

Transcription strategies related to photosynthesis and nitrogen metabolism of wheat in response to nitrogen deficiency

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Research article

Keywords: Nitrogen deficiency; Nitrogen metabolism; Photosynthesis; Transcriptome; Wheat

Posted Date: July 28th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-41826/v1>

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Version of Record: A version of this preprint was published on October 1st, 2020. See the published version at <https://doi.org/10.1186/s12870-020-02662-3>.

Abstract

Background It is a great challenge to reduce nitrogen application without yield reduction in agricultural production. Screening response gene related to important pathways will be helpful to understand the physiology, metabolism, and morphology response of wheat (*Triticum aestivum*) under nitrogen deficiency condition.

Results We conducted a hydroponic experiment with two nitrogen levels, which were complete nutrient solution (N1) and nutrient solution without nitrogen (N0). The results showed that the wheat phenotype were greatly changed under nitrogen deficiency, e.g., the decreased crop height, leaf area, root volume, photosynthetic rate, crop weight, and the increased root length, root surface area and root/shoot ratio. After a comprehensive analysis of the phenotype, transcriptome, GO pathways and KEGG pathways of wheat under nitrogen deficiency, we found that, the up-regulated Exp (24 genes) and Nrt (9 genes) families members were the positive response related to the increase of nitrogen absorption; the down-regulated Pet (3 genes), Psb (8 genes), Nar (3 genes) and Nir (1 genes) were related to the limitation of photosynthesis and nitrogen metabolism.

Conclusions This study provides 48 reference genes for improving photosynthesis and nitrogen metabolism of wheat under nitrogen deficiency, which can provide molecular markers for gene editing breeding.

Background

Many countries in the world, such as China, are facing the problems of excessive nitrogen application and low nitrogen utilization efficiency in winter wheat production[1]. Excessive application of nitrogen fertilizer is the main reason for the low nitrogen utilization efficiency in wheat [2]. At the same time, it causes environmental pollution and becomes a threat of the sustainable development of agriculture. How to reduce nitrogen application on the premise of ensuring crop yield is an urgent problem to be solved. To tap the potential of wheat physiology, metabolism and morphology using molecular breeding methods is an effective way in maintaining yield and improving nitrogen use efficiency under reduced nitrogen application [3, 4].

Under the stress conditions, such as shading, drought or nutrition deficiency, to improve crop physiological, metabolism, and architecture of root and canopy are important to maintain yield and resource utilization efficiency[5–7]. Reducing nitrogen application can modify the root morphology, and the improvement of root architecture can increase the nitrogen absorption capacity and nitrogen use efficiency under reducing nitrogen application [8]. However, under the condition of nutrient deficiency, photosynthesis and some metabolic processes often become worse [9, 10]. It is very important to find evidence related to crop physiological and metabolism at transcriptional level, so as to improve wheat adaptability under nitrogen deficiency.

Under the condition of nitrogen deficiency, the gene expression of plants changed. Previous study showed that the up-regulated expression of HvNiR1, HvGS2, HvGLU2 in shoots, the down-regulated expression of HvASN1 in shoots, and the up-regulated expression of HvGLU2 in roots, could benefit adaptation to nitrogen deficiency in barley under low-nitrogen condition [11]. The up-regulated expression of alternative oxidase (AOX) consumes excess sugars, and then the induced AOX balanced the carbon and nitrogen under nitrogen deficiency [12–14]. GmCZ-SOD1 gene was highly induced in soybean root under nitrogen deficiency [2]. The transcriptome showed 1799 maize differentially expressed genes (DEGs) involving multiple pathways under nitrogen deficiency [11]. Although large amount of studies on the transcription level have been carried out, but there is still a lack of study on understanding which genes are associated with crop physiological and metabolism of wheat under nitrogen deficiency. It is urgently to found genes related to physiology and metabolism of wheat under nitrogen deficiency [15].

We therefore conducted experiment which aimed to: (i) explore the physiological, metabolic and morphological changes of wheat under nitrogen deficiency condition; (ii) screen the differentially expressed genes (DEGs) from wheat transcriptome under nitrogen deficiency; (iii) after comprehensive analysis of transcription, metabolic pathway and phenotype of important physiological and metabolic processes, we try to find out the potential genes which can be promote wheat growth under nitrogen deficiency.

Result

Morphological and physiological changes of wheat under nitrogen deficiency

The morphological and physiological changes of wheat were shown in Fig. 1. The crop height of N0 was 0.75 times lower than that of N1; the leaf area per plant of N0 was 0.70 times lower than that of N1 (6.77 cm²); the specific leaf area of N0 and N1 had no significant difference; the net photosynthetic rate (Pn) of N0 was 0.47 times lower than that of N1; the shoot fresh wight of N0 was 0.61 times lower than that of N1.

The root length per plant of N0 was 1.61 times higher than that of N1; the root volume per plant of N0 was 0.61 times lower than that of N1; the root surface area per plant of N0 was 1.04 times higher than that of N1; the root fresh wight of N0 was 0.82 times lower than that of N1. The root shoot ratio of N0 was 1.36 times higher than that of N1.

Global analysis of RNA-seq data resulting from nitrogen deficiency

The number of genes expressed in different regions were calculated, and stacked histogram was drawn (Fig. 2a). There were 72487–78729 genes expressed in wheat shoots, and 17116–22418 genes had Fragments Per Kilobase of transcript per Million fragments mapped (FPKM) values greater than 1. There were 63273–64413 genes expressed in wheat roots, and 27785–29233 genes had FPKM values greater than 1.

Principal component analysis (PCA) was applied to explore the relationship between samples by locating the samples at different dimensions (Fig. 2b). The closer the clustering distance was, the more similar the samples were. The results of PCA analysis showed that PCA1 reflected the difference of root and shoot, accounting for 99.41% of the total variation; PCA2 reflected shoot transcription difference under N0 and N1, accounting for 0.21% of the total variation; PCA3 reflected root transcription difference under N0 and N1, accounting for 0.11% of the total variation.

The volcano plot (Fig. 3ab) and cluster map (Fig. 3 cd) of P value and \log_2 FC were applied to screen the differentially expressed genes (DEGs) under nitrogen deficiency treatment (N0) as compared to control (N1). There were 3949 DEGs in shoot, 1535 of them were up-regulated, 2414 of them were down-regulated. There were 3911 DEGs were screened in roots, 1236 of them were up-regulated, 2675 were down-regulated (Fig. 3e). The venn map (Fig. 3f) revealed that 1535 DEGs were up-regulated and 2414 were down regulated in both shoot and root. There were 372 DEGs differentially expressed in roots and shoot.

Functional analysis of DEGs identified under nitrogen deficiency

The gene ontology classification (Fig. 4) enriched 1205 up-regulated genes and 1888 down-regulated genes in shoots, while 961 up-regulated genes and 1883 down-regulated genes in roots. The enriched genes were classified into 3 major classes and 64 sub-classes. Some genes belongs to the two or more classifications. The largest four classifications (more than 980 DEGs) were cellular process, metabolic process, binding, and catalytic activity (Table 1).

Table 1

The number of differentially expressed genes (DEGs) in the four pathways with the largest number of genes under nitrogen deficiency

	GO_classify 1	GO_classify 2	DEGs-Up	DEGs-Down
Shoot	Biological process	Cellular process	385	947
	Biological process	Metabolic process	498	1029
	Molecular function	Binding	672	1031
	Molecular function	Catalytic activity	560	854
Root	Biological process	Cellular process	312	669
	Biological process	Metabolic process	390	891
	Molecular function	Binding	527	893
	Molecular function	Catalytic activity	429	936
Analysis of gene families associated with cellular process				
Expansin family members were mainly belonged to the cellular process in gene ontology (GO) classification. Under nitrogen deficiency, there were 3 DEGs (Fig. 5) of wheat Expansin family in shoot, including TreasCS2B02G411700 (up-regulated), TreasCS1A02G30020 (down-regulated) and TreasCS1B02G310300 (down-regulated); there were 6 down-regulated wheat Expansin family members (TreasCS6A02G307900 and so on) and 24 up-regulated wheat Expansin family members (TreasCS5B02G528400 and so on) in root.				

Analysis of gene families associated with metabolic process

Pet and Psb family members were important proteins in photosystem in wheat shoot, which mainly belonged to metabolic process in gene ontology (GO) classification. Under nitrogen deficiency (N0), there were 3 down-regulated DEGs (Fig. 6) of wheat Pet family (TreasCS7A02G325500 and so on); there were 8 down-regulated wheat Psb family members (TreasCS3D02G523300 and so on) and 1 up-regulated wheat Psb family members (TreasCS6B02G412100).

Nar and Nrt family members had functions related to nitrogen metabolism, and mainly belonged to metabolic process in gene ontology (GO) classification. Under nitrogen deficiency, there were 3 down-regulated DEGs (Fig. 7) of wheat Nar family (TreasCS6A02G326200, TreasCS6B02G356800, and TreasCS6D02G306000) in both shoot and root of wheat; but there were other 2 up-regulated wheat Nar family members (TreasCS6A02G210000 and TreasCS6D02G193100) in root; there were 9 up-regulated DEGs of wheat Nrt family in root (TreasCS6A02G031100).

Discussion

The evidence of wheat morphology, metabolism and physiology changes can be found from transcriptome[19–21]. In the photosynthesis pathway (Fig. 8a), the protein of Pet and Psb gene families were important parts in cytochrome b6/f complex, photosynthetic electron transport and photosystem II[22–26]. The former viewpoint is that the inhibition of these proteins will lead to the decline of photosynthetic system performance [27, 28]. In our research, the down-regulated expression of these two family members under nitrogen deficiency of wheat caused the inhibited photosynthetic electron transport and Photosystem II pathway, thus reducing photosynthetic rate and energy supply.

In the pathway of nitrogen metabolism (Fig. 8bd), Nar (nitrate reductase) family members participated in the pathway of nitrate-N reduction to nitrite-N [29, 30]; Nir (Nitrite reductase) gene family members participated in the pathway of nitrite-N reduction to ammonium-N [31, 32]; Nrt family participated in the process of nitrogen transport from extracellular to intracellular [33]. Moreover, the Nrt family was involved in root growth, flowering time and many other physiological processes by regulating transcriptional level, hormone and nitrate signaling[34–37]. The up-regulated levels of Nir genes and Nrt genes can be regarded as promoting the adaptability of crops to nutrient uptake[38–40]. Under the condition of nitrogen deficiency, the expression of Nar and Nir genes in the nitrogen metabolism pathway of wheat shoot were down-regulated, and the expression of Nir gene in root was down-regulated, which inhibited the nitrogen metabolism pathway in both shoot and root; while the expression of Nrt family in root was up-regulated, which might accelerate the movement of extracellular nitrogen into cells.

In the pathway of extracellular region (Fig. 8c), expansin gene family can increase the extensibility of cell wall[41–43]. Previous studies have shown that overexpression of expansin family members can cause the changes in crop morphology and improve the crop adaptability of stress resistance or low nutrition conditions [44, 45]. For example, the up-regulated of the TaEXPB23 changed root system architecture of transgenic tobacco so as to improve the low phosphorus adaptability [8]. In this study, under the condition of nitrogen deficiency, the expression of expansin gene family members in root was up-regulated, which may be the reason for the increase of root length and root surface area. The increased root length and root surface area were helpful for nitrogen absorption under nitrogen deficiency.

When the external environment changes, the transcription level of crops will respond quickly [42, 46], and then affect the protein level and metabolism process, resulting in matter accumulation and morphological changes [8, 47]. Some of the above responses may improve the adaptability of crops to the environment. Under the condition of nitrogen deficiency, the up-regulated expression of expansin and Nrt families in wheat root, which could increase the root surface area, can be regarded as the adaptive strategy of wheat to promote nitrogen absorption (Fig. 9). The expression of Pet, Psb, Nar, Nir family were down-regulated, which inhibited photosynthesis and nitrogen assimilation, and then negatively affected biomass accumulation, resulting in the decrease of shoot height, leaf area and root volume. By using the genetic engineering, the down-regulated genes in these four families (Pet, Psb, Nar, Nir) can be increased to make up for the short plate of photosynthesis and nitrogen metabolism, so as to improve the matter accumulation and growth condition of crops under nitrogen deficiency.

Conclusion

Under nitrogen deficiency as compared to control, the crop height, leaf area, root volume, photosynthetic rate and crop weight of wheat were decreased, while the root length, root surface area and root/shoot ratio were increased. 3949 (2414 down-regulated, 1535 up-regulated) differentially expressed genes (DEGs) were screened in shoot, while 3911 (2675 down-regulated, 1536 up-regulated) DEGs were screened in roots.

Then, the transcriptome, GO pathways and KEGG pathways of wheat under nitrogen deficiency were analyzed, 24 expansin genes (such as *TreasCS5B02G528400*) and 9 Nrt genes (such as *TreasCS6A02G031100*) were related to the increase of N absorption of wheat; 3 Pet genes (such as *TreasCS7B02G226200*) and 8 Psb genes (such as *TreasCS3D02G523300*) were related to the inhibition of photosynthetic pathway; 3 Nar genes (such as *TreasCS6A02G326200*) and 1 Nir gene (*TreasCS6D02G333900*) were related to the inhibition of nitrogen metabolism pathway.

Methods

Experimental design

The wheat variety Shannong 29, a high yield cultivar which was commonly applied in wheat production in Huang Huai Hai region of China, was used in this research. The seeds of Shannong 29 were obtained from ShanDong Shofine Seed Technology Co., Ltd.. Two nutrient solution with different nitrogen concentrations (NH_4NO_3) were set: complete nutrient solution (N1) with $5 \text{ mmol L}^{-1} \text{NH}_4\text{NO}_3$; nutrient solution without nitrogen (N0) with $0 \text{ mmol L}^{-1} \text{NH}_4\text{NO}_3$ (N0). The formula of nutrient solution was referred to Hoagland solution (N0 treatment without nitrogen addition). The concentrations of other nutrients in each treatment were as follows: $2 \text{ mmol L}^{-1} \text{CaCl}_2$; $1.8 \text{ mmol L}^{-1} \text{KCl}$; $0.2 \text{ mmol L}^{-1} \text{KH}_2\text{PO}_4$; $0.5 \text{ mmol L}^{-1} \text{MgSO}_4$; $0.1 \text{ mmol L}^{-1} \text{FeEDTA}$; $0.5 \mu\text{mol L}^{-1} \text{KI}$; $1 \mu\text{mol L}^{-1} \text{H}_3\text{BO}_3$; $1 \mu\text{mol L}^{-1} \text{MnSO}_4$; $1 \mu\text{mol L}^{-1} \text{ZnSO}_4$; $1 \mu\text{mol L}^{-1} \text{Na}_2\text{MoO}_4$; $0.1 \mu\text{mol L}^{-1} \text{CuSO}_4$; $0.1 \mu\text{mol L}^{-1} \text{CoCl}_2$. The pH was maintained at 6.8 ± 0.3 . The wheat seeds were sterilized with 75% alcohol for 30 seconds, and then washed with sterilized distilled water three times. After sterilizing the seeds of winter wheat, the seedling were cultured to 1 leaf and 1 heart stage. Each treatment contained 100 seedlings and was repeated three repetitions. The seedlings were transplanted to the nutrient solution with different treatments, and fixed by sponges. The seedlings were cultured in an artificial incubator with a cycle of 8 hours dark and 16 hours light and 70% relative humidity.

Experimental measurements

Morphological index

At 3 days after transplanting seedlings into nutrient solution, 10 plants in each treatment (repeated in three repetitions) were sampled for measuring leaf area, plant height, root length, root surface area and

root volume. In addition, another 10 plants were sampled for determining fresh weight of root and shoot.

The method for measuring root length, surface area and volume was followings: artificially rinse the roots, remove impurities and miscellaneous roots, absorb the surface water of the roots, spread the roots in the glass dish of the root scanner (0.24 × 0.32 m), and save the photos as 600 DPI pixels by the root scanner (HP Scanjet 8200; Hewlett-Packard, Palo Alto, CA, USA). The root analysis software (Delta-T Area Meter Type AMB2; Delta-T Devices Ltd., Cambridge, UK) was used for data analysis.

Physiological index

At 3 days after transplanting seedlings into nutrient solution, the net photosynthetic rate (P_n), stomatal conductance (G_s) and intercellular carbon dioxide concentration (C_i) of top leaves were measured by using LI-6400 portable photosynthesizer (LI-COR, USA) with a red-blue light source and a light quantum density of $1400 \mu\text{mol m}^2 \text{s}^{-1}$.

Transcriptome sequencing

1 day after the seedling was moved to the nutrient solution, 20 plants in each repetitions were quickly sampled and divided into roots and shoots, and then, put the samples into liquid nitrogen for quick freezing.

Total RNA was extracted using the mirVana miRNA Isolation Kit (Ambion) following the manufacturer's protocol. RNA integrity was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The samples with RNA Integrity Number (RIN) ≥ 7 were subjected to the subsequent analysis. The libraries were constructed using TruSeq Stranded mRNA LTSample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Then these libraries were sequenced on the Illumina sequencing platform (HiSeqTM 2500 or Illumina HiSeq X Ten) and 125 bp/150 bp paired-end reads were generated.

Data analysis

After obtaining the differentially expressed genes (DEGs), we enriched and analyzed the DEGs with GO [16] and described their functions (combined with the results of GO annotation). The genome and mRNA database used ftp://ftp.ensemblgenomes.org/pub/plants/release-45/fasta/triticum_aestivum/dna/Triticum_aestivum.IWGSC.dna.toplevel.fa.gz. The methods of GO functional enrichment analysis (<http://geneontology.org/>) were: all protein coding genes/transcripts were used as background lists, and differential protein coding genes/transcripts were used as candidate lists screened from background lists. The p-value representing the significant enrichment of GO functional set in differential protein coding genes/transcripts was calculated by hypergeometric distribution test, and then the p-value was tested by Benjamin & Hochberg's multiple tests.

We used KEGG [17, 18] database (<http://www.genome.jp/kegg/>) to analyze the differential protein coding genes (combined with KEGG annotation results), and used the hypergeometric distribution test to calculate the significance of differential gene enrichment in each pathway entry. The calculated results will return a significant P value of enrichment, and a small P value indicated that the differential gene had

been enriched in the pathway. Through the pathway analysis of differential genes, we can find the pathway items that enrich differential genes, and found out which cell pathway changes may be related to the differential protein coding genes of different samples.

The experimental data were represented as the mean value from three replicates. Statistical calculations were performed with SPSS statistical software (version 19.0, SPSS Inc., Chicago, USA). Comparisons among different arrangements were conducted using one way ANOVA and Duncan's multiple range test (DMRT). $P < 0.05$ was considered statistically significant.

Abbreviations

Psb: Photosystem II reaction center protein;

Pet: Cytochrome b6/f complex subunit 4;

Nar: Nitrate reductase;

Nrt: High affinity nitrate transporter;

Exp: Expansin;

Pn: Net photosynthetic rate;

Gs: Stomatal conductance;

Ci: Intercellular carbon dioxide concentration;

DEG: Differentially expressed gene

KEGG: [Kyoto Encyclopedia of Genes and Genomes](#)

GO: Gene ontology

PCA: Principal component analysis

FPKM: Fragments Per Kilobase of transcript per Million fragments mapped

Declarations

Acknowledgements

We thank Xingju Zhang in ShanDong Shofine Seed Technology Co., Ltd. for providing the seeds of Shannong 29.

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

The transcriptome data associated with this article were uploaded for supplementary data. Other datasets are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 31901457), China Postdoctoral Science Foundation (Grant No. 2019M652454), Earmarked fund for National Modern Agro-industry Technology Research System of China (Grant No. CARS-27) and Qingchuang Science and Technology Support Project of Shandong Colleges and Universities (Grant No. 2019KJF020).

Authors' Contributions

LX, MZQ and YCM initiated and designed the experiments. LX, YCM, XL, JWT, and XSZ performed the experiments and collected the data. LX analyzed the data and wrote the manuscript. LX, MZQ and YCM revised the manuscript. All authors read and approved the final manuscript.

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Figures

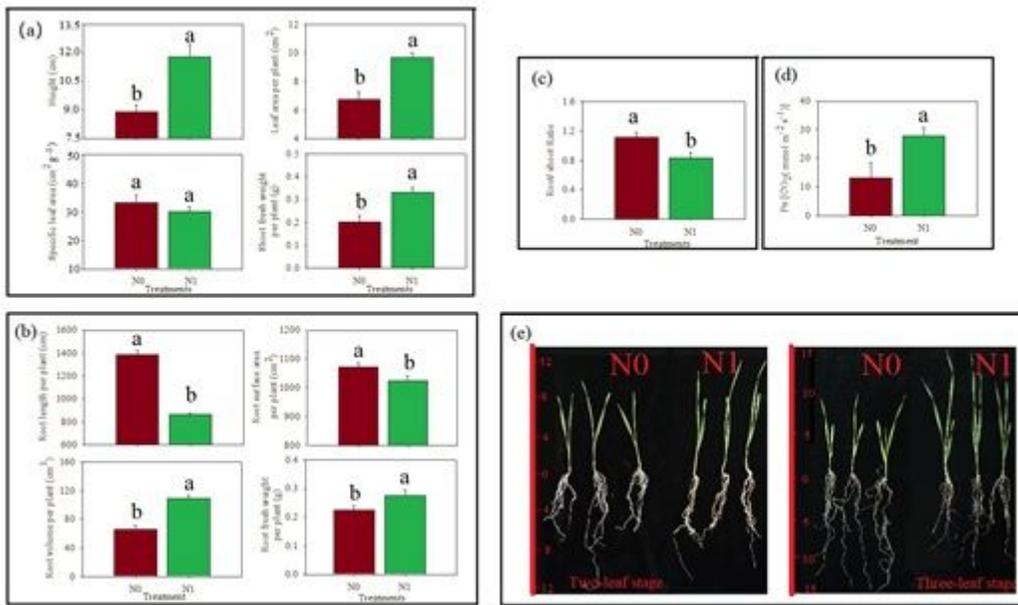


Figure 1

The shoot morphology, including crop height, leaf area per plant, specific leaf area, shoot fresh weight (a); root morphology, including root length per plant, root surface area, root volume per plant, root fresh weight per plant (b); root/shoot ratio (c); net photosynthetic rate (d); phenotype (e) under normal nitrogen treatments (N1), and nitrogen deficiency treatments (N0). The root length per plant, root surface area, root volume per plant, root fresh weight per plant were the sum of all roots of one plant. Significance levels of differences between N0 and N1 were estimated using two-tailed t-test method. Different lowercase letters indicate significant differences.

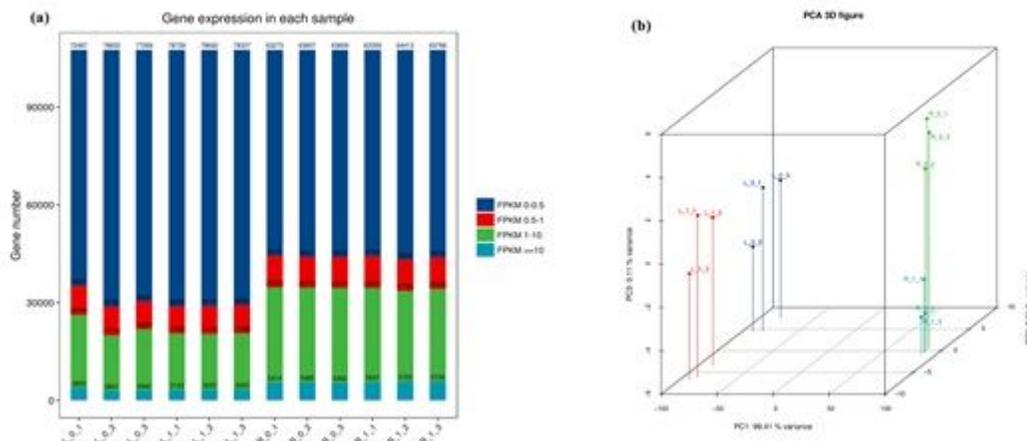


Figure 2

Difference of wheat gene expression value in each sample (a) and PCA component analysis chart (b) under nitrogen deficiency

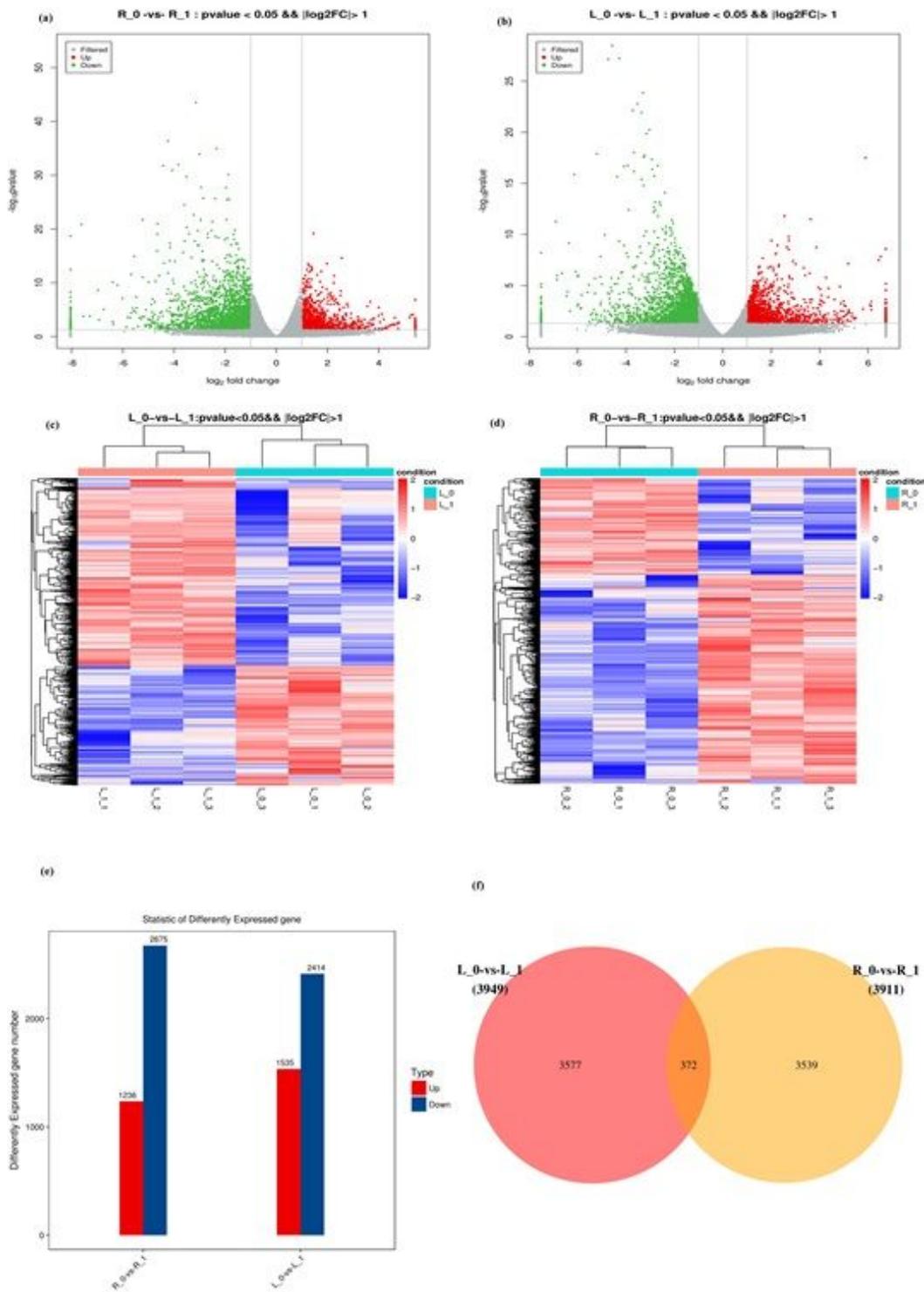


Figure 3

Volcanogram (a for root, b for shoot), cluster map (c for shoot, d for root), number of wheat differentially expressed genes (e) and venn map (f) under nitrogen deficiency. R_0 and L_0 indicated the root and shoot of N0 (nutrition solution without nitrogen), respectively; R_1 and L_1 indicated the root and shoot of N1 (complete nutrition solution), respectively. In volcanogram (a, b), gray points were the genes with non significant difference, red and green points were the genes with significant difference; X axis was the

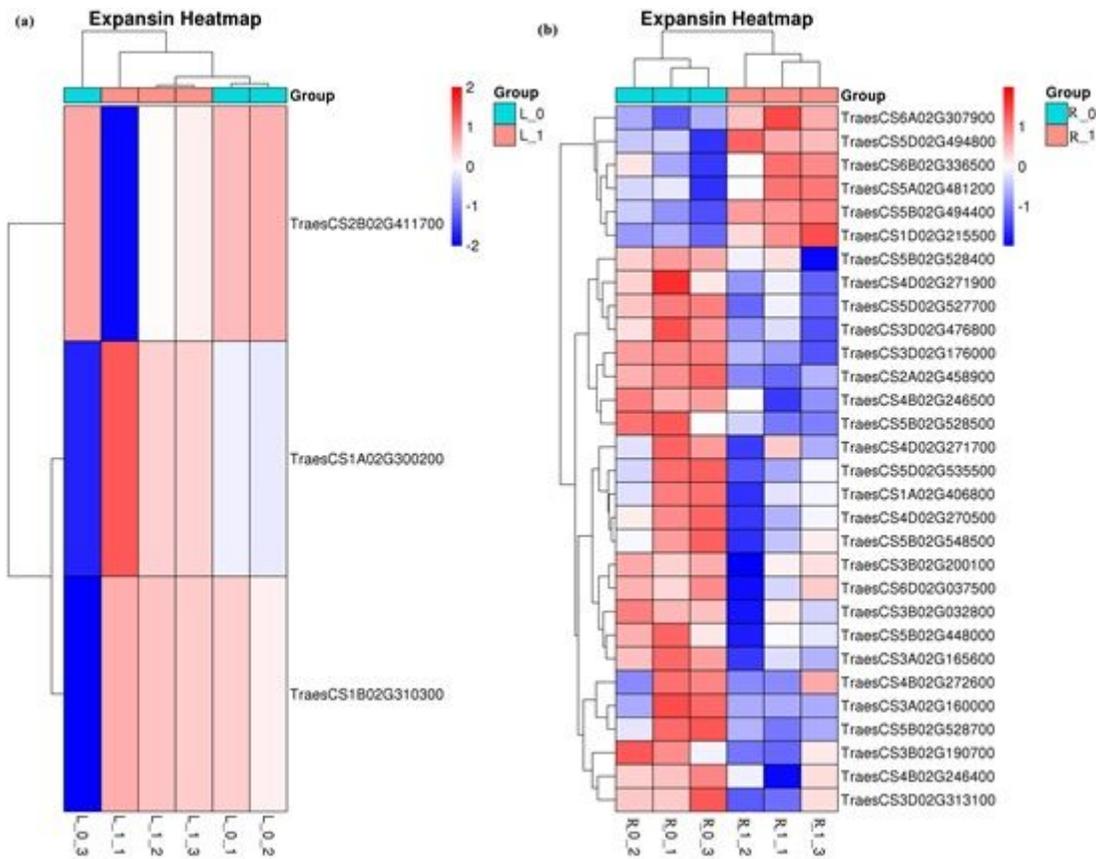


Figure 5

The heatmaps of differentially expressed genes (DEGs) of wheat Expansin family in shoot (a) and root (b) under nitrogen deficiency. The DEGs were selected by $P \text{ value} \leq 0.05$ and $-1 \leq \log_2 \text{FC} \leq 1$. R_0 and L_0 indicated the root and shoot of N0 (nutrition solution without nitrogen), respectively; R_1 and L_1 indicated the root and shoot of N1 (complete nutrition solution), respectively.

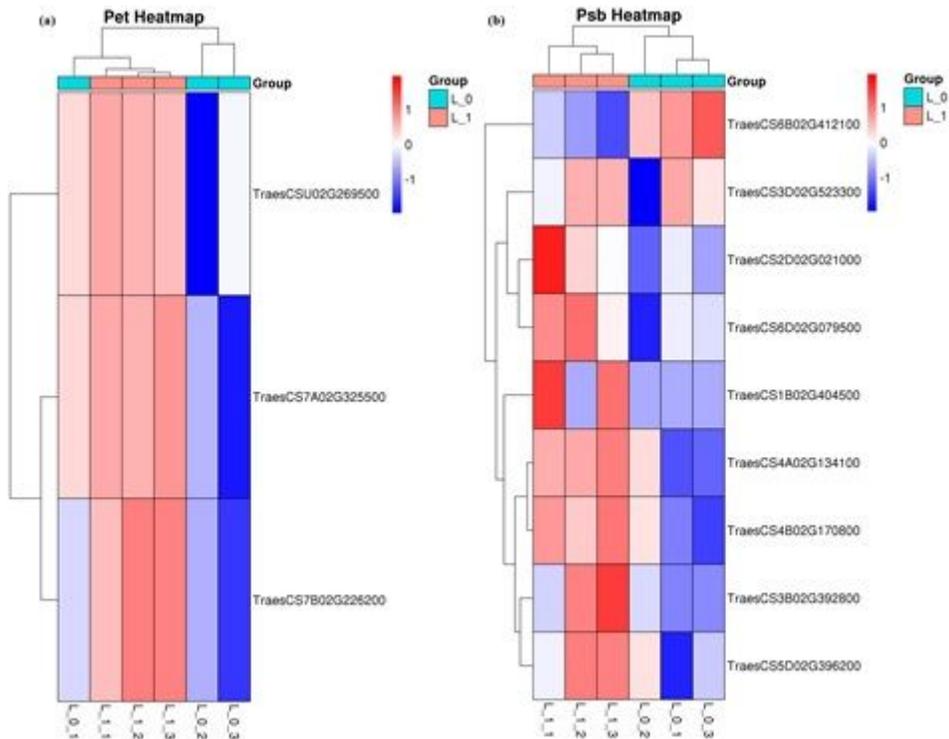


Figure 6

The heatmaps of differentially expressed genes (DEGs) of wheat Pet and Psb family in shoot under nitrogen deficiency. The DEGs were selected by $P \text{ value} \leq 0.05$ and $-1 \leq \log_2 FC \leq 1$. L_0 indicated the shoot of N0 (nutrition solution without nitrogen); L_1 indicated the shoot of N1 (complete nutrition solution).

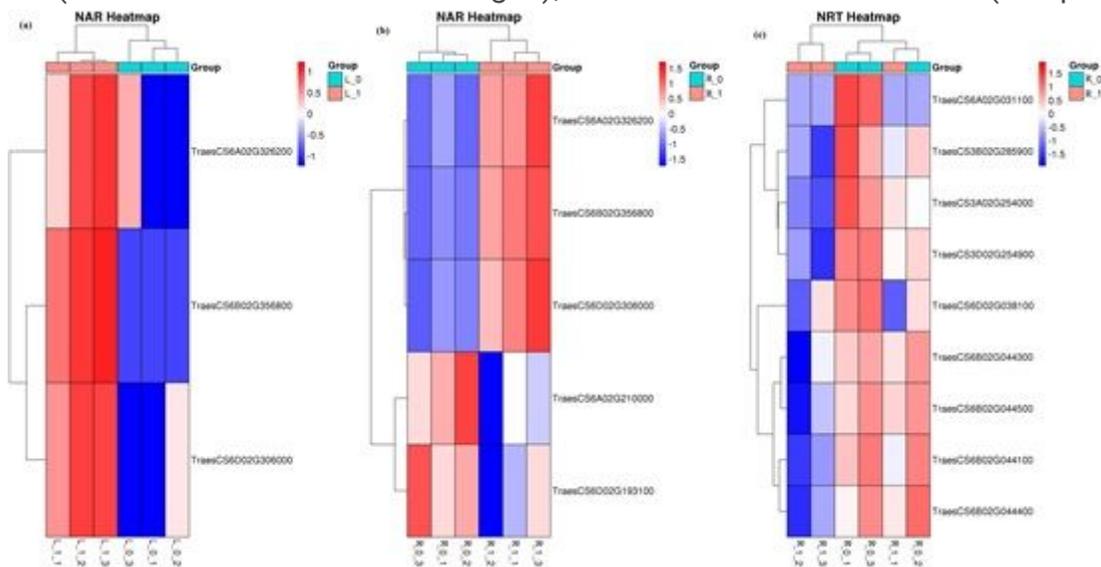


Figure 7

The heatmaps of differentially expressed genes (DEGs) in wheat Nar (a) in shoot, Nar in root (b) and Nrt in root (c) under nitrogen deficiency. The DEGs were selected by $P \text{ value} \leq 0.05$ and $-1 \leq \log_2 FC \leq 1$. R_0 and L_0 indicated the root and shoot of N0 (nutrition solution without nitrogen), respectively; R_1 and L_1 indicated the root and shoot of N1 (complete nutrition solution), respectively.

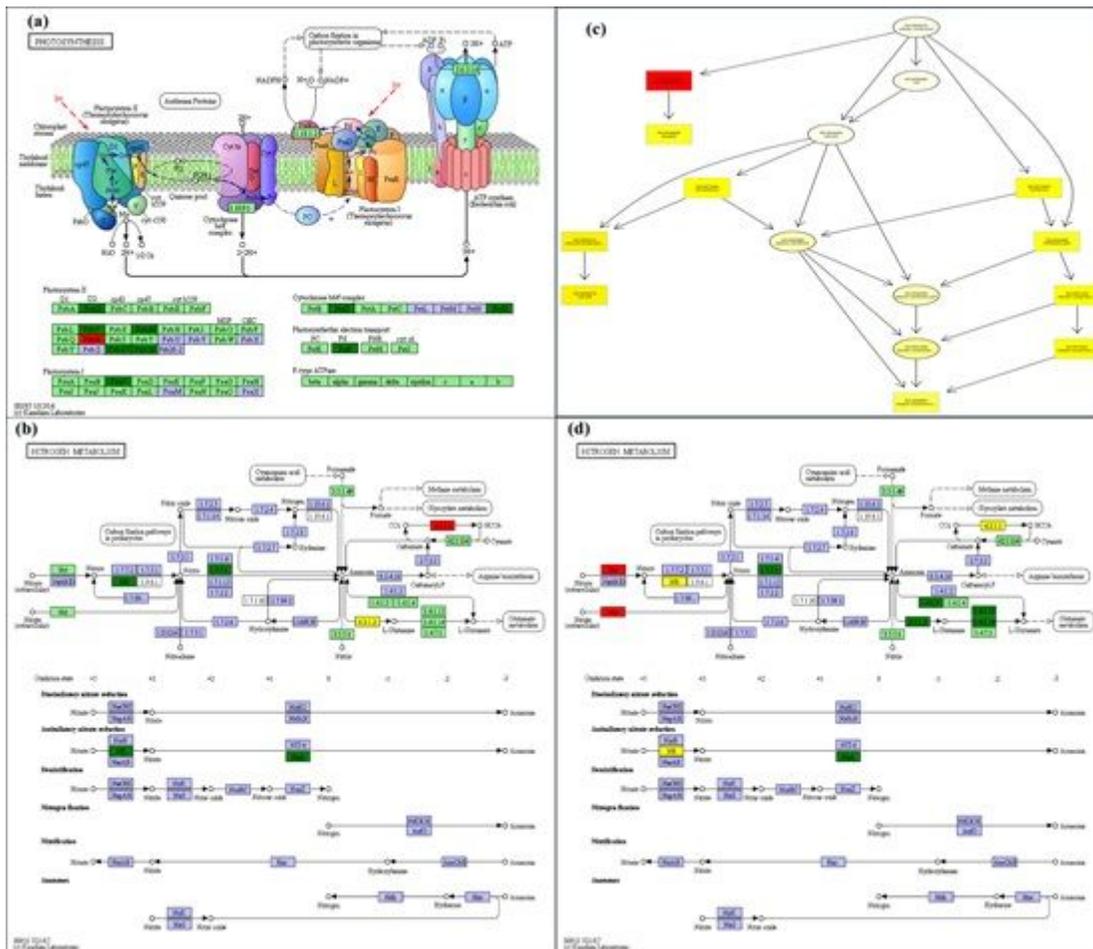


Figure 8

The photosynthesis pathway by KEGG analysis (a) and nitrogen metabolism pathway by KEGG analysis in shoot (b); the extracellular pathway by GO analysis (c) and nitrogen metabolism pathway by KEGG analysis (d) in root. The red frame indicated the up-regulated genes; the green frame indicated the down-regulated genes.

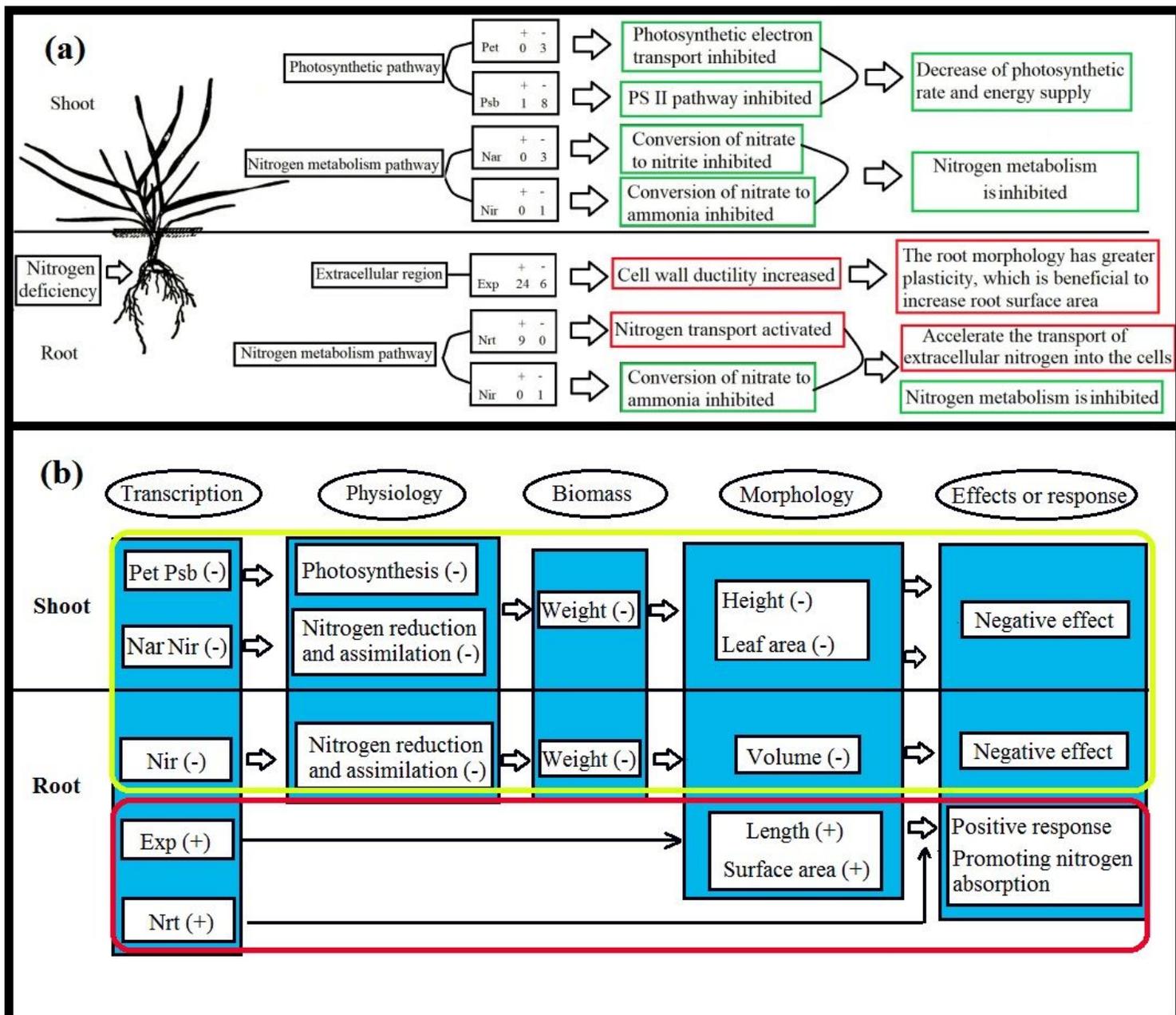


Figure 9

The chart of response pathways under nitrogen deficiency (a); the chart of the relationship of gene transcription, physiology, biomass, morphology and their effects or response under nitrogen deficiency (b). "+" means up-regulated or increased; "-" means down-regulated or decreased.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [ALLvsDEG.KEGGClassification1.xls](#)
- [ALLvsDEG.KEGGClassification.xls](#)

- enrichmentgoR0vsR1Total.xls
- enrichmentgoL0vsL1Total.xls
- fpkmanno.xls
- report.fpkm.xls
- L0vsL1diffpval0.05FC2.gene.xls
- R0vsR1diffpval0.05FC2.gene.xls