

Article

Integrated Transcriptomic and Phenotypic Analyses Reveal Tissue-Specific Nitrogen Responses and Candidate Genes for Low-Nitrogen Tolerance in *Sorghum*

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Abstract

Sorghum (*Sorghum bicolor* (L.) Moench) is an important crop with remarkable tolerance to adverse environments, including nitrogen deficiency. To investigate the mechanisms underlying sorghum tolerance to low-nitrogen (LN) stress, we integrated phenotypic evaluation, transcriptome profiling, weighted gene co-expression network analysis (WGCNA), and haplotype analysis. Two accessions, the LN-tolerant ‘Liaonian B-1’ and the LN-sensitive ‘Yikeerli’, were examined under hydroponic and field conditions. Under LN conditions, Liaonian B-1 showed increases of 32% in root length, 4.3-fold in root fresh weight, and 91% in root dry weight, whereas the LN-sensitive accession showed severe reductions in shoot biomass and a decrease in root fresh weight, with root dry weight remaining relatively stable. Transcriptomic analysis revealed more shared differentially expressed genes and stronger enrichment of N metabolism pathways in shoots, whereas roots showed enrichment of ATP-binding cassette (ABC) transporter and fatty acid elongation pathways. WGCNA identified 500 hub genes in roots and shoots. Five genes in the glutamine synthetase/glutamate synthase (GS-GOGAT) pathway were significantly associated with nitrogen-related traits. *SORBI_3001G116400*, which encodes glutamate synthase, showed the strongest haplotype effect in 232 sorghum accessions. The A allele (*Hap1*) was significantly associated with increased plant height, SPAD value, grain number, nitrogen accumulation, and biomass under LN stress, suggesting that *SORBI_3001G116400* as a candidate gene requiring functional validation and testing in additional genetic backgrounds.

Keywords: sorghum; nitrogen responses; transcriptomic; WGCNA; haplotype analysis; candidate genes



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1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is an important crop used globally for grain, forage, and bioenergy [1]. Sorghum possesses multiple beneficial traits, including tolerance to drought, waterlogging, salinity, high temperatures, and low fertility [2]. These characteristics make sorghum suitable for cultivation in nutrient-poor regions and marginal lands. Its tolerance to nitrogen deficiency, drought, and other stressors enables stable

yield under low-input soil conditions. Therefore, as a key stress-tolerant crop, sorghum remains important for identifying genetic resources for low-nitrogen (LN) and low-fertility tolerance, which could help to alleviate pressure on arable land in China and improve the utilization efficiency of marginal lands [3,4].

Nitrogen is an essential macronutrient for plant growth and influences crop quality and yield [5]. Nitrogen deficiency restricts plant growth, leading to reduced quality and yield. As a major agricultural country, China consumes 30% of the world's nitrogen fertilizer [6]. Driven by population growth, China has rapidly increased its nitrogen fertilizer use in recent decades. Although this has significantly boosted grain yield, it has also led to reduced fertilizer efficiency and environmental problems [7,8]. China's N use efficiency stands at about 40%, which is well below the 60–70% reported in developed countries [9]. The long-standing high-input, high-yield model may have selected for genotypes with ultra-high N responsiveness, at the cost of losing valuable low-fertility tolerance genes [10]. To identify LN tolerance genes and understand their underlying mechanisms, researchers have examined LN responses in multiple crops using transcriptome sequencing, including Arabidopsis [11], rice [12], maize [13–15], and wheat [16]. These studies have provided critical information on the molecular networks that control nitrogen use efficiency (NUE). Mailun et al. reported that under LN conditions, *Oryza sativa* HRS1 HOMOLOG3 (*OsHHO3*), a transcriptional repressor, was downregulated to promote ammonium uptake and transport in rice. In addition, overexpression of *Oryza sativa* NAC domain-containing protein 6 (*OsNAC6*), an NAC transcription factor gene associated with NUE, significantly increased both NUE and grain weight under nitrogen deficiency but had no significant effect under normal nitrogen (NN) conditions [12]. Similarly, Tang et al. found that the *Oryza sativa* NAC transcription factor 42 (*OsNAC42*) transcription factor activates the nitrate transporter *Oryza sativa* nitrate transporter/peptide transporter family 6.1 (*OsNPF6.1*), and that the *HapB* haplotype of this gene confers high NUE in rice, providing a basis for breeding [17].

Comparative transcriptomic analysis of LN responsive maize inbred lines under LN stress revealed distinct responses. In Qi319, a tolerant line, LN-responsive genes in roots were enriched in metabolic pathways, suggesting that roots generate more energy to cope with LN conditions. In contrast, in Ye478, a sensitive line, photosynthesis-related genes were downregulated in shoots, leading to reduced photosynthetic efficiency [12]. Another comparative root transcriptomic analysis in maize seedlings identified genes such as *NPF7.3*, *GlpT4*, *HAK24*, *HAK5*, *MRP2*, *bZIP77*, and *bZIP53* that regulate root growth under nitrogen, phosphorus, and potassium deficiency [15].

Despite significant progress in understanding LN tolerance mechanisms in various crops, the molecular regulatory network underlying LN tolerance in sorghum remains unclear. Systematic studies on the coordinated regulation of tissue-specific transcriptional responses and nitrogen metabolism across different sorghum accessions, as well as on the relationship between haplotype polymorphisms of key candidate genes and field performance, are still lacking. In this study, two sorghum accessions with contrasting nitrogen responses, the LN-tolerant (T) accession and the LN-sensitive (S) accession, were selected. By combining phenotypic analysis and transcriptome sequencing, we systematically compared transcriptional responses to LN stress in roots and shoots. Subsequently, we used Weighted Gene Co-expression Network Analysis (WGCNA) to identify key gene modules and genes related to nitrogen traits. Haplotype analysis of 232 sorghum accessions revealed favorable haplotypes of key LN tolerance genes. Our findings support gene discovery and marker-assisted breeding for LN tolerance in sorghum, contributing to sustainable marginal land use, reduced fertilizer inputs, and maintenance of farmland yield.

2. Materials and Methods

2.1. Plant Materials and Experimental Treatments

Two sorghum accessions with different nitrogen responses, the T accession (Liaonian B-1) and the S accession (Yikeerli), were used in this experiment. These two accessions were selected from a natural population of 232 sorghum accessions based on hydroponic screening under LN conditions (Table S1). The natural population comprised 156 accessions originating from China, 30 from India, 19 from the United States, 13 from Africa, and 14 from other regions. These accessions were categorized into three distinct subgroups, in line with previous characterizations [18].

The hydroponic experiment was carried out in a controlled-environment greenhouse at the Sorghum Research Institute, Shanxi Agricultural University, under a 16 h light/8 h dark photoperiod, with daytime and nighttime temperatures of 28 °C and 20 °C, respectively, and a relative humidity of 70%. Sorghum accession seeds were selected based on uniform size and plumpness, then sterilized for 20 min in 10% (*sv/v*) sodium hypochlorite (available chlorine $\geq 5.2\%$, analytical grade, Shanghai Lingfeng Chemical Reagent Co., Ltd., Shanghai, China) solution. The sterilized seeds were washed thrice with distilled water and germinated in the dark at 28 °C until the two-leaf stage. Once the embryos were removed, the seedlings were transplanted into black hydroponic incubators (50 × 34 × 14 cm) containing half-strength Hoagland nutrient solution, with two seedlings per hole. Seedlings underwent a 3-day acclimation period in half-strength Hoagland nutrient solution before being transferred to the experimental hydroponic culture and divided into LN and NN groups for N treatment. Ammonium nitrate (NH_4NO_3 , $\geq 99.0\%$, analytical grade, Guangdong Jingzhun Chemical Co., Ltd., Guangdong, China) served as the nitrogen source, with concentrations set at 2 mmol/L for the NN condition and 0.02 mmol/L for the LN condition. The hydroponic nutrient solution was prepared according to a previously established protocol [18]. Three biological replicates per treatment were used in each experiment.

A field experiment was conducted in 2024 with 232 sorghum accessions grown at Dongbai experimental station of the Sorghum Research Institute, Shanxi Agricultural University, Yuci District, Jinzhong, Shanxi, China (37.61° N, 112.73° E). The experiment included NN and LN treatments, each with three replications, giving a total of 6 independent plots. Within each plot, 232 sorghum accessions were grouped by plant height and then randomly distributed, with a row spacing of 45 cm and a plant spacing of 18.5 cm. In the NN treatment, fertilizers were applied at 225 kg ha⁻¹ N, 90 kg ha⁻¹ P, and 60 kg ha⁻¹ K. The LN treatment received the same management, except that no N fertilizer was applied. The LN field had received no nitrogen fertilizer for 10 consecutive years before the experiment. Soil total nitrogen content was measured before sowing and fertilization. The measured soil total nitrogen values were 1.01 g/kg (NN) and 0.44 g/kg (LN).

2.2. Phenotypic Data

Phenotypic traits were measured under both LN and NN conditions in the hydroponically grown T and S accessions. The hydroponic seedlings were grown under nitrogen-deficient conditions for 20 d. Three biological replicates, each with three seedlings, were used. Plants were oriented naturally upright, and plant height and root length were recorded from the stem base to the apical meristem using a ruler. To determine relative chlorophyll content (SPAD), the second fully expanded leaf from the top was selected. Measurements were taken at five distinct points per leaf, avoiding the main veins, using a SPAD-502 m (Konica Minolta, Tokyo, Japan), and the average value was recorded. Fresh shoots and roots were separated and weighed immediately to obtain shoot fresh weight and root fresh weight. To determine dry mass, the dissected plant parts were heated to 105 °C for 30 min and then dried at 80 °C for 8 h until a constant weight was achieved. Shoot dry

weight and root dry weight were measured to the nearest 0.0001 g. Dried shoot and root samples were pulverized through a 100-mesh sieve, and their total nitrogen content was analyzed with a FOSS Kjeltec™ 8400 analyzer (FOSS Analytical A/S, Höganäs, Sweden) using the standard Kjeldahl technique [19]. Nitrogen accumulation in shoots and roots was calculated as dry weight $\times$ nitrogen content.

At maturity, five representative plants were randomly sampled of each plot and pooled as one biological replicate. All phenotypic measurements and statistical analyses were performed at the plot level ($n = 3$). The following agronomic and physiological traits were recorded: plant height, SPAD, grain number per panicle, grain weight per panicle, straw nitrogen accumulation, grain nitrogen accumulation, total aboveground nitrogen accumulation, panicle fresh weight, straw fresh weight, total aboveground fresh weight, panicle dry weight, straw dry weight, grain length, and grain width. SPAD was measured on the second fully expanded leaf from the top, with an average of three readings per leaf. Fresh weight was measured immediately after harvest in the field, with samples processed in batches according to maturity stage. The samples were cut into approximately 5 cm segments, transferred to nylon mesh bags, oven-dried at 105 °C for 30 min, and then dried at 60 °C to constant weight. Dry weight was determined, and the dried tissues were milled and passed through a 100-mesh sieve before nitrogen content measurement. The total nitrogen concentration in the straws and grains was measured as described for the hydroponic experiment. Straw nitrogen accumulation and grain nitrogen accumulation were calculated as dry weight $\times$ nitrogen content, and total aboveground nitrogen accumulation was calculated as straw nitrogen accumulation + grain nitrogen accumulation. To assess relative performance under LN stress, the nitrogen response coefficient (NRC) was calculated for each trait as the ratio of the value under LN to that under NN.

Statistical analyses were performed using R version 4.3.0 (R Core Team, 2023). Two-way analysis of variance (ANOVA), with NN-LN treatment and accession as fixed factors, was applied, followed by Tukey's honestly significant difference post hoc test, with $p < 0.05$ considered significant. All measurements were performed in triplicate per treatment.

2.3. Total RNA Extraction, Illumina Sequencing, and Data Analysis

Fresh shoots and roots of T and S sorghum accessions were collected separately after 20 days of LN and NN treatment in hydroponic experiments. Three biological replicates were harvested per genotype (T and S accessions), N treatment (LN and NN), and tissue type (shoot and root), yielding 24 samples in total, organized into 8 groups: LN-SS (S accession shoots under LN), LN-TS (T accession shoots under LN), NN-SS (S accession shoots under NN), NN-TS (T accession shoots under NN), LN-SR (S accession roots under LN), LN-TR (T accession roots under LN), NN-SR (S accession roots under NN), NN-TR (T accession roots under NN). All 24 samples were immediately frozen in liquid nitrogen upon collection and stored at -80 °C until RNA extraction.

Total RNA was extracted using TRIzol® Reagent (Thermo Fisher Scientific, Waltham, MA, USA), and the samples were subsequently treated with RNase-free DNase I (TaKaRa, Kusatsu, Japan) to remove any remaining genomic DNA. A NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine RNA concentration and purity, and RNA integrity was assessed using RNA-specific agarose gel electrophoresis (agarose, biotechnology grade, Sangon Biotech, Shanghai, China).

Using the NEBNext Ultra™ RNA Library Prep Kit (NEB, Ipswich, MA, USA), 24 cDNA libraries were successfully established. High-throughput sequencing was performed using the Illumina NovaSeq X Plus platform in PE150 mode. The clean reads were aligned to the sorghum reference genome (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/003/195/GCF_000003195.3_Sorghum_bicolor_NCBIv3, accessed on 14 April 2025)) using HISAT2

(v2.1.0). Gene expression levels were calculated as fragments per kilobase of transcript per million mapped reads (FPKM) using HTSeq (v0.9.1). Finally, differentially expressed genes (DEGs) between LN and NN conditions and between T and S accessions were identified using DESeq2 (v1.38.3), with strict cutoff criteria of $|\log_2FC| > 1$ and $p < 0.05$.

2.4. Functional Enrichment Analysis of DEGs

Functional annotation of DEGs responding to LN was performed using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (www.geneontology.org; www.kegg.jp). Using ClusterProfiler (v4.6.0), we assessed the statistical enrichment of DEGs against background genes using a hypergeometric test. Terms with $p < 0.05$ were considered significant.

2.5. Construction and Analysis of Gene Co-Expression Modules and Candidate Genes

We performed WGCNA (v1.72) on shoot and root tissues to identify co-expression patterns and potential hub genes. Before network construction, genes with an average FPKM > 1 across three biological replicates were retained. The soft-thresholding power (β) was set to 8 to ensure that the network adhered to a scale-free topology ($R^2 > 0.85$). Using the dynamic tree-cut algorithm, we partitioned the gene hierarchy into distinct modules and assigned each module a distinct color. To connect gene modules with physiological traits, we calculated module eigengenes, defined as the first principal component, and assessed their Pearson correlations with phenotypic traits, including plant height, root length, and nitrogen accumulation. Modules showing robust correlations ($|r| \geq 0.7$, $p < 0.05$) were identified as significantly trait-associated.

To further narrow down candidate genes, the module showing the strongest correlation with phenotypic traits was selected for shoots and roots. Within each selected module, genes with high module membership (kME, defined as the correlation between a gene's expression profile and the module eigengene) > 0.95 were retained. Candidate genes from shoots and roots were combined and duplicates removed, yielding 860 non-redundant genes. From this pool, only genes that were differentially expressed in at least one of the 8 comparison groups were retained as the final set of 500 hub genes for subsequent analyses.

2.6. Haplotype Analysis of Key Hub Genes Associated with LN Tolerance

We further performed haplotype analysis of key hub genes to elucidate the genetic basis of LN tolerance. Our panel comprised 232 natural sorghum accessions that were subjected to whole-genome resequencing. After extracting high-quality genomic DNA from fresh leaves, we constructed paired-end libraries with approximately 350 bp inserts and sequenced them on an Illumina NovaSeq 6000 platform, ensuring an average coverage of $10\times$. Clean reads were mapped to the *Sorghum bicolor* v3.1 reference genome. From this dataset, we identified 3,629,268 high-quality single-nucleotide polymorphisms (SNPs). We managed the Variant Call Format (VCF) files using VCFtools (v0.1.16) and BCFtools (v1.9) and imputed missing data points using the KNNi method implemented in TASSEL 5.0. Distinct haplotypes for each gene were defined by focusing on nonsynonymous SNPs in the exons. The functional significance of these haplotypes was tested against phenotypic traits using ANOVA and Tukey's HSD post hoc tests, with $p < 0.05$ considered significant.

3. Results

3.1. Effect of LN on Growth, Physiological, and Nutritional Traits in Sorghum Seedlings

Under LN and NN treatments, growth, biomass, and nitrogen accumulation in sorghum seedlings differed significantly between T and S accessions. As shown in Figure 1, the S accession showed more severe growth inhibition under LN stress than the T acces-

sion. Plant height decreased under LN treatment in both accessions compared with that under NN treatment. However, plant height in S accession decreased significantly by 54% ($p < 0.05$), whereas the decrease in the T accession was not significant (Figure 2A). The pattern of change in SPAD was consistent with that of plant height (Figure 2C). In contrast, root length increased in the T accession but remained unchanged in S accession (Figure 2B). In Yikeerli, shoot fresh weight and shoot dry weight decreased by 68.3% and 47.6%, respectively, under LN treatment, whereas no significant changes were observed in the T accession (Figure 2D). Root responses differed between the accessions. In the T accession, both root fresh weight and root dry weight increased, indicating enhanced root growth under nitrogen deficiency. In contrast, root fresh weight decreased under LN, whereas root dry weight remained relatively stable in the S accession (Figure 2E).

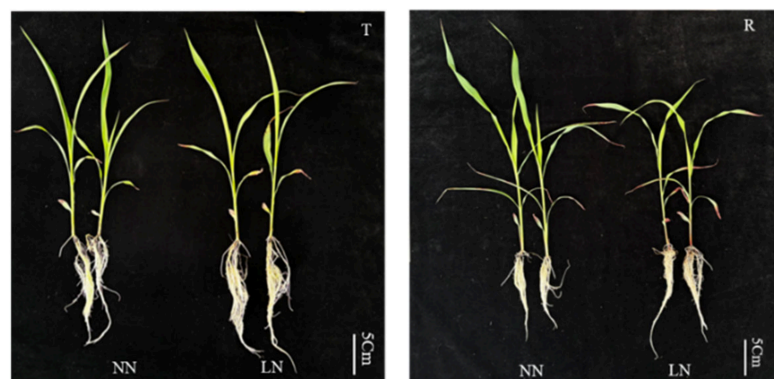


Figure 1. Growth performance of T and S sorghum accessions under NN and LN conditions.

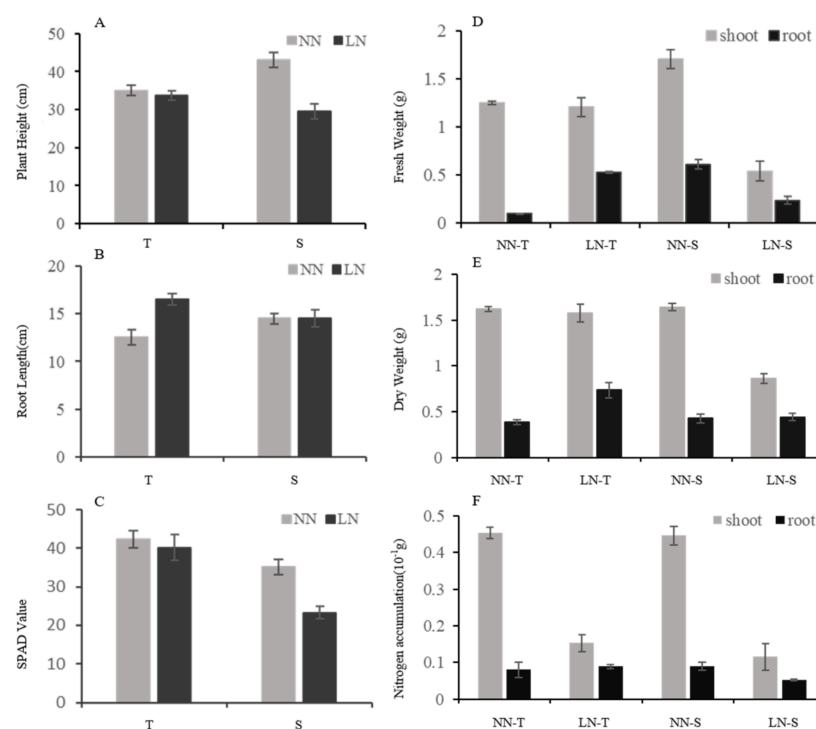


Figure 2. Morphological and physiological characteristics of sorghum seedlings under LN conditions. (A) Plant height. (B) Root length. (C) SPAD value. (D) Fresh weight of shoots and roots. (E) Dry weight of shoot and roots. (F) Nitrogen accumulation in shoots and roots. The numbers in the histograms represent the average values of each trait.

Furthermore, shoot nitrogen accumulation under LN treatment decreased in both accessions compared with that under NN treatment, with reductions of 74.1% in the S

accession and 66.2% in the T accession. Root nitrogen accumulation also decreased by 41.58% in the S accession under LN treatment but showed no significant change in the T accession (Figure 2F). These results suggest that the T accession maintained better nitrogen uptake and adaptive root growth under LN conditions, whereas the S accession showed severe growth inhibition and reduced nitrogen acquisition.

3.2. Analysis of DEGs

Transcriptome sequencing was performed on shoot and root tissues of T and S accessions under LN and NN treatments. High-quality clean reads were obtained from 24 samples. The sequencing data showed $Q20 \geq 98.11\%$ and $Q30 \geq 94.77\%$, indicating high sequencing quality for downstream analysis. After quality control, the alignment rates with the sorghum reference genome exceeded 90% for all samples (Table S2). A total of 12,547 DEGs were identified across eight pairwise comparisons (Tables S3–S11): four in shoots, namely LN-SS vs. LN-TS, NN-SS vs. NN-TS, LN-TS vs. NN-TS, and LN-SS vs. NN-SS; and four in roots, namely LN-SR vs. LN-TR, NN-SR vs. NN-TR, LN-TR vs. NN-TR, and LN-SR vs. NN-SR.

The DEG heatmap showed that the T and S accessions clustered into two distinct groups, indicating different expression patterns between the two accessions (Figure 3A, Table S12). Within each accession, expression patterns differed between LN and NN treatments, suggesting that LN stress induced distinct transcriptional responses in the two accessions. Under NN conditions, most genes were differentially expressed between SS (S accessions shoots) and TS (T accessions shoots). The comparison of NN-SS vs. NN-TS revealed 2552 upregulated and 4797 downregulated genes. In roots under NN conditions, the comparison of NN-SR vs. NN-TR revealed 2130 upregulated and 4372 downregulated genes. Under LN treatment, the comparison of LN-SR vs. LN-TR revealed 1989 upregulated and 4320 downregulated genes in roots, whereas LN-SS vs. LN-TS revealed 1751 upregulated and 3846 downregulated genes in shoots (Figure 3B). Across comparisons, the S accession consistently showed a greater number of DEGs than the T accession. These results indicate that more genes were repressed in the S accession under LN stress.

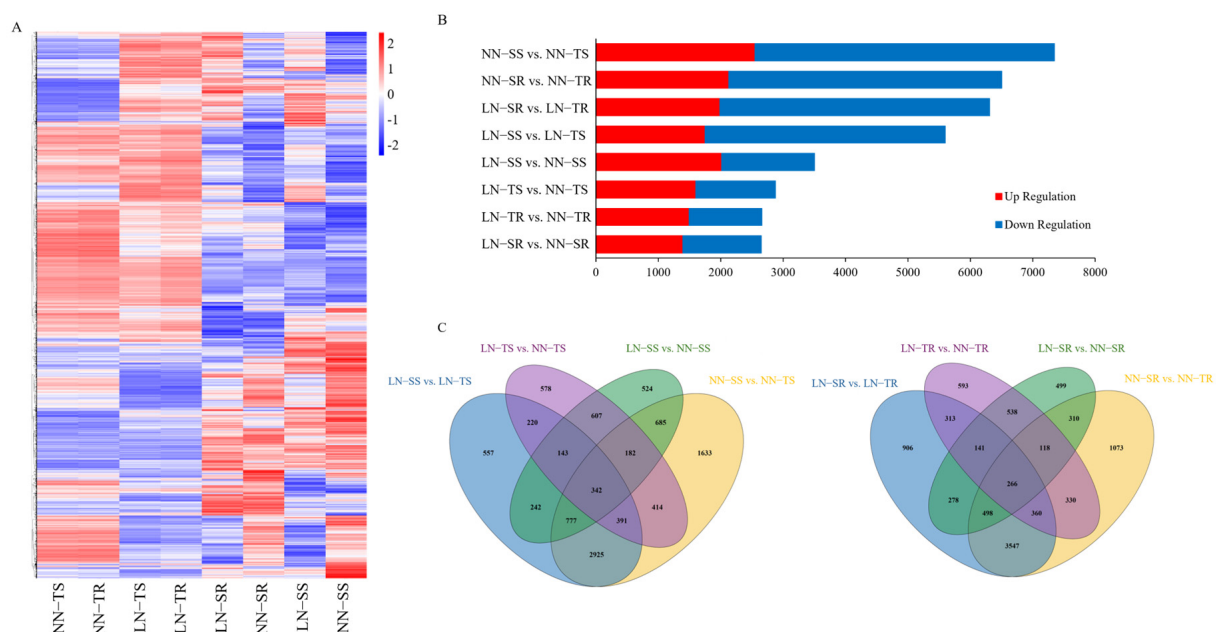


Figure 3. Transcriptional responses of roots and shoots to LN stress in T and S sorghum accessions. (A) Heatmap of all DEGs in the eight comparisons. (B) Upregulated and downregulated DEGs in the eight root and shoot comparisons. (C) Venn analyses of DEGs in roots and shoots.

Venn analysis of the four shoot and root groups revealed substantial numbers of shared and specific DEGs. In shoots, 342 genes were common to all four shoot groups, with the highest number of specific DEGs found in NN-SS vs. NN-TS. In roots, 278 common genes were detected among all four root groups (Figure 3C). Shoot comparisons consistently yielded more shared DEGs than root comparisons, which may reflect a more pronounced transcriptional response in shoots under the experimental conditions tested.

3.3. DEGs Classification and GO Functional Annotation

GO enrichment analysis demonstrated that the DEGs were most significantly enriched in “carbohydrate metabolic process” in the biological process category. In addition, “membrane,” “membrane part,” “intrinsic component of membrane,” and “integral component of membrane” were enriched in the cellular component category. In the molecular function category, “catalytic activity” was enriched (Figure 4A, Table S13).

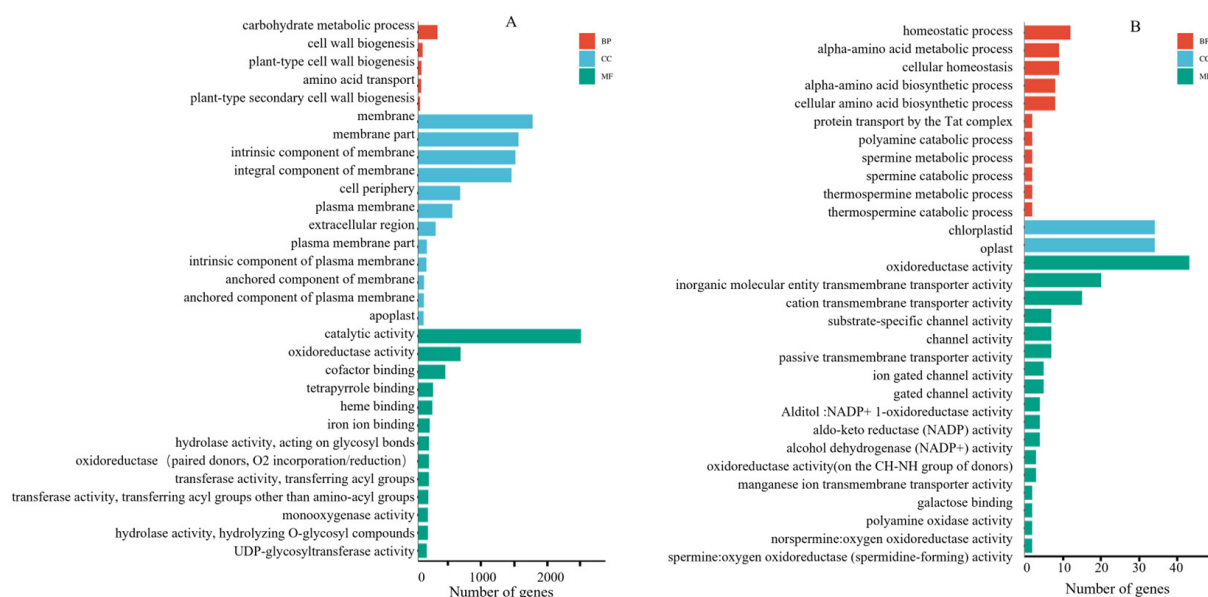


Figure 4. GO enrichment of DEGs in shoots and roots under LN stress. (A) GO enrichment terms of all DEGs. (B) GO enrichment terms of the 500 hub genes.

The selected 500 hub genes were mainly enriched in GO terms related to “oxidoreductase activity,” “chloroplast,” “plastid,” “inorganic molecular entity transmembrane transporter activity,” and “cation transmembrane transporter activity.” These results indicate that, under LN stress, sorghum seedlings exhibited coordinated changes in multiple biological processes, particularly carbohydrate and cell wall metabolism, membrane-associated transport, and oxidoreductase activity (Figure 4B, Table S14).

3.4. Significant KEGG Pathways of DEGs in Roots and Shoots

The significantly enriched KEGG pathways ($p < 0.05$) are summarized in Table 1. Thirty pathways were significantly enriched in shoots. The most significantly enriched pathway was “Phenylpropanoid biosynthesis,” followed by “flavonoid biosynthesis,” “starch and sucrose metabolism,” “cutin, suberine and wax biosynthesis,” and “amino sugar and nucleotide sugar metabolism”. Additionally, “nitrogen metabolism” and “plant hormone signal transduction” pathways were enriched in shoots. These results indicate that secondary metabolism was extensively reprogrammed in shoots under LN conditions.

In roots, 36 pathways were significantly enriched. As in shoots, the most significantly enriched pathways were “phenylpropanoid biosynthesis,” “flavonoid biosynthesis,” “starch and sucrose metabolism,” and “cutin, suberine, and wax biosynthesis.” In addition,

roots showed significant enrichment of “stilbenoid, diarylheptanoid, and gingerol biosynthesis,” “fatty acid elongation,” “cyanoamino acid metabolism,” and “ABC transporters.”

Pathways such as phenylpropanoid biosynthesis, flavonoid biosynthesis, starch and sucrose metabolism, cutin/suberine/wax biosynthesis, amino sugar metabolism, linoleic acid metabolism, and circadian rhythm were enriched in both shoots and roots. Notably, “nitrogen metabolism” showed stronger enrichment in shoots, whereas “fatty acid elongation” and “ABC transporters” showed stronger enrichment in roots, which may reflect tissue-specific responses under LN conditions, although this interpretation requires further experimental validation (Figure S2).

Table 1. Most enriched KEGG pathways in shoots and roots.

Pathway	Pathway-ID	Shoots	Roots
Phenylpropanoid biosynthesis	sbi00940	1.42×10^{-14}	3.92×10^{-16}
Flavonoid biosynthesis	sbi00941	4.50×10^{-8}	8.42×10^{-12}
Starch and sucrose metabolism	sbi00500	5.69×10^{-6}	4.15×10^{-7}
Cutin, suberine and wax biosynthesis	sbi00073	2.24×10^{-5}	8.86×10^{-8}
Amino sugar and nucleotide sugar metabolism	sbi00520	7.74×10^{-5}	4.81×10^{-5}
Stilbenoid, diarylheptanoid and gingerol biosynthesis	sbi00945	1.60×10^{-4}	1.01×10^{-6}
Linoleic acid metabolism	sbi00591	1.16×10^{-4}	2.46×10^{-4}
Cyanoamino acid metabolism	sbi00460	5.24×10^{-4}	1.60×10^{-5}
Fatty acid elongation	sbi00062	4.54×10^{-3}	3.03×10^{-5}
Circadian rhythm—plant	sbi04712	3.53×10^{-4}	3.14×10^{-4}
Diterpenoid biosynthesis	sbi00904	5.19×10^{-4}	5.55×10^{-2}
Ubiquinone and other terpenoid-quinone biosynthesis	sbi00130	1.90×10^{-2}	2.63×10^{-4}
Monoterpenoid biosynthesis	sbi00902	1.86×10^{-3}	1.15×10^{-2}
Tyrosine metabolism	sbi00350	2.60×10^{-3}	6.19×10^{-3}
Plant hormone signal transduction	sbi04075	3.59×10^{-3}	3.58×10^{-4}
Pentose phosphate pathway	sbi00030	3.19×10^{-3}	1.30×10^{-2}
Isoquinoline alkaloid biosynthesis	sbi00950	1.04×10^{-2}	9.69×10^{-4}
Alanine, aspartate and glutamate metabolism	sbi00250	3.67×10^{-2}	1.86×10^{-3}
Cysteine and methionine metabolism	sbi00270	3.78×10^{-3}	7.79×10^{-2}
Fatty acid biosynthesis	sbi00061	3.49×10^{-2}	3.94×10^{-3}
Phenylalanine metabolism	sbi00360	2.08×10^{-2}	5.69×10^{-3}
Ribosome	sbi03010	6.13×10^{-3}	1.00×10^0
Ascorbate and aldarate metabolism	sbi00053	4.72×10^{-2}	6.19×10^{-3}
DNA replication	sbi03030	1.04×10^{-2}	2.46×10^{-2}
ABC transporters	sbi02010	8.73×10^{-2}	6.61×10^{-3}
Pantothenate and CoA biosynthesis	sbi00770	1.13×10^{-2}	3.88×10^{-2}
Plant–pathogen interaction	sbi04626	2.49×10^{-2}	9.79×10^{-3}
Glutathione metabolism	sbi00480	1.15×10^{-2}	1.86×10^{-1}
Nitrogen metabolism	sbi00910	1.35×10^{-2}	1.03×10^{-1}
Biotin metabolism	sbi00780	8.12×10^{-2}	1.45×10^{-2}
beta-Alanine metabolism	sbi00410	8.94×10^{-2}	1.56×10^{-2}
MAPK signaling pathway—plant	sbi04016	2.15×10^{-2}	3.82×10^{-2}
alpha-Linolenic acid metabolism	sbi00592	1.01×10^{-1}	1.75×10^{-2}
Carbon fixation in photosynthetic organisms	sbi00710	3.17×10^{-1}	1.86×10^{-2}
Tropane, piperidine and pyridine alkaloid biosynthesis	sbi00960	3.12×10^{-1}	1.92×10^{-2}
Fructose and mannose metabolism	sbi00051	4.13×10^{-2}	5.58×10^{-2}
Sulfur metabolism	sbi00920	2.04×10^{-1}	2.48×10^{-2}
Benzoxazinoid biosynthesis	sbi00402	4.63×10^{-2}	3.41×10^{-2}
Phenylalanine, tyrosine and tryptophan biosynthesis	sbi00400	2.24×10^{-1}	3.07×10^{-2}
Sesquiterpenoid and triterpenoid biosynthesis	sbi00909	6.00×10^{-1}	3.32×10^{-2}
Flavone and flavonol biosynthesis	sbi00944	6.04×10^{-2}	3.71×10^{-2}
Limonene and pinene degradation	sbi00903	6.18×10^{-2}	4.02×10^{-2}
Photosynthesis	sbi00195	2.85×10^{-1}	4.52×10^{-2}

3.5. Identification of a Weighted Gene Co-Expression Network

To identify gene modules associated with related phenotypic traits, including plant height, SPAD, dry weight of shoot and root, and nitrogen accumulation in shoots and roots, WGCNA was performed using 8000 high-variance genes. As shown in the hierarchical clustering dendrogram, seven co-expressed modules were identified in shoots after merging (Figure 5A). The yellow module, including 888 genes, showed the strongest positive correlation with shoot nitrogen accumulation ($r = 0.96$, $p = 1 \times 10^{-6}$), as well as significant positive correlations with SPAD ($r = 0.71$, $p = 0.01$) and plant height ($r = 0.60$, $p = 0.04$). The green module, including 573 genes, and the red module, including 142 genes, were significantly negatively correlated with the four traits. Except for the correlation between the green module and plant height, all other module–trait correlations were significant (Figure 5B).

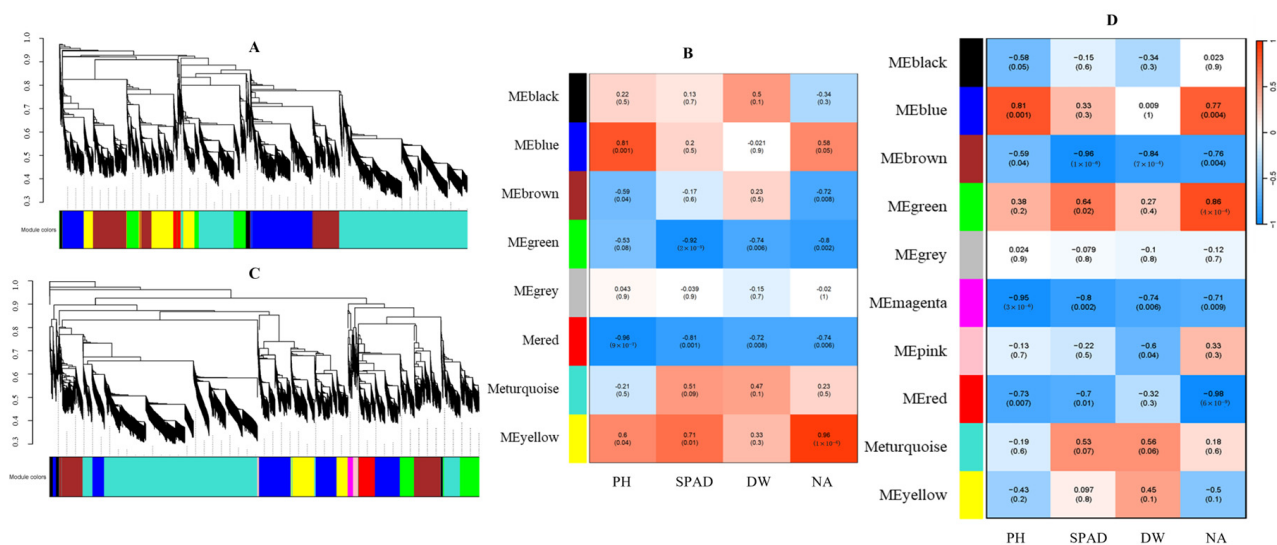


Figure 5. Weighted gene co-expression network analysis in sorghum shoots and roots. (A) Hierarchical clustering dendrogram showing co-expression modules in shoots. (B) Module–trait correlation heatmap in shoots. (C) Hierarchical clustering dendrogram showing co-expression modules in roots. (D) Module–trait correlation heatmap in roots. All traits were measured under hydroponic conditions. Abbreviations: PH—plant height; SPAD—chlorophyll content; DW—dry weight of shoot and root; NA—nitrogen accumulation of shoot and root.

In roots, WGCNA identified ten distinct co-expression modules (Figure 5C). The red module showed the strongest negative correlation with LN-related traits. The correlation coefficients with root nitrogen accumulation, plant height, and SPAD were -0.98 , -0.73 , and -0.70 , respectively, all of which were statistically significant ($p < 0.05$). The brown and magenta modules also exhibited negative correlations with LN-related traits, whereas the blue and green modules exhibited positive correlations (Figure 5D).

3.6. Hub Genes and DEGs Related to Nitrogen Absorption and Utilization

We integrated the WGCNA and DEG results from shoots and roots. The three trait-associated shoot modules, yellow, green, and red, were further analyzed for hub genes defined by high module membership. In total, 512 high-connectivity hub genes were identified across the three modules: 317 in the yellow module, 164 in the green module, and 31 in the red module (Table S15). The red, blue, green, brown, and magenta trait-associated modules in roots were further analyzed for hub genes. A total of 1350 high-connectivity hub genes were identified, with 242 in the red module, 452 in the blue module, 331 in the green module, 287 in the brown module, and 38 in the magenta module (Table S16).

A total of 500 candidate hub genes, consisting of both high-connectivity hub genes and DEGs from shoots and roots, were identified as core components of the LN response network (Table S17). The heatmap revealed two distinct expression clusters, indicating diverse transcriptional responses to LN. Each cluster displayed distinct expression patterns under LN and NN conditions (Figure 6A).

The top 14 enriched pathways are shown in Figure 6B and included nitrogen metabolism, carbohydrate metabolism, amino acid metabolism, and cofactor biosynthesis (Table S18). “Nitrogen metabolism” (sbi00910) was the most significantly enriched pathway ($p = 0.0016$), containing five hub genes: *SORBI_3003G258800* (K00264, nitrite reductase), *SORBI_3003G306700* (K01455, carbonic anhydrase), *SORBI_3003G234200* (K01673, glutamine synthetase), and two copies of *SORBI_3001G451500/SORBI_3001G116400* (K01915, glutamate synthase). These genes are directly involved in nitrate reduction, ammonium assimilation, and nitrogen recycling, suggesting their potential involvement in NUE under LN stress.

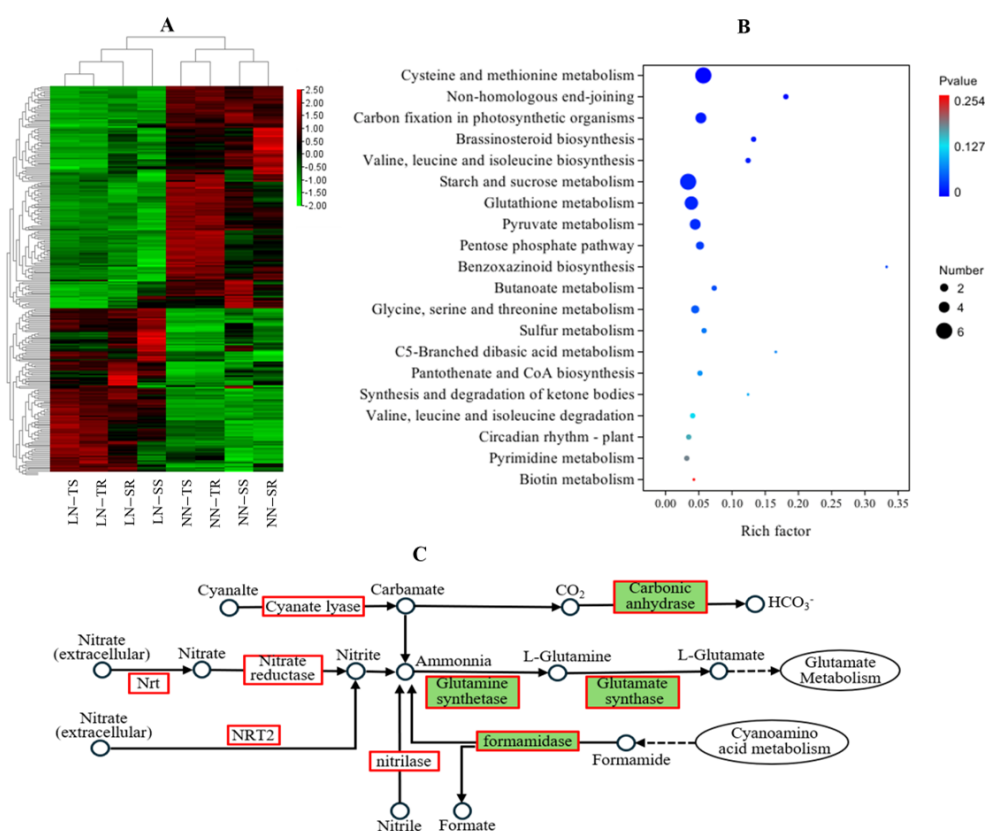


Figure 6. Expression patterns and KEGG pathway enrichment of the 500 hub genes. (A) Heatmap of the expression profiles of the 500 hub genes. (B) KEGG pathway enrichment analysis of the 500 hub genes. (C) Nitrogen metabolism pathway. Red borders indicate upregulated genes under LN and green background indicates enriched hub genes.

The “starch and sucrose metabolism” pathway and amino acid metabolism pathways were also significantly enriched. These pathways are essential for nitrogen reallocation, synthesis of stress-related amino acids, and production of secondary metabolites, such as glutathione and polyamines (Figure 6C).

3.7. Verification of Candidate Hub Genes via Haplotype Analysis in a Natural Population

SORBI_3003G258800, *SORBI_3003G306700*, and *SORBI_3001G116400* contained non-synonymous SNPs with a minor allele frequency $\geq 5\%$. Haplotype analysis of the candidate genes was performed using a natural population of 232 sorghum accessions under both

LN and NN field conditions in 2024. Each gene exhibited two major haplotypes, *Hap1* and *Hap2*, in the population (Table S19). The nonsynonymous SNPs at position 59,662,486 (C/T) of *SORBI_3003G258800* defined two haplotypes. *Hap2*, with a frequency of 17.67%, was associated with significantly higher SPAD, grain number, total aboveground fresh weight, total aboveground dry weight and grain length under LN stress. The major C allele, *Hap1*, of *SORBI_3003G306700* was associated with significantly higher plant height, SPAD, grain number, and total aboveground nitrogen accumulation, indicating that it conferred enhanced nitrogen stress tolerance (Table 2).

SORBI_3001G116400 exhibited the strongest haplotype effect on the measured phenotypic traits. In *SORBI_3001G116400*, a nonsynonymous SNP at position 9,072,758 (A/G) defined *Hap1*, with a frequency of 42.24%, and *Hap2*, with a frequency of 50.00%; the remaining 7.76% of accessions carried heterozygous genotypes and were excluded from the haplotype analysis. *Hap1* carriers consistently outperformed *Hap2* carriers across most measured traits, including plant height (0.95 vs. 0.87, $p < 0.05$), SPAD (0.90 vs. 0.81, $p < 0.05$), grain number per panicle (0.96 vs. 0.65, $p < 0.05$), grain weight per panicle (0.67 vs. 0.56, $p < 0.05$), total aboveground nitrogen accumulation (0.67 vs. 0.51, $p < 0.05$), total fresh weight (0.84 vs. 0.60, $p < 0.05$), and total dry weight (0.83 vs. 0.62, $p < 0.05$). The only exception was grain length, for which the G allele showed a higher value than the A allele (0.96 vs. 0.90). These findings suggest that the A allele of *SORBI_3001G116400* may be associated with improved NUE in sorghum under LN conditions (Figure 7).

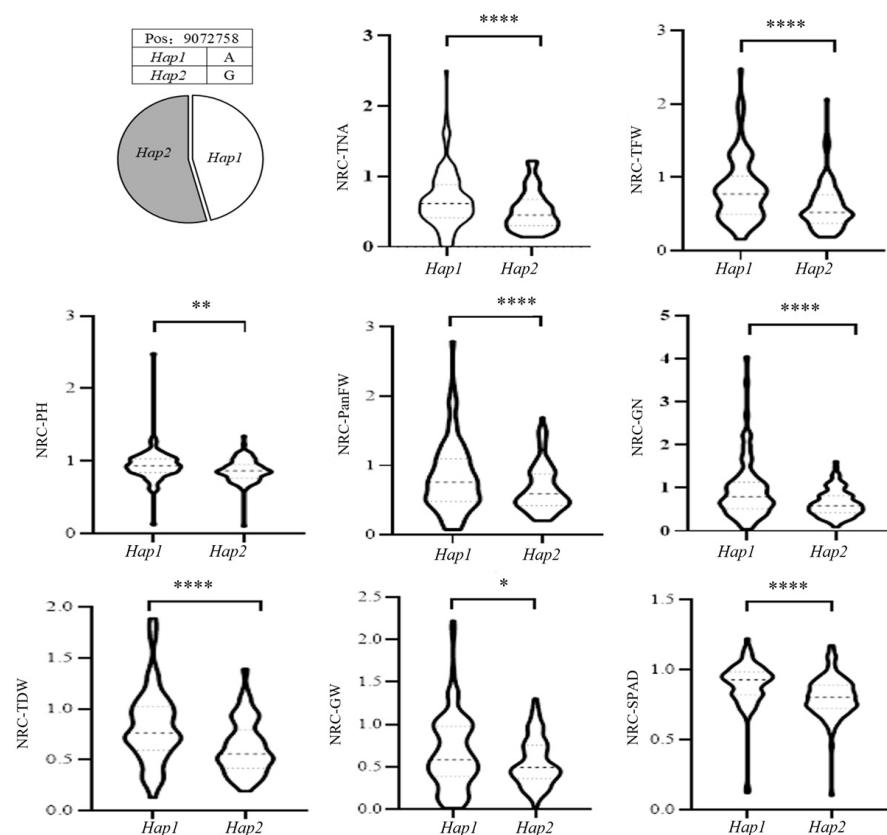


Figure 7. Phenotypic variation associated with haplotypes of *SORBI_3001G116400* under LN stress. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. PH—plant height; SPAD—chlorophyll content; GN—grain number per panicle; GW—grain weight per panicle; TNA—total aboveground nitrogen accumulation; PanFW—panicle fresh weight; TFW—total aboveground fresh weight; TDW—total aboveground dry weight.

Table 2. Haplotype analysis of candidate genes identified across the population.

Candidate Gene	Variation Pattern	Freq	NRC-PH	NRC-SPAD	NRC-GN	NRC-GW	NRC-TNA	NRC-TFW	NRC-TDW	NRC-GL
SORBI_3003G258800	C	79.31%	0.90a	0.83a	0.72a	0.61a	0.56a	0.68a	0.69a	0.96a
	T	17.67%	0.91a	0.92b	1.06b	0.57a	0.68a	0.90b	0.85b	0.86b
SORBI_3003G306700	C	60.78%	0.93a	0.87a	0.85a	0.62a	0.64a	0.79a	0.79a	0.92a
	T	38.79%	0.87b	0.81b	0.69b	0.59a	0.52b	0.63b	0.63b	0.98b
SORBI_3001G116400	A	42.24%	0.95a	0.90a	0.96a	0.67a	0.67a	0.84a	0.83a	0.90a
	G	50.00%	0.87b	0.81b	0.65b	0.56b	0.51b	0.60b	0.62b	0.96b

All listed traits were measured in the field under NN and LN treatments. PH—plant height; SPAD—chlorophyll content; GN—grain number per panicle; GW—grain weight per panicle; TNA—total aboveground nitrogen accumulation; TFW—total aboveground fresh weight; TDW—total aboveground dry weight; GL—grain length. Different letters within the same gene indicate significant differences between haplotypes at $p < 0.05$.

4. Discussion

4.1. Physiological Responses to LN Stress

Our study revealed distinct response patterns to LN stress between the T and S accessions. The T accession maintained plant height, SPAD value, and shoot biomass under LN conditions, whereas the S accession showed marked reductions in these traits, consistent with previous reports of genotypic variation in sorghum nitrogen stress tolerance [20]. The result suggests that, as a C4 crop, sorghum may possess inherently strong tolerance mechanisms to nitrogen limitation [21,22].

Root responses to LN stress differed markedly between the T and S sorghum accessions. Under LN stress, the T accession substantially increased root length, dry weight and fresh weight, whereas the S accession showed no such enhancement. This divergence implies that root biomass expansion serves as an effective adaptive strategy in the T accession, possibly facilitating continued N uptake under LN. Similar root responses to nitrogen deficiency have been reported in other cereals [22–24].

The nitrogen accumulation further supports functional relevance of this root response. In the T accession, root nitrogen accumulation remained stable under LN stress despite a marked reduction in shoot nitrogen accumulation, suggesting that roots may serve as a temporary nitrogen storage pool, ensuring nitrogen availability for remobilization during later reproductive stages [25]. Wherever it was located, the S accession showed significant reductions in both root and shoot nitrogen accumulation, indicating the S accession was unable to mount an active adaptive response to LN stress.

Collectively, these physiological observations suggest that root biomass expansion and nitrogen accumulation in root tissues represent two interconnected components of the T accession's strategy for adapting to LN stress. Furthermore, the strong relationship between crop nitrogen status at anthesis and grain yield highlights the universal importance of assessing nitrogen status for yield prediction [23,24].

4.2. Tissue-Specific Transcriptomic Reprogramming in Response to LN Stress

As a highly stress-tolerant C4 crop, sorghum adapts to LN stress through morphological changes and fine-scale transcriptional reprogramming [26,27]. Our transcriptomic data indicated that LN stress induced substantial differences in gene expression, with strong tissue specificity. Under LN conditions, the S accession Yikeerli showed a greater number of repressed genes than the T accession. These results support the findings of Gelli et al. (2014), who reported that nitrogen-sensitive genotypes undergo more extensive transcriptional reprogramming under stress [28]. More importantly, a greater number of shared DEGs were observed in shoots than in roots, implying that shoots are the primary

organs for nitrogen assimilation and nutrient accumulation and exhibiting a more focused response to changes in nitrogen nutrition [29].

In our study, GO enrichment analysis identified “carbohydrate metabolic process” as the most significantly enriched term among biological processes, consistent with the recognized role of carbon–nitrogen coordination in NUE [27,30]. KEGG analysis further revealed that “phenylpropanoid biosynthesis” and “flavonoid biosynthesis” were commonly enriched in both shoots and roots, supporting the hypothesis that phenylpropanoid and flavonoid biosynthesis play coordinated roles in stress adaptation across plant organs [31,32]. The “nitrogen metabolism” pathway showed stronger enrichment in shoots than in roots, suggesting that sorghum prioritizes the maintenance of nitrogen assimilation capacity in aboveground tissues—a strategy consistent with the observed stability of shoot biomass in the T accession. This is consistent with findings in rice, where genes involved in nitrogen metabolism (AMTs, NRTs, and the GS GOGAT cycle) showed stronger responses in shoots under both LN and high-nitrogen conditions [27].

However, the “ABC transporter” and “fatty acid elongation” pathways were significantly enriched in roots. ABC transporters play established roles in nutrient uptake and stress responses [33]. Enrichment of the fatty acid elongation pathway may be linked to root membrane remodeling. Shoots prioritize nitrogen assimilation efficiency, whereas roots prioritize nutrient uptake and signal perception. This division of labor represents a strategy by which sorghum copes with nitrogen stress [30] and may partly explain its tolerance to LN conditions. Carbon metabolism provides carbon skeletons and energy for nitrogen metabolism [27]. The significant enrichment of the “starch and sucrose metabolism” pathway in both shoots and roots underscores the fundamental role of carbon metabolism in supporting nitrogen assimilation under LN conditions, further highlighting its importance in adaptation to nitrogen stress [34].

4.3. Hub Genes, Nitrogen Metabolism Candidates, and Haplotype Validation

The integration of WGCNA and differential expression analysis provides a powerful framework for identifying core genes underlying complex traits [35]. In the present study, a complex LN response transcriptional network was elucidated by identifying co-expression modules that were significantly correlated with nitrogen-related traits. This led to the identification of 500 high-confidence candidate genes that are likely involved in the LN response network. These genes were mainly enriched in GO terms, including “oxidoreductase activity,” “chloroplast,” “plastid,” “inorganic ion transmembrane transporter activity,” and “cation transmembrane transporter activity.” Oxidoreductase activity is closely linked to antioxidant systems and nitrate reduction [36]. The enrichment of “chloroplast” and “plastid” terms points to the importance of photosynthetic apparatus maintenance in nitrogen stress adaptation, consistent with findings in wheat [37].

Through KEGG pathway enrichment analysis, the “nitrogen metabolism” pathway (sbi00910) was significantly enriched and contained five hub genes: *SORBI_3003G258800*, *SORBI_3003G306700*, *SORBI_3003G234200*, *SORBI_3001G451500*, and *SORBI_3001G116400*. These genes are central to the GS-GOGAT cycle, which is responsible for primary ammonium assimilation and the production of glutamine and glutamate, the building blocks for all nitrogenous compounds in plants. This finding confirmed the pivotal role of the GS-GOGAT cycle in sorghum NUE. This cycle converts ammonium into glutamine and glutamate, which serve as precursors for all nitrogen-containing compounds [38]. This finding highlights the importance of the GS GOGAT cycle in sorghum NUE. Notably, GS and GOGAT enzymes play important roles in NUE in rice and maize, and natural variation in these genes is often associated with yield performance under LN conditions [39]. In rice, overexpression of *OsGOGAT1* has been shown to enhance NUE and grain yield [40].

The positive association of the A allele of *SORBI_3001G116400* with improved phenotypic performance in our study is consistent with these observations, although direct functional evidence in sorghum is still needed.

Using genotypic and phenotypic data from a natural population under field conditions, we performed haplotype analysis of the candidate genes. Both *SORBI_3003G258800* and *SORBI_3003G306700* showed significant haplotype associations with nitrogen-related traits under LN stress; however, their specific roles in nitrogen metabolism remain poorly understood, making them promising candidates for future functional studies. *SORBI_3001G116400* exhibited the most pronounced and consistent haplotype effects. GOGAT functions together with GS to assimilate ammonium into amino acids. In rice, natural variations in *OsGOGAT1* have been associated with grain yield and NUE, and its overexpression enhances nitrogen utilization [40]. Similarly, *ZmGOGAT* is upregulated under LN conditions and contributes to efficient nitrogen recycling in maize [31]. Our results suggest that the A allele of *SORBI_3001G116400* may be associated with improved nitrogen assimilation and agronomic performance under LN stress, but this interpretation remains correlative and requires functional validation. Transcriptomic, co-expression, and haplotype evidence suggest that *SORBI_3001G116400* is a candidate gene potentially involved in LN response, although further studies are needed to clarify its regulatory role. Whether this gene could serve as a target for genome editing to improve nitrogen efficiency requires additional validation in independent populations, diverse environments, and functional experiments.

5. Conclusions

In conclusion, to adapt to nitrogen deficiency, the T accession ‘Liaonian B-1’ enhanced root elongation and root biomass, whereas the S accession ‘Yikeerli’ exhibited more extensive gene repression. Compared with roots, shoot tissues exhibited greater sensitivity to nitrogen deficiency, with stronger enrichment of nitrogen metabolism pathways. KEGG pathway enrichment analysis revealed that “phenylpropanoid biosynthesis” and “flavonoid biosynthesis” were significantly enriched in both roots and shoots, whereas “nitrogen metabolism”, was preferentially enriched in shoots and “ABC transporters,” and “fatty acid elongation” was preferentially enriched in roots. Among the hub genes identified by WGCNA in shoots and roots, five genes involved in the GS-GOGAT nitrogen assimilation pathway—*SORBI_3003G258800*, *SORBI_3003G306700*, *SORBI_3003G234200*, *SORBI_3001G451500*, and *SORBI_3001G116400*—were significantly associated with nitrogen-related phenotypes. Haplotype analysis of a natural population of 232 sorghum accessions revealed that *SORBI_3001G116400*, which encodes GOGAT, exhibited the strongest haplotype effects on phenotypic traits. The A allele (*Hap1*) was significantly associated with higher plant height, SPAD value, grain number, nitrogen accumulation, and biomass under LN stress, suggesting that it may represent a favorable allele potentially contributing to nitrogen use efficiency.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy16151498/s1>, Figure S1: GO enrichment of DEGs in shoots and roots under LN stress. (A) GO enrichment terms of all DEGs. (B) GO enrichment terms of 500 hub genes; Figure S2: KEGG enrichment analysis of differentially expressed genes; Table S1: List of 232 sorghum accessions and their phenotypic traits; Table S2: Quality statistics of transcriptome; Table S3: DEGs of LN-SS vs. LN-TS; Table S4: DEGs of NN-SS vs. NN-TS; Table S5: DEGs of LN-TS vs. NN-TS; Table S6: DEGs of LN-SS vs. NN-SS; Table S7: DEGs of LN-SR vs. LN-TR; Table S8: DEGs of NN-SR vs. NN-TR; Table S9: DEGs of LN-TR vs. NN-TR; Table S10: DEGs of LN-SR vs. NN-SR; Table S11. All DEGs of 8 compasion (before filtering); Table S12: Filtered DEGs used for heatmap; Table S13: The top 30 GO term of DEGs; Table S14: The top 30 GO term of hub genes; Table S15: Genes

in significantly associated modules in shoot; Table S16: Genes in significantly associated modules in root; Table S17: Expression levels of the 500 hub genes; Table S18: KEGG terms of the 500 hub genes; Table S19: Haplotype associations of candidate genes with all measured phenotypic traits.

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Conflicts 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.

Abbreviations

LN: low nitrogen; NUE: nitrogen use efficiency; NN: normal nitrogen; *OsHHO3*: *Oryza sativa* HRS1 HOMOLOG3; *OsNAC6*: *Oryza sativa* NAC domain-containing protein 6; *OsNAC42*: *Oryza sativa* NAC transcription factor 42; *OsNPF6.1*: *Oryza sativa* nitrate transporter/peptide transporter family 6.1; T accession: LN-tolerant accession; S accession: the LN-sensitive accession; WGCNA: Weighted Gene Co-expression Network Analysis; SPAD: chlorophyll content; NRC: the nitrogen response coefficient; ANOVA: Two-way analysis of variance; LN-SS: S accession shoots under LN; LN-TS: T accession shoots under LN; NN-SS: S accession shoots under NN; NN-TS: T accession shoots under NN; LN-SR: S accession roots under LN; LN-TR: T accession roots under LN; NN-SR: S accession roots under NN; NN-TR: T accession roots under NN; FPKM: fragments per kilobase of transcript per million mapped reads; DEGs: differentially expressed genes; GO: #Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; SNPs: single-nucleotide polymorphisms; VCF: variant call format.

References

1. Paterson, A.H.; Bowers, J.E.; Bruggmann, R.; Dubchak, I.; Grimwood, J.; Gundlach, H.; Haberer, G.; Hellsten, U.; Mitros, T.; Poliakov, A. The sorghum bicolor genome and the diversification of grasses. *Nature* **2009**, *457*, 551–556. [[CrossRef](#)] [[PubMed](#)]
2. Prasad, V.B.R.; Govindaraj, M.; Djanaguiraman, M.; Djalovic, I.; Shailani, A.; Rawat, N.; Singla-Pareek, S.L.; Pareek, A.; Prasad, P.V.V. Drought and high temperature stress in sorghum: Physiological, genetic, and molecular insights and breeding approaches. *Int. J. Mol. Sci.* **2021**, *22*, 9826. [[CrossRef](#)] [[PubMed](#)]
3. Chen, V.; Tang, Y.; Zhang, L.; Zhou, Y.; Yu, M.; Shi, G.; Wang, X.; Li, Y.; Gao, S.; Lu, X.; et al. The green revolution of Chinese grain hybrid sorghum. *Sci. Agric. Sin.* **2025**, *58*, 1494–1507. [[CrossRef](#)]
4. Meng, X.; Li, L.; Qian, Q.; Jiang, L.; Kong, Z. Hybrid sorghum breeding in China: A historical review and perspectives. *J. Integr. Plant Biol.* **2025**. [[CrossRef](#)] [[PubMed](#)]
5. Guo, H.; Pu, X.; Jia, H.; Zhou, Y.; Ye, G.; Yang, Y.; Na, T.; Wang, J. Transcriptome analysis reveals multiple effects of nitrogen accumulation and metabolism in the roots, shoots, and leaves of potato (*Solanum tuberosum* L.). *BMC Plant Biol.* **2022**, *22*, 282. [[CrossRef](#)] [[PubMed](#)]
6. Liu, Y.; Wang, H.; Jiang, Z.; Wang, W.; Xu, R.; Wang, Q.; Zhang, Z.; Li, A.; Liang, Y.; Ou, S. Genomic basis of geographical adaptation to soil nitrogen in rice. *Nature* **2021**, *590*, 600–605. [[CrossRef](#)] [[PubMed](#)]

7. Chen, X.; Cui, Z.; Fan, M.; Vitousek, P.; Zhao, M.; Ma, W.; Wang, Z.; Zhang, W.; Zhang, W.; Yan, X.; et al. Producing more grain with lower environmental costs. *Nature* **2014**, *514*, 486–489. [[CrossRef](#)] [[PubMed](#)]
8. Liu, X.; Zhang, F. Nitrogen fertilizer induced greenhouse gas emissions in China. *Curr. Opin. Environ. Sustain.* **2011**, *3*, 407–413. [[CrossRef](#)]
9. Ju, X.; Kou, C.; Zhang, F.; Christie, P. Nitrogen balance and groundwater nitrate contamination: Comparison among three intensive cropping systems on the North China Plain. *Environ. Pollut.* **2006**, *143*, 117–125. [[CrossRef](#)] [[PubMed](#)]
10. Tilman, D.; Cassman, K.G.; Matson, P.A.; Naylor, R.; Polasky, S. Agricultural sustainability and intensive production practices. *Nature* **2002**, *418*, 671–677. [[CrossRef](#)] [[PubMed](#)]
11. Du, Q.; Yang, J.; Shah, S.M.S.; Yang, R.; Yu, J.; Li, W. Comparative transcriptome analysis of different nitrogen responses in low-nitrogen sensitive and tolerant maize genotypes. *J. Integr. Agric.* **2021**, *8*, 2043–2055. [[CrossRef](#)]
12. Mailun, Y.; Yasuhito, S.; Shuichi, Y. Down-regulation of the rice HRS1 HOMOLOG3 transcriptional repressor gene due to N deficiency directly co-activates ammonium and phosphate transporter genes. *J. Exp. Bot.* **2024**, *76*, 461–477. [[CrossRef](#)] [[PubMed](#)]
13. Li, Y.; Tong, L.; Lin, Y.; Su, Z.; Bao, H.; Wang, F.; Liu, J.; Qu, J.; Hu, S.; Sun, J.; et al. Investigation of low nitrogen tolerance of ZmCCT10 in maize. *Sci. Agric. Sin.* **2023**, *56*, 1035–1044. [[CrossRef](#)]
14. Goche, T.; Mavindidze, P.; Zenda, T. Advances in functional genomics for exploring abiotic stress tolerance mechanisms in cereals. *Plants* **2025**, *14*, 2459. [[CrossRef](#)] [[PubMed](#)]
15. Guo, T.; Yang, J.; Li, D.; Sun, K.; Luo, L.; Xiao, W.; Wang, J.; Liu, Y.; Wang, S.; Wang, H.; et al. Integrating GWAS, QTL, mapping and RNA-seq to identify candidate genes for seed vigor in rice (*Oryza sativa* L.). *Mol. Breed.* **2019**, *39*, 87. [[CrossRef](#)]
16. Yan, H.; Shi, H.; Hu, C.; Luo, M.; Xu, C.; Wang, S.; Li, N.; Tang, W.; Zhou, Y.; Wang, C.; et al. Transcriptome differences in response mechanisms to low-nitrogen stress in two wheat varieties. *Int. J. Mol. Sci.* **2021**, *22*, 12278. [[CrossRef](#)] [[PubMed](#)]
17. Tang, W.; Ye, J.; Yao, X.; Zhao, P.; Xuan, W.; Tian, Y.; Zhang, Y.; Xu, S.; An, H.; Chen, G.; et al. Genome-wide associated study identifies NAC42-activated nitrate transporter conferring high nitrogen use efficiency in rice. *Nat. Commun.* **2019**, *10*, 5279. [[CrossRef](#)] [[PubMed](#)]
18. Fan, F.; Wang, Y.; Cheng, X.; Liu, R.; Wang, Y.; Ju, L.; Yan, H.; Niu, H.; Lv, X.; Chu, J.; et al. Genome-wide association study and transcriptome analysis identify QTL and candidate genes involved in nitrogen response mechanisms in sorghum. *Agronomy* **2025**, *15*, 2250. [[CrossRef](#)]
19. Gautam, V.P.; Mishra, S.; Ahmed, H. Comparison of total nitrogen estimation by Kjeldahl method and CHNS analyzer in dry tropical grassland. *Int. J. Plant Environ.* **2023**, *9*, 180–182. [[CrossRef](#)]
20. Grzybowski, M.W.; Zwiener, M.; Jin, H.; Wijewardane, N.K.; Atefi, A.; Naldrett, M.J.; Alvarez, S.; Ge, Y.; Schnable, J. Variation in morpho-physiological and metabolic responses to low nitrogen stress across the sorghum association panel. *BMC Plant Biol.* **2022**, *22*, 433. [[CrossRef](#)] [[PubMed](#)]
21. Tu, M.; Du, C.; Yu, B.; Wang, G.; Deng, Y.; Wang, Y.; Chen, M.; Chang, J.; Yang, G.; He, G.; et al. Current advances in the molecular regulation of abiotic stress tolerance in sorghum via transcriptomic, proteomic, and metabolomic approaches. *Front. Plant Sci.* **2023**, *14*, 1147328. [[CrossRef](#)] [[PubMed](#)]
22. Liu, C.; Gu, W.; Liu, C.; Shi, X.; Li, B.; Zhou, Y. Comparative phenotypic and transcriptomic analysis reveals genotypic differences in nitrogen use efficiency in sorghum. *Plant Physiol. Biochem.* **2024**, *215*, 109028. [[CrossRef](#)] [[PubMed](#)]
23. Jiang, S.; Sun, J.; Tian, Z.; Hu, H.; Michel, E.; Gao, J.; Jiang, D.; Cao, W.; Dai, T. Root extension and nitrate transporter up-regulation induced by nitrogen deficiency improves nitrogen status and plant growth at the seedling stage of winter wheat (*Triticum aestivum* L.). *Environ. Exp. Bot.* **2017**, *141*, 28–40. [[CrossRef](#)]
24. Zhou, Y.; Huang, J.; Li, Z.; Wang, Q.; Li, Y.; Zhang, Y.; Zhang, X.; Wu, Y. Optimal nitrogen management for high yield and N use efficiency of ratoon sorghum. *Sci. Rep.* **2024**, *14*, 119566. [[CrossRef](#)] [[PubMed](#)]
25. Rodriguez, I.M.; Lacasa, J.; Lemaire, G.; Zhao, B.; Ata-Ul-Karim, S.T.; Ciampitti, I.A. Crop nitrogen status and yield formation: A cross-species comparison for maize, rice, and wheat field crops. *Field Crop. Res.* **2024**, *316*, 109515. [[CrossRef](#)]
26. Yang, N.; Li, S.; Wang, S.; Li, Q.; Xu, F.; Shi, L.; Wang, C.; Ye, X.; Cai, H.; Ding, G. Dynamic transcriptome analysis indicates extensive and discrepant transcriptomic reprogramming of two rapeseed genotypes with contrasting NUE in response to nitrogen deficiency. *Plant Soil* **2020**, *456*, 369–390. [[CrossRef](#)]
27. Xin, W.; Zhang, L.; Zhang, W.; Gao, J.; Yi, J.; Zhen, X.; Li, Z.; Zhao, Y.; Peng, C.; Zhao, C. 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.* **2019**, *20*, 2349. [[CrossRef](#)] [[PubMed](#)]
28. Gelli, M.; Duo, Y.; Konda, A.R.; Zhang, C.; Holding, D.; Dweikat, I. Identification of differentially expressed genes between sorghum genotypes with contrasting nitrogen stress tolerance by genome-wide transcriptional profiling. *BMC Genom.* **2014**, *15*, 179. [[CrossRef](#)] [[PubMed](#)]
29. Sevanthi, A.M.; Sinha, S.K.; Sureshkumar, V.; Rani, M.; Saini, M.R.; Kumari, S.; Kaushik, M.; Prakash, C.; Venkatesh, K.; Singh, G.P.; et al. Integration of dual stress transcriptomes and major QTLs from a pair of genotypes contrasting for drought and chronic nitrogen starvation identifies key stress responsive genes in rice. *Rice* **2021**, *14*, 49. [[CrossRef](#)] [[PubMed](#)]

30. Zhang, Z.; Gao, S.; Chu, C. Improvement of nutrient use efficiency in rice: Current toolbox and future perspectives. *Theor. Appl. Genet.* **2020**, *133*, 1365–1384. [[CrossRef](#)] [[PubMed](#)]
31. Ramarosan, M.-L.; Koutouan, C.; Helesbeux, J.-J.; Le Clerc, V.; Hamama, L.; Geoffriau, E.; Briard, M. Role of Phenylpropanoids and Flavonoids in Plant Resistance to Pests and Diseases. *Molecules* **2022**, *27*, 8371. [[CrossRef](#)] [[PubMed](#)]
32. Xu, W.; Dou, Y.; Geng, H.; Fu, J.; Dan, Z.; Liang, T.; Cheng, M.; Zhao, W.; Zeng, Y.; Hu, Z.; et al. OsGRP3 enhances drought resistance by altering phenylpropanoid biosynthesis pathway in rice (*Oryza sativa* L.). *Int. J. Mol. Sci.* **2022**, *23*, 7045. [[CrossRef](#)] [[PubMed](#)]
33. Li, P.; Luo, T.; Pu, X.; Zhou, Y.; Yu, J.; Liu, L. Plant transporters: Roles in stress responses and effects on growth and development. *Plant Growth Regul.* **2021**, *93*, 253–266. [[CrossRef](#)]
34. Gao, Y.; Xu, Z.; Zhang, L.; Li, S.; Wang, S.; Yang, H.; Liu, X.; Zeng, D.; Liu, Q.; Qian, Q.; et al. *Myb61* is regulated by *grf4* and promotes nitrogen utilization and biomass production in rice. *Nat. Commun.* **2020**, *11*, 5219. [[CrossRef](#)] [[PubMed](#)]
35. Li, W.; Yang, S.; Liu, H.; Cao, Z.; Xu, F.; Ning, C.; Zhang, Q.; Wang, D.; Tang, H. Identification of key lncRNAs and mRNAs associated with intramuscular fat in pig via WGCNA. *BMC Genom.* **2025**, *26*, 233. [[CrossRef](#)] [[PubMed](#)]
36. Zhou, X.; Yu, S.; Tang, L.; Liu, X.; Lei, Q.; Yu, Y.; Xiong, Y.; Ye, D.; Wang, W.; Cao, K.; et al. Transcriptomic analysis reveals the role of the molybdate transporter2 in mediating nitrate stress tolerance in tomato seedlings. *BMC Plant Biol.* **2025**, *25*, 118. [[CrossRef](#)] [[PubMed](#)]
37. Wang, Y.; Li, P.; Zhu, Y.; Shang, Y.; Wu, Z.; Tao, Y.; Wang, H.; Li, D.; Zhang, C. Transcriptome profiling reveals the gene network responding to low nitrogen stress in wheat. *Plants* **2024**, *13*, 371. [[CrossRef](#)] [[PubMed](#)]
38. Bernard, S.M.; Habash, D.Z. The importance of cytosolic glutamine synthetase in nitrogen assimilation and recycling. *New Phytol.* **2009**, *182*, 608–620. [[CrossRef](#)] [[PubMed](#)]
39. Fortunato, S.; Nigro, D.; Lasorella, C.; Marcotuli, I.; Gadaleta, A.; Pinto, M.C. The role of glutamine synthetase (GS) and glutamate synthase (GOGAT) in the improvement of nitrogen use efficiency in cereals. *Biomolecules* **2023**, *13*, 1771. [[CrossRef](#)] [[PubMed](#)]
40. Lee, S.; Marmagne, A.; Park, J.; Fabien, C.; Yim, Y.; Kim, S.; Kim, T.H.; Lim, P.O.; Masclaux-Daubresse, C.; Nam, H.G. Concurrent activation of *OsAMT1;2* and *OsGOGAT1* in rice leads to enhanced nitrogen use efficiency under nitrogen limitation. *Plant J.* **2020**, *102*, 1052–1068. [[CrossRef](#)] [[PubMed](#)]

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