length (RLR) under low and high nitrogen treatments was not significant (Additional file 3: Table S2).



**Fig. 1** Phenotype distribution of root morphology traits of 295 rice varieties under low and high nitrogen treatments. **A** HRD: root diameter under high-nitrogen treatment. LRD: root diameter under low-nitrogen treatment. **B** HRL: root length under high-nitrogen treatment. LRL: root length under low-nitrogen treatment. **C** RDR: relative value of root diameter under low and high nitrogen treatments. **D** RLR: relative value of root length under low and high nitrogen treatments

### QTLs Identification

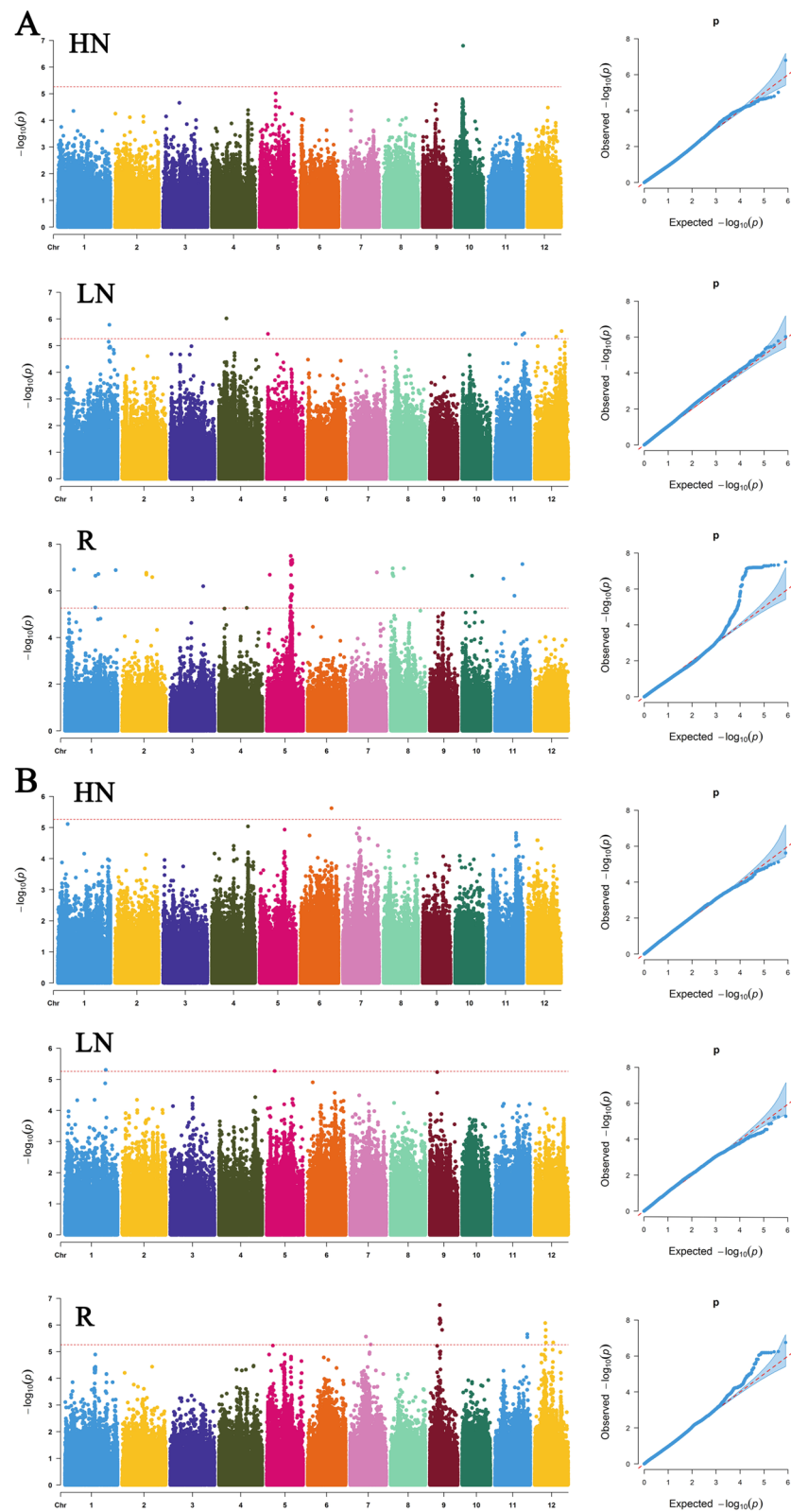
The Manhattan and Q-Q plots of the GWAS results are shown in Fig. 2. GWAS identified 35 leading SNPs in the whole panel (Additional file 4: Table S3). Five significant leading SNPs associated with LRD were identified on chromosomes 1, 4, 5, 11, 12 and named *qLRD1*, *qLRD4*, *qLRD5*, *qLRD11* and *qLRD12*, respectively. One significant leading SNP associated with HRD was detected on chromosomes 10 and named *qHRD10*. Two significant leading SNPs associated with LRL were mapped on chromosomes 1, 5 and named *qLRL1* and *qLRL5*, respectively. One significant leading SNP associated with HRL was identified on chromosomes 6 and named *qHRL6*. Nineteen significant leading SNPs associated with RDR were detected on chromosomes 1, 2, 3, 4, 5, 7, 8, 10, 11 and named *qRDR1-1*, *qRDR1-2*, *qRDR1-3*, *qRDR1-4*, *qRDR2-1*, *qRDR2-2*, *qRDR3*, *qRDR4*, *qRDR5-1*, *qRDR5-2*, *qRDR7*, *qRDR8-1*, *qRDR8-2*, *qRDR8-3*, *qRDR8-4*, *qRDR10*, *qRDR11-1*, *qRDR11-2* and *qRDR11-3*, respectively. Seven significant leading SNPs associated with RLR were mapped on chromosomes 7, 9, 11, 12 and named

*qRLR7-1*, *qRLR7-2*, *qRLR9-1*, *qRLR9-2*, *qRLR11*, *qRLR12-1* and *qRLR12-2*, respectively (Fig. 2 and Additional file 4: Table S3). There were 493 genes in the 35 QTL intervals (Additional file 5: Table S4). These leading SNPs are essential for rice in mediating root growth and development in response to nitrogen at seedling stage.

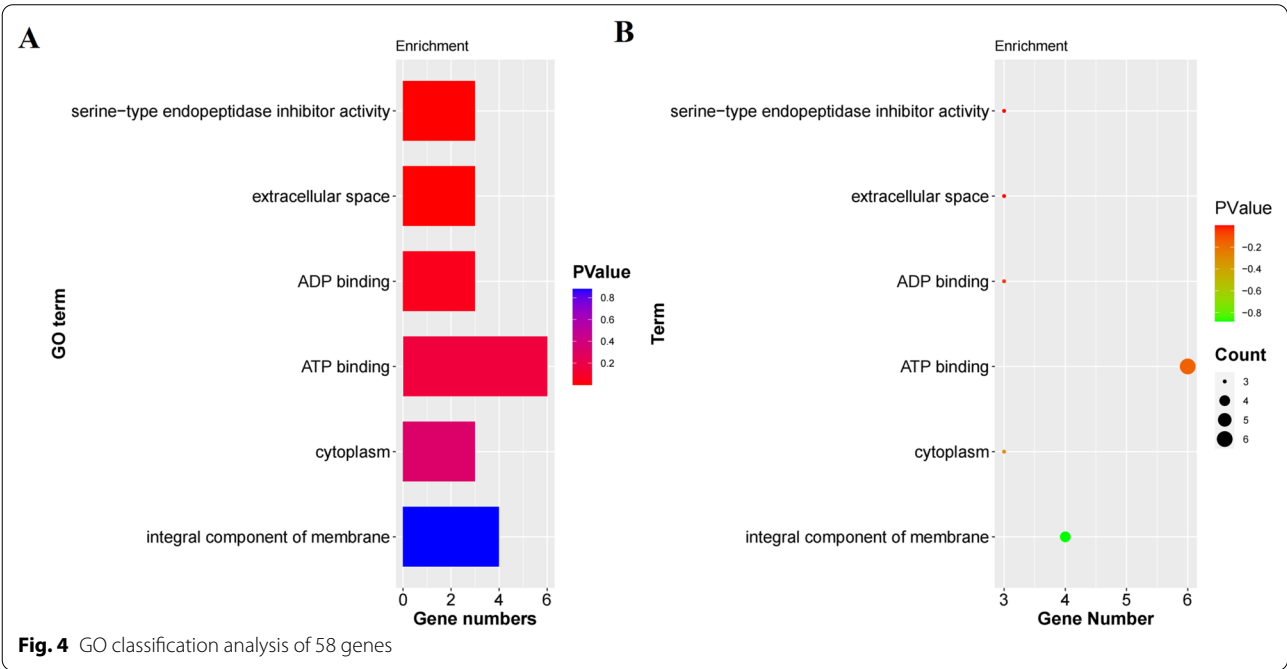
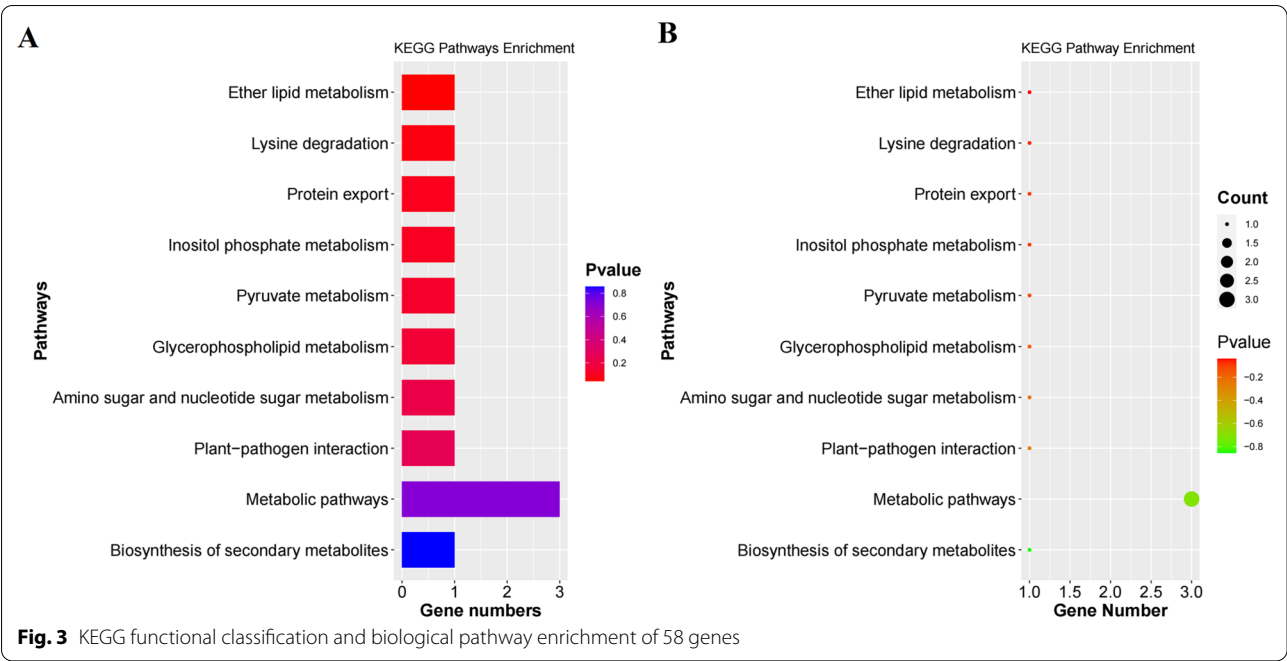
### Candidate Gene Mining

Among these 493 genes, 174 genes showed different haplotype patterns, with 96 genes having 2 haplotypes, 54 genes having 3 haplotypes, 18 genes having 4 haplotypes, 4 genes having 5 haplotypes, and 2 genes having 6 haplotypes, respectively. According to the phenotypic data of 295 accessions, there were significant phenotype differences among different haplotypes of 58 genes (Additional file 5: Table S4). Therefore, we hypothesized that these 58 genes were candidate genes for low nitrogen tolerance related to root morphology.

KEGG enrichment analysis and GO classification analysis were conducted for these 58 genes (Figs. 3, 4). These 58 genes were mainly distributed in the



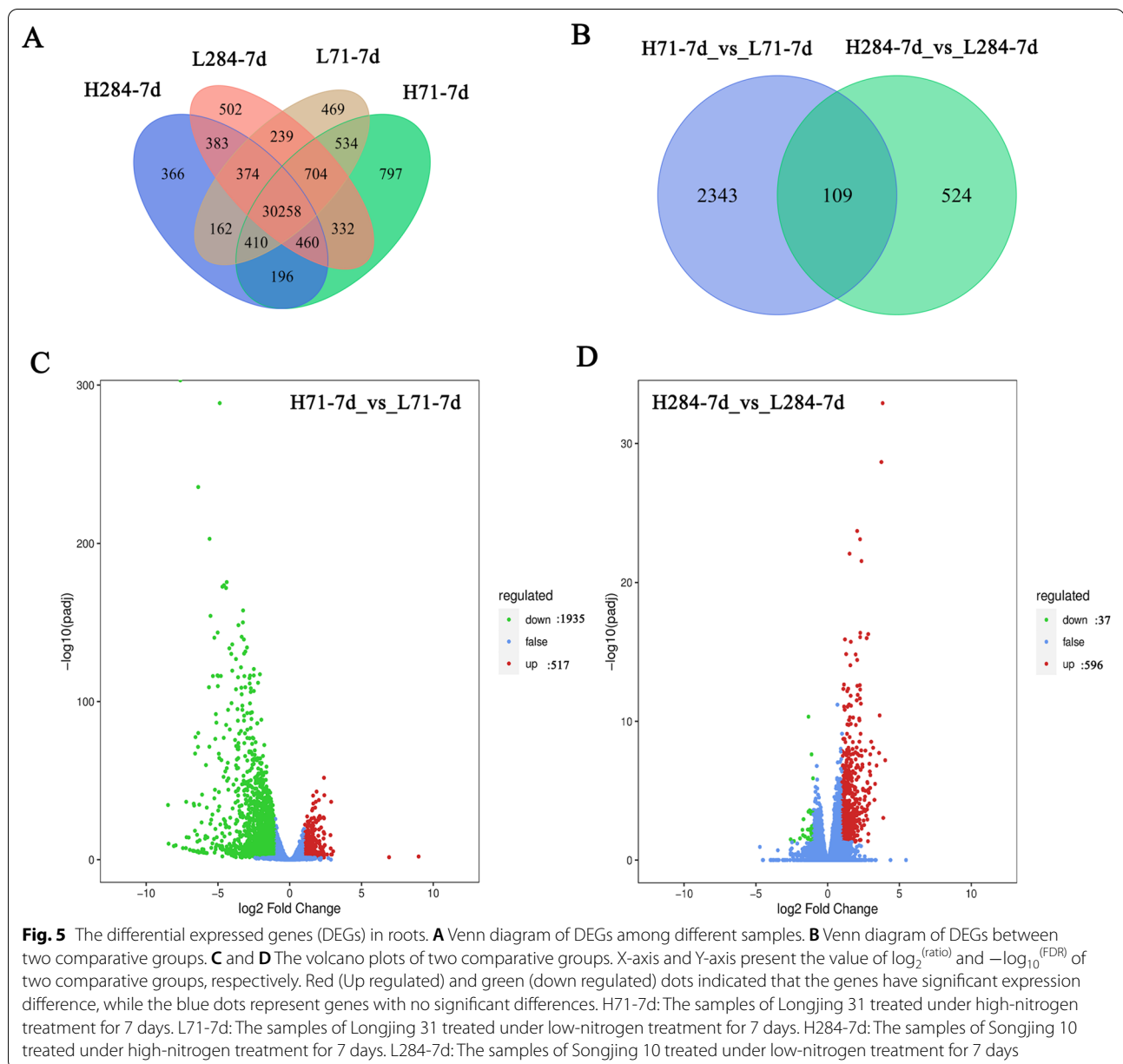
**Fig. 2** Manhattan plots and quantile–quantile (Q–Q) plots of root morphology traits. **A** GWAS assays of root diameter under high and low nitrogen treatments. **B** GWAS assays of root length under high and low nitrogen treatments. LN: low-nitrogen. HN: high-nitrogen. R: relative value of traits under low and high nitrogen treatments



metabolic category. The number of gene in metabolic pathways is the most (3 genes). A total of 6 functional groups were annotated among these 58 genes, including 3 cellular component categories and 3 molecular function categories. There were 6 genes in the molecular function of ATP binding.

### RNA-Seq Statistics

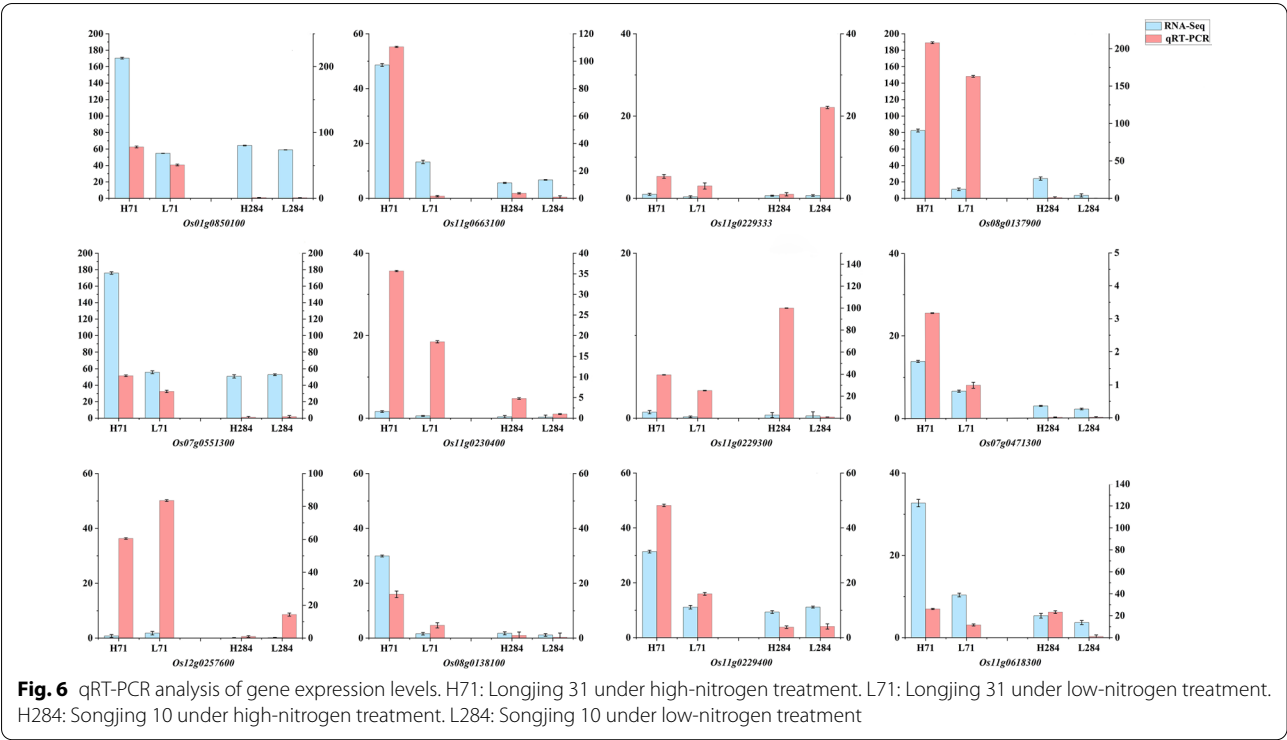
The transcriptome analysis was conducted to clarify the expression differences of these 58 genes (Additional file 6: Table S5). Two comparative groups (H71 vs. L71 and H284 vs. L284) were set up for the two rice cultivar under different treatments. The letter H, letter L, number



71 and number 284 were designated to represent high-nitrogen, low-nitrogen, Longjing 31 (low-nitrogen tolerant variety) and Songjing 10 (low-nitrogen sensitive variety), respectively. As shown in Fig. 5A, gene expression levels differed greatly among different treatments and among different varieties. There were 2452 and 633 significant differential expressed genes (DEGs) in the two comparative groups, indicating significant differences of the gene expression level within the varieties and the treatments (Fig. 5B). Among the 2452 DEGs between the treatment (low-nitrogen) and control (high-nitrogen) of Longjing 31, 517 genes were up-regulated

and 1935 genes were down-regulated. The number of up-regulated and down-regulated DEGs was 596 and 37 respectively, among the 633 DEGs between the treatment and control of Songjing 10 (Fig. 5C and D). Obviously, the DEGs quantity of Longjing 31 was more than that of Songjing 10. This result indicated that the effect of nitrogen stress on the low-nitrogen tolerant variety was greater than that on the low-nitrogen sensitive variety. Among these 3085 DEGs, 14 genes were associated with nitrogen utilization (Additional file 7: Table S6) according to the Rice Genome Annotation Project (<http://rice.plantbiology.msu.edu/>). These 14 DEGs included 7 different





**Table 1** Candidate genes expressed differentially under low nitrogen conditions

Candidate genes	Regulated	QTLs	Annotation
<i>Os07g0471300</i>	Down	<i>qRLR7-2</i>	AGO protein
<i>Os11g0230400</i>	Down	<i>qRDR11-1</i>	Serin domain containing protein, putative, expressed
<i>Os11g0229300</i>	Down	<i>qRDR11-1</i>	Disease resistance protein <i>RPM1</i> , putative, expressed
<i>Os11g0229400</i>	Down	<i>qRDR11-1</i>	Disease resistance protein <i>RPM1</i> , putative, expressed
<i>Os11g0618300</i>	Down	<i>qLRD11</i>	Protein kinase family protein, putative, expressed
<i>Os11g0229333</i>	Down	<i>qRDR11-1</i>	Hypothetical gene

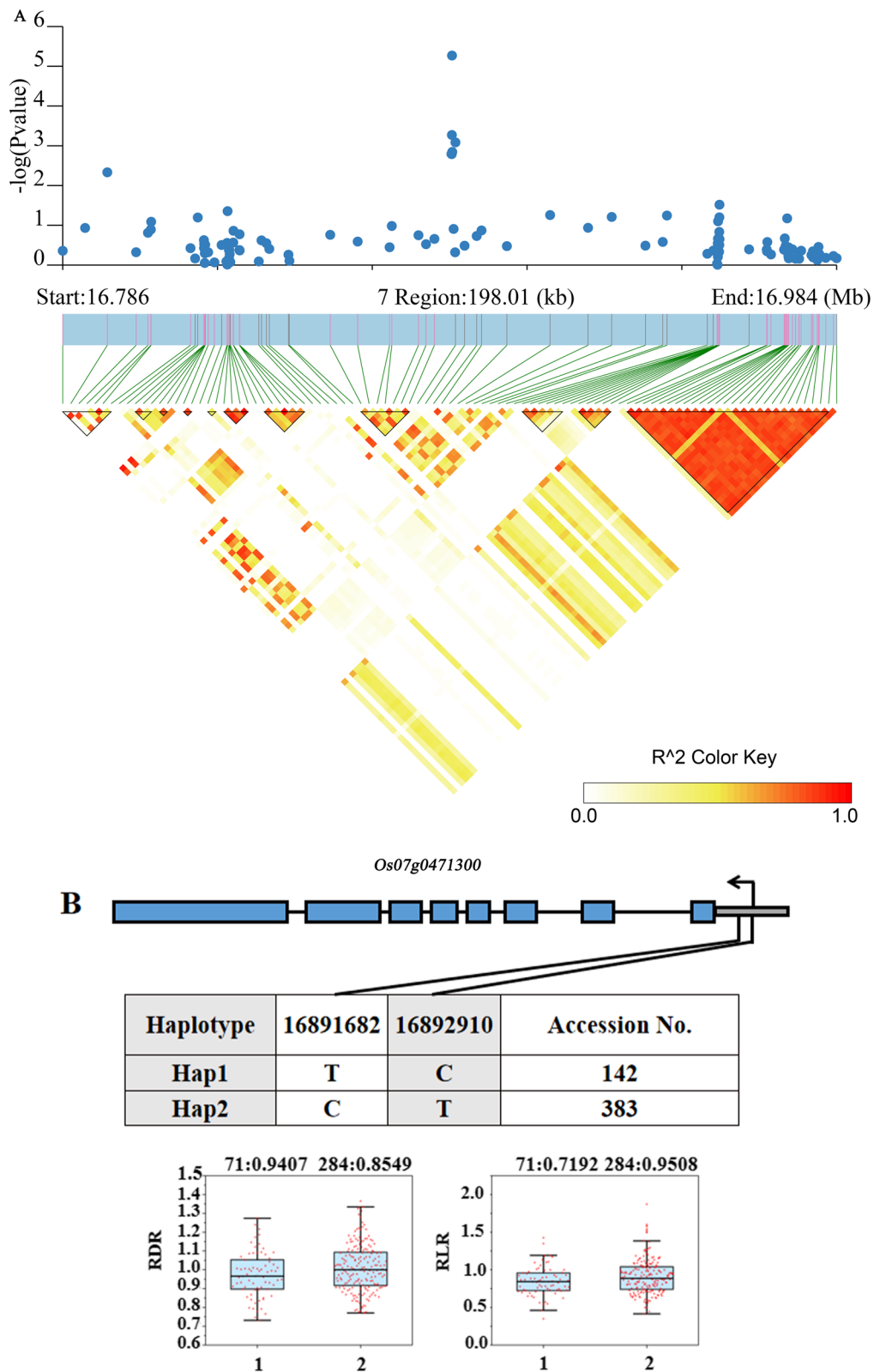
types, which were peptide transporter, nitrate reductase, nitrate transporter, POT family protein, proton-dependent oligopeptide transport, ammonium transporter, and NADH-glutamate synthase.

To confirm the accuracy and reproducibility of Illumina RNA-Seq results, 12 genes were randomly selected to verify their expression levels between Longjing 31 and Songjing 10 under low and high nitrogen treatments by qRT-PCR. Relative expression trends of these 12 genes were consistent between the qRT-PCR results and RNA-seq data, but the absolute expression levels showed some differences (Fig. 6).

**Expression Analysis of Candidate Genes**

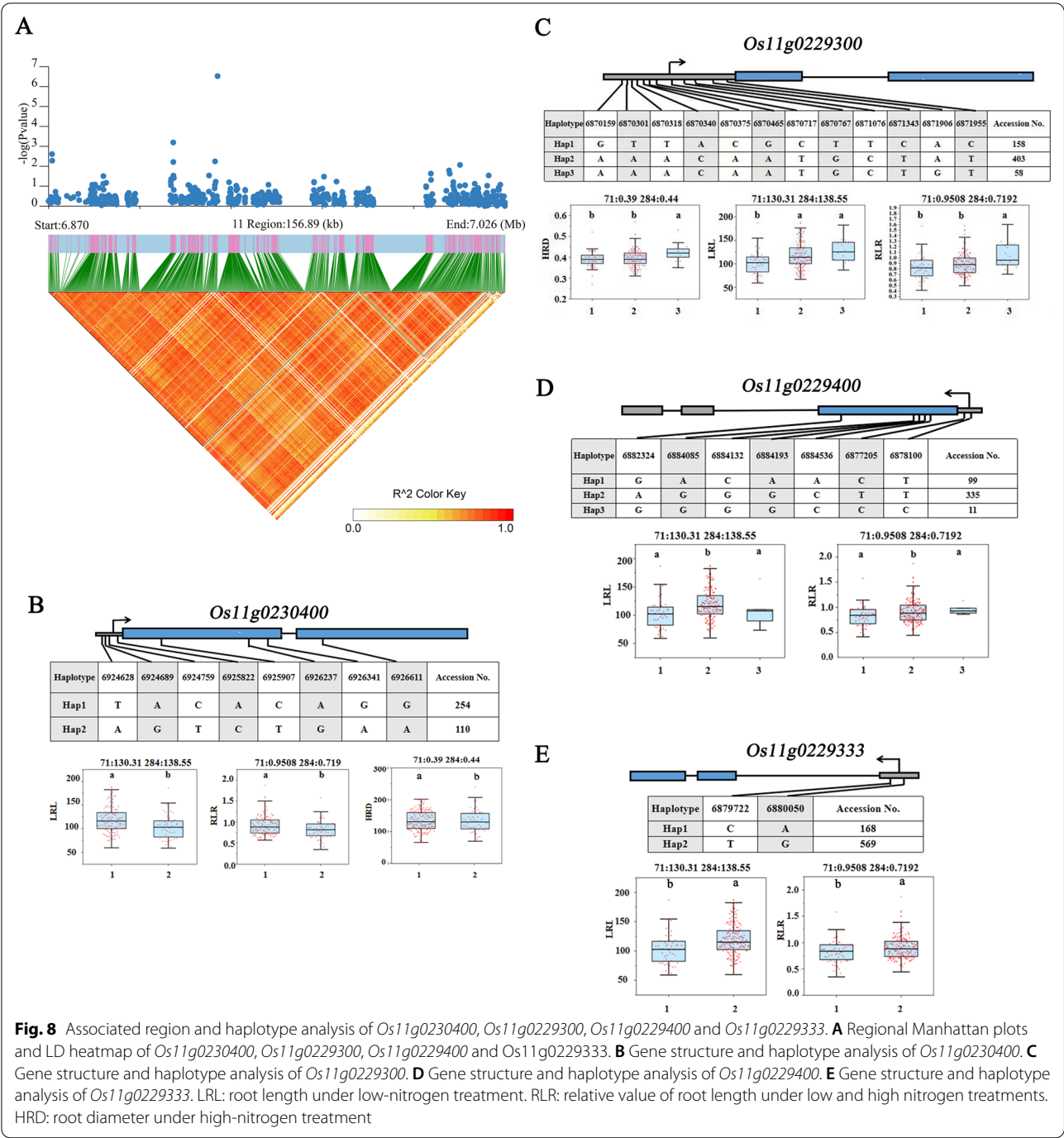
According to the results of transcriptome sequencing, six genes were down-regulated and four genes were up-regulated between the treatment (low-nitrogen)

and control (high-nitrogen) of Longjing 31 and Songjing 10, respectively (Additional file 8: Table S7). Therefore, these 6 genes which expressed differentially in Longjing 31, *Os07g0471300*, *Os11g0230400*, *Os11g0229300*, *Os11g0229400*, *Os11g0618300* and *Os11g0229333*, were defined as more valuable candidate genes among the 58 genes with haplotype differences for this study (Table 1). The linkage disequilibrium (LD) of these six candidate genes were showed in Figs. 7A, 8A and 9A. Two SNPs located in the promoter region of *Os07g0471300* formed two haplotypes, while no nonsynonymous SNP or InDel was found in CDS (Fig. 7B). In the two haplotypes of *Os11g0230400*, a total of 4 SNPs were detected in the promoter region, and 4 nonsynonymous SNPs or InDels were found in CDS (Fig. 8B). Three haplotypes of *Os11g0229300* included 12 SNPs in the

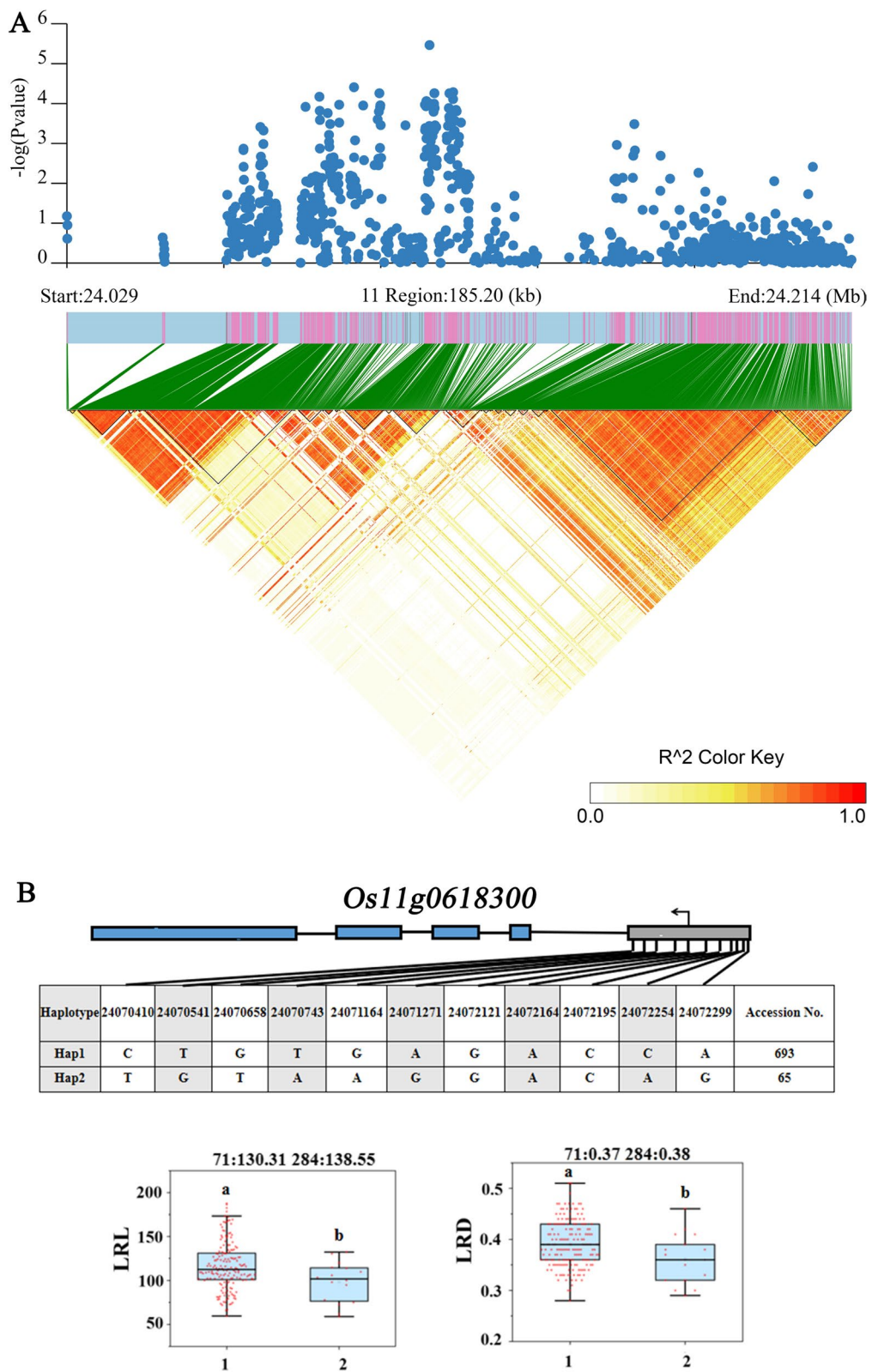


**Fig. 7** Associated region and haplotype analysis of *Os07g0471300*. A, Regional Manhattan plots and LD heatmap of *Os07g0471300*. B, Gene structure and haplotype analysis of *Os07g0471300*. RDR: relative value of root diameter under low and high nitrogen treatments. RLR: relative value of root length under low and high nitrogen treatments





promoter region (Fig. 8C). The haplotype analysis of *Os11g0229400* indicated that 2 SNPs were identified in the promoter region and 5 nonsynonymous SNPs or InDels were detected in CDS (Fig. 8D). Two haplotypes of each gene were defined by 2 and 11 SNPs located in the promoter region of *Os11g0229333* and *Os11g0618300*, respectively (Figs. 8E and 9B).



**Fig. 9** Associated region and haplotype analysis of *Os11g0618300*. **A** Regional Manhattan plots and LD heatmap of *Os11g0618300*. **B** Gene structure and haplotype analysis of *Os11g0618300*. LRD: root diameter under low-nitrogen treatment. LRL: root length under low-nitrogen treatment

## Discussion

Nitrogen is an important factor regulating growth and development of rice root system. To promote plants absorption of nitrogen can help reduce the application of fertilizers, which is a very important factor in cutting agricultural costs, controlling pollution and ensuring food safety. Plants have evolved with various response strategies to low-nitrogen stress so as to complete their life cycles and reproduce offspring in nitrogen deficient environment. The study also found that crop adaptation to low-nitrogen was positively correlated to yield (Kosgey et al. 2013). Therefore, the genetic improvement of crops low-nitrogen tolerance is not only feasible in theory, but also practical in reality. Roots play an essential role in the absorption of water and nutrients. Understanding the genetic basis of rice root development under low-nitrogen condition is conducive to breeding new varieties with low-nitrogen tolerance and enhancing resource utilization efficiency. Different ways of nitrogen treatment will directly affect the development of rice root. Previous studies showed that low-nitrogen condition had improved root morphology traits of rice and maize, such as the total root length, main root length, lateral root length and the ratio of root dry weight to whole plant dry weight (Chun et al. 2005; Wang et al. 2005; Potarzycki 2011). In this study, the effects of low-nitrogen on root morphology traits were studied by measuring root length and root diameter.

Most of the previous mapping studies were based on classical QTL mapping (Sudarshan et al. 2017; Zhang et al. 2021a; Singh et al. 2021; Weng et al. 2008; Wan et al. 2006; Maeda et al. 2004), which utilizes the limited genetic variations between two specific parents. However, GWAS takes advantage of extensive variations in natural population (Wang et al. 2020a). In the past decade, GWAS has successfully mapped plenty of potential loci in rice and identified several critical genes, which have been further conformed in subsequent functional experiments (Chen et al. 2014; Si et al. 2016; Yano et al. 2016). This study was conducted by Genome-wide association study on a basis of two root morphological traits (root length and root diameter) and 788,396 SNPs of a natural population of 295 rice varieties. There were 493 genes in the 35 QTL intervals identified in this study. Among these 493 genes, 58 genes with haplotype differences had significant phenotypic differences among all samples (Additional file 5: Table S4). Therefore, we hypothesized that these 58 genes were candidate genes for low nitrogen tolerance related to root morphology.

The genome-wide investigation of gene expression using RNA-seq is an effective approach to analyze gene regulatory networks of the complex agronomic trait. Transcriptome analysis can be used to characterize the

response of plant to environmental stresses (Wang et al. 2009). Under low-nitrogen condition, many genes show differential expressions, which are crucial in rice adaptation to low-nitrogen stress (Subudhi et al. 2020). Through transcriptome sequencing, 3085 differential expressed genes (DEGs) were identified between the treatment (low-nitrogen) and control (high-nitrogen) of Longjing 31 and Songjing 10. Among these 3085 DEGs, 14 genes were associated with nitrogen utilization (Additional file 7: Table S6) according to the Rice Genome Annotation Project (<http://rice.plantbiology.msu.edu/>). Among the 14 genes, 6 genes have been studied completely by predecessors. *OsNRT2.1* (*Os02g0112100*) encoded a high-affinity nitrate transporter, highly expressed throughout roots and mainly functioned in nitrate uptake under low nitrate concentrations (Feng et al. 2011). *OsPTR6* (*Os04g0597800*) encoded a small peptide transporter and expressed in both roots and stems of over-expressed plants. The up-regulate expression of *OsPTR6* could be activated by the increase of  $\text{NO}_3^-$  concentration in roots (Fan et al. 2014). *OsNPF4.5* (*Os01g0748950*) encoded a nitrate transporter. Increasing the expression abundance of *NPF4.5* can significantly improve the nitrogen uptake efficiency and promote rice growth (Wang et al. 2020b). *OsNRT2.2* (*Os02g0112600*) encoded a high-affinity nitrate transporter. The expression of *OsNRT2.2* was promoted by nitrate and inhibited by  $\text{NH}_4^+$  at a high temperature of 37 °C (Feng et al. 2011). It is reported that *OsAMT1;3* (*Os02g0620500*, encoding an ammonium transporter) regulated the uptake of ammonium nitrogen cooperatively under low-nitrogen condition (Konishi and Ma, 2021). *OsNADH-GOGAT2* (*Os05g0555600*) is a NADH-glutamate synthase gene. According to previous reports, the total nitrogen content of senescent leaves of *OsNADH-GOGAT2* mutant is only about half of that of wild type plant (Tamura et al. 2011). Among the 14 genes, 8 genes have not been studied thoroughly by predecessors. They may have the function of affecting rice nitrogen use efficiency, which is worthy of further researches.

Low-nitrogen tolerance is a complex trait controlled by multiple genes. In recent years, many low-nitrogen tolerance genes have been identified, such as *TOND1*, *OsNAC42*, *OsNPF6.1* and *OsTCP19* (Zhang et al. 2015; Tang et al. 2019; Liu et al. 2021). With the development of high-throughput sequencing technology, the research method combining GWAS and RNA-seq has provided possibilities to quickly analyze the genetic basis of complex traits, such as lncRNAs response to low-nitrogen, seed germination ability and seedlings root length under drought stress (Guo et al. 2020; Ma et al. 2022). Our previous research also identified many saline-alkaline tolerant candidate genes (*OsIRO3*, *OsSAP16*) by integrating GWAS/BSA and transcriptome data analysis (Li et al.

2019; Lei et al. 2020). In this study, the transcriptome of low-nitrogen tolerant variety and low-nitrogen sensitive variety were sequenced. By combining the amount of gene expression, six genes which expressed differentially in low-nitrogen tolerant variety were defined as more valuable candidate genes among the 58 genes with haplotype differences (Table 1). Haplotype analysis revealed that these genes showed different haplotype patterns in association panel. There were significant phenotype differences among different haplotypes of these genes. As far as we know, there is no report on the breeding value of these six candidate genes. The results showed six favorable alleles including Hap2 of *Os07g0471300*, Hap1 of *Os11g0230400*, Hap3 of *Os11g0229300*, Hap2 of *Os11g0229400*, Hap1 of *Os11g0618300* and Hap2 of *Os11g0229333* (Figs. 7, 8, 9). The combination of GWAS and transcriptome was proved to be an efficient method to mine favorable alleles in improved varieties.

## Conclusion

In this study, GWAS was conducted on a basis of two root morphological traits (root length and root diameter) and 788,396 SNPs of a natural population of 295 rice varieties. The transcriptome of low-nitrogen tolerant variety (Longjing 31) and low-nitrogen sensitive variety (Songjing 10) were sequenced between low and high nitrogen treatments. There were 493 genes in the 35 QTL intervals mapped in this study. 3085 differential expressed genes were identified. Haplotype analysis revealed that 174 out of the 493 genes showed different haplotype patterns in the association panel. There were significant phenotype differences among different haplotypes of 58 genes with haplotype differences. These 58 genes were hypothesized as candidate genes for low nitrogen tolerance related to root morphology. The expression levels of these 58 genes were analyzed and six genes which expressed differentially in low-nitrogen tolerant variety were defined as more valuable candidate genes among the 58 genes. However, further study was needed to investigate the regulation mechanism of these candidate genes mediating low-nitrogen tolerance in rice. These results will provide references for the cloning and molecular breeding of low-nitrogen tolerance gene of rice.

## Materials and Methods

### Plant Materials

The 295 rice varieties from Northeast Agricultural University were adopted in this experiment. Plump seeds were kept at incubator at 48 °C for 48 h to break their dormancy. Then sixteen seeds of each accession were sowed in one row of two 96-well PCR plates respectively with one seed in one well. The seeds were soaked

and sterilized in 1% sodium hypochlorite solution for 30 min. After 3 times of washing with distilled water, the sterilized seeds were placed in incubator at 31 °C and germinated in the following 2 days. The germinated seeds were cultured in the greenhouse (23.8 °C/22.4 °C, 10 h in the day/14 h at night).  $\text{NH}_4\text{NO}_3$  was chosen as nitrogen source. The low and high nitrogen treatments were adopted as 8 ppm and 40 ppm, respectively. Firstly, sixteen seedling of each accession were cultured under high-nitrogen treatment (40 ppm) for 5 days; then, eight seedlings were kept in the same solution for 21 days, while the other eight seedlings were transplanted into low-nitrogen treatment (8 ppm) for 21 days. Hydroponic nutrient solution was prepared by referring to IRRI's conventional nutrient solution formula (Xin et al. 2019). The nutrient solution was replaced every seven days.

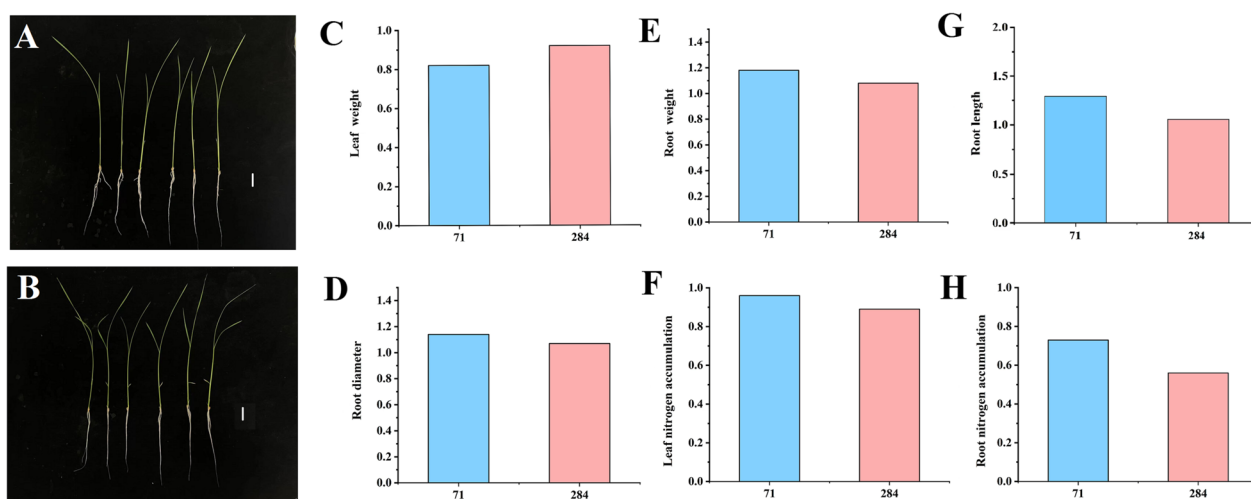
### Phenotype Data

Root length (RL) and root diameter (RD) were measured by LA-S plant root analysis system (Microtek Scan Makeri800) after 21 days of treatment. The above-ground and underground parts of seedlings were harvested and dried in oven at 105 °C for 30 min, respectively. After the samples were dried to constant weight, their dry matter weight was measured. Nitrogen content in aboveground and underground parts was analyzed by an element analyzer (Primacs SNC 100-IC-E), and nitrogen accumulation were calculated. The relative value of each trait was represented by the quotient of phenotypic value under low-nitrogen treatment divided by the value under high-nitrogen treatment.

### GWAS

Plink 2.0 software (Chang et al. 2015) was used to screen 788,396 SNPs developed by re-sequencing 295 rice varieties (Li et al. 2019). GWAS was conducted via the mixed linear model (MLM) of Tassel 5.0 (Bradbury et al. 2007). The threshold of SNP significantly associated with trait was set as  $P < 5.46 \times 10^{-6}$ . The Manhattan map was drawn by the CMplot package of R (Yin 2017). In order to identify the leading SNPs with the lowest  $P$ -value, redundant SNPs were filtered in a least-distance interval. The annotation information of the genes in the QTL interval was checked from the ensembl genome database (<https://plants.ensembl.org/>). The pairwise  $R^2$  value between any two SNPs within the interval of leading SNPs  $\pm 2$  Mb were calculated by LDBlockShow software (Dong et al. 2021). The average of top 10%  $R^2$  value within the interval from leading SNPs + 1.5 Mb to leading SNPs + 2 Mb was recorded as the background value of linkage disequilibrium (LD) attenuation. The background value of LD attenuation plus 0.2 was adopted to define the LD attenuation interval of the leading SNPs.





**Fig. 10** Root morphology traits of low-nitrogen tolerant variety (Longjing 31) and low-nitrogen sensitive variety (Songjing 10) under low and high nitrogen treatments. **A** The seedling of Longjing 31 under high (three on the left) and low (three on the right) nitrogen treatments. **B** The seedling of Songjing 10 under high (three on the left) and low (three on the right) nitrogen treatments. Scale bar represented 2 cm. **C** The relative value of the leaf weight under low and high nitrogen treatments. **D** The relative value of the root diameter under low and high nitrogen treatments. **E** The relative value of the root weight under low and high nitrogen treatments. **F** The relative value of the leaf nitrogen accumulation under low and high nitrogen treatments. **G** The relative value of the root length under low and high nitrogen treatments. **H** The relative value of the root nitrogen accumulation under low and high nitrogen treatments. 71: Longjing 31. 284: Songjing 10

### RNA-Seq

According to phenotype data of 295 rice accessions, Longjing 31 and Songjing 10 were selected as low-nitrogen tolerant variety and low-nitrogen sensitive variety, respectively (Additional file 9: Table S8 and Fig. 10). Firstly, sixteen seedling of each variety (Longjing 31 and Songjing 10) were cultured under high-nitrogen treatment (40 ppm) for 5 days; then, eight seedlings were kept in the same solution for 7 days, while the other eight seedlings were transplanted into low-nitrogen treatment (8 ppm) for 7 days. After 12 days, 12 root samples (three replicates of each treatment) were collected. Total RNA was extracted from the 12 samples using the TransZol Up RNA Kit (TransGen Biotech, Beijing, China). Complementary DNA was synthesized from total RNA using the HiFiScript cDNA Synthesis Kit (CWBio, Beijing, China). An Illumina library was constructed according to the manufacturer's instructions (Illumina, San Diego, CA, USA). High-throughput RNA sequencing was performed using the Illumina HiSeq 2500 platform. HISAT v2.1.0 was adopted to construct the index, and to map clean reads to the reference genome. The gene alignment and FPKM were calculate by using featureCounts v1.6.2 (Yang et al. 2014). The  $P < 0.05$  and  $|\log_2FC| > 1$  were adopt as the threshold to identify the differential expressed genes between any two comparative groups by edgeR v3.24.3 (Robinson et al. 2010).

### Quantitative Real-Time PCR

QRT-PCR analysis were performed to verify the expression levels of 12 genes selected randomly in Longjing 31 and Songjing 10 under low and high nitrogen treatments. Roche LightCycler96 was applied to conduct qRT-PCR following the manufacturers' instructions. All primers were listed in Additional file 10: Table S9.

### Candidate Gene Prediction

The genes in the Leading SNP intervals were integrated to predict candidate genes. The haplotypes of candidate genes were screened and analyzed. Combined with the amount of gene expression, genes which expressed differentially in low-nitrogen tolerant variety (Longjing 31) were adopted to identify more valuable candidate genes.

### Data Analysis

The density distribution graph of each trait was plotted using "ggplot2" in R software. Phenotypic data were processed with IBM SPSS Statistics 26.0 software (SPSS Inc., Chicago, IL, USA.) for correlation analysis and descriptive statistical analysis, including mean, range and coefficient of variation.

### Abbreviations

SNPs: Single nucleotide polymorphisms; QTL: Quantitative trait locus; DEGs: Differential expressed genes; RL: Root length; RD: Root diameter; RDR: The relative value of root diameter; RLR: The relative value of root length; HN:

High-nitrogen treatment; LN: Low-nitrogen treatment; LRD: Root diameter under low-nitrogen treatment; HRD: Root diameter under high-nitrogen treatment; LRL: Root length under low-nitrogen treatment; HRL: Root length under high-nitrogen treatment; qRT-PCR: Real time quantitative polymerase chain reaction; InDel: Insertion deletion; BSA: Bulk segregant analysis; CDS: Sequence coding for amino acids in protein.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12284-022-00597-x>.

**Additional file 1: Figure S1.** Phenotype distribution of root morphology traits of 295 rice varieties under low and high-nitrogen treatments. A, HRL: root length under high-nitrogen treatment. B, LRL: root length under low-nitrogen treatment. C, HRD: root diameter under high-nitrogen treatment. D, LRD: root diameter under low-nitrogen treatment.

**Additional file 2: Table S1.** Descriptive statistics of phenotypic traits in natural populations.

**Additional file 3: Table S2.** Phenotype analysis of natural populations.

**Additional file 4: Table S3.** Significant associated loci.

**Additional file 5: Table S4.** Annotation and haplotype analysis of associated genes.

**Additional file 6: Table S5.** Summary of RNA-seq data.

**Additional file 7: Table S6.** RNA-seq data of the 14 differential expressed genes associated with nitrogen utilization.

**Additional file 8: Table S7.** RNA-seq data of 58 genes.

**Additional file 9: Table S8.** Phenotypic data after 21 days of treatment.

**Additional file 10: Table S9.** Sequences of primers used in this study.

## Acknowledgements

Thank for the funding from the National Natural Science Foundation of China (grant no. 32101748), Innovation of Specific *Japonica* Rice Varieties in North-east China, Key Research & Development Program of Heilongjiang Province (GA21B002) and Heilongjiang Province postdoctoral research startup fund (LBH-Q20066).

## Author contributions

JL, WX, DZ and JW designed the experiments; HZ, LY, HL and YJ prepared the experimental materials; JL, WW, SZ, LX, XJ and YD carried out the experiments; JL, WX and JW analyzed the data and wrote the manuscript. All authors read and approved of the final manuscript.

## Funding

This research is financially supported by the National Natural Science Foundation of China (Grant No. 32101748), Innovation of Specific *Japonica* Rice Varieties in Northeast China, Key Research & Development Program of Heilongjiang Province (GA21B002) and Heilongjiang Province postdoctoral research startup fund (LBH-Q20066).

## Availability of Data and Materials

The datasets supporting the conclusions of this article are included within the additional files.

## Declarations

## Ethics Approval and Consent to Participate

Not applicable.

## Consent for Publication

Applicable.

## Competing interests

The authors declare that they have no competing interests.

Received: 4 May 2022 Accepted: 29 September 2022

Published online: 15 October 2022

## References

- Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 23(19):2633–2635. <https://doi.org/10.1093/bioinformatics/btm308>
- Challa S, Neelapu NRR (2018) Genome-wide association studies (GWAS) for abiotic stress tolerance in plants. *Biochem Physiol Mol Avenues Combat Abiotic Stress Tolerance Plants* 9:135–150. <https://doi.org/10.1016/B978-0-12-813066-7.00009-7>
- Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience* 4:7. <https://doi.org/10.1186/s13742-015-0047-8>
- Chen W, Gao YQ, Xie WB, Gong L, Lu K, Wang WS, Li Y, Liu XQ, Zhang HY, Dong HX, Zhang W, Zhang LJ, Yu SB, Wang GW, Lian XM, Luo J (2014) Genome-wide association analyses provide genetic and biochemical insights into natural variation in rice metabolism. *Nat Genet* 46(7):714–721. <https://doi.org/10.1038/ng.3007>
- Chen GD, Li XL, Wang Q, Qi KJ, Yin H, Qiao X, Wang P, Zhang SL, Wu JY, Huang Z (2018) Dynamic transcriptome analysis of root nitrate starvation and re-supply provides insights into nitrogen metabolism in pear (*Pyrus bretschneideri*). *Plant Sci Int J Exp Plant Biol* 277:322–333. <https://doi.org/10.1016/j.plantsci.2018.10.007>
- Chun L, Mi GH, Li JS, Chen FJ, Zhang FS (2005) Genetic analysis of maize root characteristics in response to low nitrogen stress. *Plant Soil* 276(1–2):369–382. <https://doi.org/10.1007/s11104-005-5876-2>
- Dong SS, He WM, Ji JJ, Zhang C, Guo Y, Yang TL (2021) LDBlockShow: a fast and convenient tool for visualizing linkage disequilibrium and haplotype blocks based on variant call format files. *Brief Bioinform* 22(4):1–6. <https://doi.org/10.1093/bib/bbaa227>
- Duan P, Ni S, Wang J, Zhang B, Xu R, Wang Y, Chen H, Zhu X, Li Y (2015) Regulation of *OsGRF4* by *OsmiR396* controls grain size and yield in rice. *Nat Plants* 2(1):15203. <https://doi.org/10.1038/nplants.2015.203>
- El HS, Backhaus A, Bendaou N, Sanchez-Garcia M, Al-Abdallat A, Tadesse W (2021) Genome-wide association study for adult plant resistance to yellow rust in spring bread wheat (*Triticum aestivum* L.). *Euphytica* 217(5):87. <https://doi.org/10.1007/s10681-021-02803-1>
- Fan XR, Xie D, Chen JG, Lu HY, Xu YL, Ma C, Xu GH (2014) Over-expression of *OsPTR6* in rice increased plant growth at different nitrogen supplies but decreased nitrogen use efficiency at high ammonium supply. *Plant Sci* 227:1–11. <https://doi.org/10.1016/j.plantsci.2014.05.013>
- Fan XR, Feng HM, Tan YW, Xu YL, Miao QS (2016) A putative 6-transmembrane nitrate transporter *OsNRT1.1b* plays a key role in rice under low nitrogen. *J Integr Plant Biol* 58:590–599. <https://doi.org/10.1111/jipb.12382>
- Feng HM, Yan M, Fan XR, Li BZ, Shen QR, Miller AJ, Xu GH (2011) Spatial expression and regulation of rice high-affinity nitrate transporters by nitrogen and carbon status. *J Exp Bot* 62(7):2319–2332. <https://doi.org/10.1093/jxb/erq403>
- Gao ZY, Wang YF, Chen G, Zhang AP, Yang SL, Shang LG, Wang DY, Ruan BP, Liu CL, Jiang HZ, Dong GJ, Zhu L, Hu J, Zhang GH, Zeng DL, Guo LB, Xu GH, Teng S, Harberd NP, Qian Q (2019) The indica nitrate reductase gene *OsNR6* allele enhances rice yield potential and nitrogen use efficiency. *Nat Commun* 10(1):1–10. <https://doi.org/10.1038/s41467-019-13110-8>
- Guo TC, Xuan HM, Yang YY, Wang LN, Wei LT, Wang YH, Kang GZ (2014) Transcription Analysis of Genes Encoding the Wheat Root Transporter *NRT1* and *NRT2* Families During Nitrogen Starvation. *J Plant Growth Regul* 33(4):837–848. <https://doi.org/10.1007/s00344-014-9435-z>
- Guo J, Li CH, Zhang XQ, Li YX, Zhang DF, Shi YS, Song YC, Li Y, Yang DG, Wang TY (2020) Transcriptome and GWAS analyses reveal candidate gene for seminal root length of maize seedlings under drought stress. *Plant Sci* 292:110380. <https://doi.org/10.1016/j.plantsci.2019.110380>
- Han K, Lee HY, Ro NY, Hur OS, Lee JH, Kwon JK, Kang BC (2018) QTL mapping and GWAS reveal candidate genes controlling capsaicinoid content in Capsicum. *Plant Biotechnol J* 16:1546–1558. <https://doi.org/10.1111/pbi.12894>
- Harb A, Krishnan A, Ambavaram MMR, Pereira A (2010) Molecular and physiological analysis of drought stress in arabidopsis reveals early responses



- leading to acclimation in plant growth. *Plant Physiol* 154(3):1254–1271. <https://doi.org/10.1104/pp.110.161752>
- Huang X, Qian Q, Liu S, Sun H, He S, Luo D, Xia G, Chu C, Li J, Fu X (2009) Natural variation at the *DEP1* locus enhances grain yield in rice. *Nat Genet* 41(4):494–497. <https://doi.org/10.1038/ng.352>
- Kant S, Bi YM, Rothstein SJ (2011) Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *J Exp Bot* 62(4):1499–1509. <https://doi.org/10.1093/jxb/erq297>
- Karthika KS, Rashmi I, Parvathi MS (2018) Biological functions, uptake and transport of essential nutrients in relation to plant growth. In: *Plant Nutrients and abiotic stress tolerance*, pp 1–49. [https://doi.org/10.1007/978-981-10-9044-8\\_1](https://doi.org/10.1007/978-981-10-9044-8_1)
- Kim SM, Reinke RF (2019) A novel resistance gene for bacterial blight in rice, *Xa43(t)* identified by GWAS, confirmed by QTL mapping using a bi-parental population. *PLoS ONE* 14(2):e0211775. <https://doi.org/10.1371/journal.pone.0211775>
- Konishi N, Ma JF (2021) Three polarly localized ammonium transporter 1 members are cooperatively responsible for ammonium uptake in rice under low ammonium condition. *New Phytol* 232(4):1778–1792. <https://doi.org/10.1111/nph.17679>
- Korte A, Farlow A (2013) The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods* 9(1):29. <https://doi.org/10.1186/1746-4811-9-29>
- Kosgey JR, Moot DJ, Fletcher AL, McKenzie BA (2013) Dry matter accumulation and post-silking N economy of “stay-green” maize (*Zea mays* L.) hybrids. *Eur J Agron* 51:43–52. <https://doi.org/10.1016/j.eja.2013.07.001>
- Latshaw SP, Vigil MF, Haley SD (2016) Genotypic differences for nitrogen use efficiency and grain protein deviation in hard winter wheat. *Agron J* 108(6):2201–2213. <https://doi.org/10.2134/agronj2016.02.0070>
- Lawlor DW (2002) Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *J Exp Bot* 53(370):773–787. <https://doi.org/10.1093/jexbot/53.370.773>
- Lawlor DW (2005) Causes of differences in response of plant species to nitrogen supply and the ecological consequences. In: *Plant responses to air pollution and global change*. Springer, Tokyo, pp 63–70. [https://doi.org/10.1007/4-431-31014-2\\_8](https://doi.org/10.1007/4-431-31014-2_8)
- Lei L, Zheng HL, Bi YL, Yang LM, Liu HL, Wang JG, Sun J, Zhao HW, Li XW, Li JM, Lai YC, Zou DT (2020) Identification of a major QTL and candidate gene analysis of salt tolerance at the bud burst stage in Rice (*Oryza sativa* L.) using QTL-Seq and RNA-Seq. *Rice* 13:55. <https://doi.org/10.1186/s12284-020-00416-1>
- Li N, Zheng HL, Cui JN, Wang JG, Liu HL, Sun J, Liu TT, Zhao HW, Lai YC, Zou DT (2019) Genome-wide association study and candidate gene analysis of alkalinity tolerance in *japonica* rice germplasm at the seedling stage. *Rice* 12:24. <https://doi.org/10.1186/s12284-019-0285-y>
- Liu LJ, Chen TT, Wang ZQ, Zhang H, Yang JC, Zhang JH (2013) Combination of site-specific nitrogen management and alternate wetting and drying irrigation increases grain yield and nitrogen and water use efficiency in super rice. *Field Crop Res* 154:226–235. <https://doi.org/10.1016/j.fcr.2013.08.016>
- Liu RX, Gong JW, Xiao XH, Zhang Z, Li JW, Liu AY, Lu QW, Shang HH, Shi YZ, Ge Q, Iqbal MS, Deng XY, Li SQ, Pan JT, Duan L, Zhang Q, Jiang X, Zou XY, Hafeez A, Chen QJ, Geng HW, Zou WK, Yuan YL (2018) GWAS analysis and QTL identification of fiber quality traits and yield components in upland cotton using enriched high-density SNP markers. *Front Plant Sci* 9:1067. <https://doi.org/10.3389/fpls.2018.01067>
- Liu YQ, Wang HR, Jiang ZM, Wang W, Xu RN, Wang QH, Zhang ZH, Li AF, Liang Y, Ou SJ, Liu XJ, Cao SY, Tong HN, Wang YH, Zhou F, Liao H, Hu B, Chu CC (2021) Genomic basis of geographical adaptation to soil nitrogen in rice. *Nature* 590(7847):600–605. <https://doi.org/10.1038/s41586-020-03091-w>
- Ma LL, Wang C, Hu Y, Dai W, Liang ZJ, Zou CY, Pan GT, Lubberstedt T, Shen Y (2022) GWAS and transcriptome analysis reveal MADS26 involved in seed germination ability in maize. *Theor Appl Genet* 135:1717–1730. <https://doi.org/10.1007/s00122-022-04065-4>
- Maeda H, Sugisawa T, Nemoto H, Sunohara Y (2004) QTL analysis for rice stripe resistance in the Japanese upland rice Kanto72. *Breed Sci* 54(1):19–26. <https://doi.org/10.1270/jsbbs.54.19>
- Meng L, Ding WX, Cai ZC (2005) Long-term application of organic manure and nitrogen fertilizer on N<sub>2</sub>O emissions, soil quality and crop production in a sandy loam soil. *Soil Biol Biochem* 37(11):2037–2045. <https://doi.org/10.1016/j.soilbio.2005.03.007>
- Nanjareddy K, Arthikala MK, Gómez BM, Blanco L, Lara M (2017) Differentially expressed genes in mycorrhized and nodulated roots of common bean are associated with defense, cell wall architecture, N metabolism, and P metabolism. *PLoS ONE* 12(8):e0182328. <https://doi.org/10.1371/journal.pone.0182328>
- Potarzycki J (2011) Effect of magnesium or zinc supplementation at the background of nitrogen rate on nitrogen management by maize canopy cultivated in monoculture. *Plant Soil Environ* 57(1):19–25. <https://doi.org/10.1080/00103624.2011.552658>
- Purwani J, Pratiwi E, Sipahutar IA (2021) The effect of different species of cyanobacteria on the rice yield and nitrogen use efficiency under different levels of nitrogen fertilizer on Alluvial West Java. *IOP Conf Ser Earth Environ Sci* 648(1):012196. <https://doi.org/10.1088/1755-1315/648/1/012196>
- Ren ML, Lu GH, Su Q, Ran QY, Zhang XN, Li XL, Wang Y (2013) Study on four typical plant root architectures and functions in arid area. *Xinjiang Agric Sci* 50(1):147–153. <https://doi.org/10.6048/j.issn.1001-4330.2013.01.024>
- Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26(1):139. <https://doi.org/10.1093/bioinformatics/btp616>
- Si LZ, Ji YC, Huang XH, Gong H, Luo JH, Hou QQ, Zhou TY, Lu TT, Zhu JJ, Shang-guan YY, Chen EW, Gong CX, Zhao Q, Jing YF, Zhao Y, Li Y, Cui LL, Fan DL, Lu YQ, Weng QJ, Wang YC, Zhan QL, Liu KY, Wei XH, An K, An G, Han B (2016) *OsSPL13* controls grain size in cultivated rice. *Nat Genet* 48(4):447. <https://doi.org/10.1038/ng.3518>
- Singh A, Dikshit HK, Ramawat N, Kumar S (2021) QTL mapping for abiotic stress in legumes. *Abiotic Stress Legumes* 15:337–370. <https://doi.org/10.1016/B978-0-12-815355-0.00017-5>
- Subudhi PK, Garcia RS, Coronejo S, Tapia R (2020) Comparative transcriptomics of rice genotypes with contrasting responses to nitrogen stress reveals genes influencing nitrogen uptake through the regulation of root architecture. *Int J Mol Sci* 21(16):5759. <https://doi.org/10.3390/ijms21165759>
- Sudarshan GP, Kulkarni M, Akhoy L, Ashe P, Shaterian H, Cloutier S, Rowland G, Wei YD, Selvaraj G (2017) QTL mapping and molecular characterization of the classical *D* locus controlling seed and flower color in *Linum usitatissimum* (flax). *Sci Rep* 7:15751. <https://doi.org/10.1038/s41598-017-11565-7>
- Tamura W, Kojima S, Toyokawa A, Watanabe H, Tabuchi-Kobayashi M, Hayakawa T, Yamaya T (2011) Disruption of a novel NADH-glutamate synthase2 gene caused marked reduction in spikelet number of rice. *Front Plant Sci* 2:57. <https://doi.org/10.3389/fpls.2011.00057>
- Tang WJ, Ye J, Yao XM, Zhao PZ, Xuan W, Tian YL, Zhang YY, Xu S, An HZ, Chen GM, Yu J, Wu W, Ge YW, Liu XL, Li J, Zhang HZ, Zhao YQ, Yang B, Jiang XZ, Peng C, Zhou C, Terzaghi W, Wang CM, Wan JM (2019) Genome-wide associated study identifies *NAC42*-activated nitrate transporter conferring high nitrogen use efficiency in rice. *Nat Commun* 10:5279. <https://doi.org/10.1038/s41467-019-13187-1>
- Tollenaar M, Wu J (1999) Yield improvement in temperate maize is attributable to greater stress tolerance. *Crop Sci* 39(6):1597–1604. <https://doi.org/10.2135/cropsci1999.3961597x>
- Tu YS, Fu LB, Wang FY, Wu DZ, Shen QF, Zhang GP (2021) GWAS and transcriptomic integrating analysis reveals key salt-responding genes controlling Na<sup>+</sup> content in barley roots. *Plant Physiol Biochem* 167:596–606. <https://doi.org/10.1016/j.plaphy.2021.08.038>
- Wan XY, Wan JM, Jiang L, Wang JK, Zhai HQ, Weng JF, Wang HL, Lei CL, Wang JL, Zhang X, Cheng ZJ, Guo XP (2006) QTL analysis for rice grain length and fine mapping of an identified QTL with stable and major effects. *Theor Appl Genet* 112(7):1258–1270. <https://doi.org/10.1007/s00122-006-0227-0>
- Wang Y, Mi G, Chen F, Zhang J, Zhang F (2005) Response of root morphology to nitrate supply and its contribution to nitrogen accumulation in maize. *J Plant Nutr* 27(12):2189–2202. <https://doi.org/10.1081/PLN-200034683>
- Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 10(1):57–63. <https://doi.org/10.1038/nrg2484>
- Wang CL, Ulloa M, Shi XY, Yuan XH, Saski C, Yu JZ, Roberts PA (2015) Sequence composition of BAC clones and SSR markers mapped to Upland cotton chromosomes 11 and 21 targeting resistance to soil-borne pathogens. *Front Plant Sci* 6:791. <https://doi.org/10.3389/fpls.2015.00791>
- Wang HM, Wei J, Li PC, Wang YY, Ge ZZ, Qian JY, Fan YY, Ni JR, Xu Y, Yang ZF, Xu CW (2019) Integrating GWAS and gene expression analysis identifies candidate genes for root morphology traits in maize at the seedling stage. *Genes* 10(10):773. <https://doi.org/10.3390/genes10100773>

- Wang Q, Tang JL, Han B, Huang XH (2020a) Advances in genome-wide association studies of complex traits in rice. *Theor Appl Genet* 133(5):1415–1425. <https://doi.org/10.1007/s00122-019-03473-3>
- Wang SS, Chen AQ, Xie K, Yang XF, Luo ZZ, Chen JD, Zeng DC, Ren YH, Yang CF, Wang LX, Feng HM, Lizbeth LD, Rafael HL, Xu GH (2020b) Functional analysis of the OsNPF4.5 nitrate transporter reveals a conserved mycorrhizal pathway of nitrogen in. *Proc Natl Acad Sci U S A* 117(28):16649–16659. <https://doi.org/10.1073/pnas.2000926117>
- Weng JF, Gu SH, Wan XY, Gao H, Guo T, Su N, Lei CL, Zhang X, Cheng ZJ, Guo XP, Wang JL, Jiang L, Zhai HQ, Wan JM (2008) Isolation and initial characterization of GW5, a major QTL associated with rice grain width and weight. *Cell Res* 18(12):1199–1209. <https://doi.org/10.1038/cr.2008.307>
- Xin W, Zhang L, Zhang WZ, Gao JP, Yi J, Zhen XX, Li Z, Zhao Y, Peng CC, Zhao C (2019) An integrated analysis of the rice transcriptome and metabolome reveals differential regulation of carbon and nitrogen metabolism in response to nitrogen availability. *Int J Mol Sci* 20(9):2349. <https://doi.org/10.3390/ijms20092349>
- Xiong SP, Wu KY, Wang XC, Zhang J, Du P, Wu YX, Ma XM (2016) Analysis of root absorption characteristics and nitrogen utilization of wheat genotypes with different N efficiency. *Sci Agric Sin* 49(12):2267–2279. <https://doi.org/10.3864/j.issn.0578-1752.2016.12.003>
- Yang L, Smyth GK, Wei S (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 7:923–930. <https://doi.org/10.1093/bioinformatics/btt656>
- Yano K, Yamamoto E, Aya K, Takeuchi H, Lo PC, Hu L, Yamasaki M, Yoshida S, Kitano H, Hirano K, Matsuoka M (2016) Genome-wide association study using whole-genome sequencing rapidly identifies new genes influencing agronomic traits in rice. *Nat Genet* 48(8):927. <https://doi.org/10.1038/ng.3596>
- Yin F, Fu BJ, Mao RZ (2007) Effects of nitrogen fertilizer application rates on nitrate nitrogen distribution in saline soil in the Hai River Basin. *China J Soils Sedim* 7(3):136–142. <https://doi.org/10.1065/jss2007.04.218>
- Yin LL (2017) CMAplot: Circle Manhattan Plot
- Zhang Z, Xu YW, Liu J, Kwok CK (2012) Identify predictive SNP groups in genome wide association study: a sparse learning approach. *Procedia Comput Sci* 11:107–114. <https://doi.org/10.1016/j.procs.2012.09.012>
- Zhang YJ, Tan LB, Zhu ZF, Yuan LX, Xie DX, Sun CQ (2015) TOND1 confers tolerance to nitrogen deficiency in rice. *Plant J* 81(3):367–376. <https://doi.org/10.1111/tpj.12736>
- Zhang J, Wang Y, Zhao Y, Zhang Y, Zhang J, Ma H, Han Y (2020) Correction: Transcriptome analysis reveals Nitrogen deficiency induced alterations in leaf and root of three cultivars of potato (*Solanum tuberosum* L.). *PLoS ONE* 16(6):e0253994. <https://doi.org/10.1371/journal.pone.0240662>
- Zhang C, Anarjan MB, Win KT, Begum S, Lee S (2021a) QTL-seq analysis of powdery mildew resistance in a Korean cucumber inbred line. *Theor Appl Genet* 134(4):1–17. <https://doi.org/10.1007/s00122-020-03705-x>
- Zhang H, Wang ML, Dang P, Jiang T, Zhao SZ, Lamb M, Chen C (2021b) Identification of potential QTLs and genes associated with seed composition traits in peanut (*Arachis hypogaea* L.) using GWAS and RNA-Seq analysis. *Gene* 769:145215. <https://doi.org/10.1016/j.gene.2020.145215>
- Zhou Q, Su Z, Li Y, Liu Y, Wang L, Lu S, Wang S, Gan T, Liu F, Zhou X, Wei M, Liu G, Chen S (2019) Genome-wide association mapping and gene expression analyses reveal genetic mechanisms of disease resistance variations in *Cynoglossus semilaevis*. *Front Genet* 10:1167. <https://doi.org/10.3389/fgene.2019.01167>
- Zibae A (2013) Rice: importance and future. *Rice Res Open Access* 1(2):e102. <https://doi.org/10.4172/jrr.1000e102>
- Zou YB, He-Jun AO, Xia B, Tang QY, Peng SB, Buresh RJ (2008) Effects of different nitrogen application on the yield and nitrogen use efficiency in hybrid rice. *Crop Res* 22(4):214–219. <https://doi.org/10.16848/j.cnki.issn.1001-5280.2008.04.003>

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Submit your manuscript to a SpringerOpen<sup>®</sup> journal and benefit from:**

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

---

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)