Photosynthetic performance and photosynthesis-related gene expression coordinated in a shade-tolerant species *Panax notoginseng* under nitrogen regimes

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Abstract

Nitrogen (N) is an essential component of photosynthetic apparatus. However, the mechanism that photosynthetic capacity is suppressed by N is not completely understood. Photosynthetic capacity and photosynthesis-related genes were comparatively analyzed in a shade-tolerant species Panax notoginseng grown under the levels of low N (LN), moderate N (MN) and high N (HN). Photosynthetic assimilation was significantly suppressed in the LN- and HN-grown plants. Compared with the MN-grown plants, the HN-grown plants showed thicker anatomic structure and larger chloroplast accompanied with decreased ratio of mesophyll conductance (gm) to Rubisco content (gm /Rubisco) and lower Rubisco activity. Meanwhile, LN-grown plants displayed smaller chloroplast and accordingly lower internal conductance (gi). LN- and HN-grown individuals allocated less N to light-harvesting system (NL) and carboxylation system (NC), respectively. N surplus negatively affected the expression of genes in Car biosynthesis (GGPS , DXR , PSY , IPI and DXS) and non-net carboxylative process (CEF-PSI). The LN individuals outperformed others with respect to non-photochemical quenching. The expression of genes (FBA, PGK, RAF2, GAPC, CAB, PsbA and PsbH) encoding enzymes of Calvin cycle and structural protein of light reaction were obviously repressed in the LN individuals, accompanying with a reduction in Rubisco content and activity. Correspondingly, the expression of genes encoding RAF2 , RPI4 , CAB and PetE were repressed in the HN-grown plants. LN-induced depression of photosynthetic capacity might be caused by the deceleration on Calvin cycle and light reaction of photosynthesis, and HN-induced depression of ones might derive from an increase in the form of inactivated Rubisco and the deprivation of photoprotection.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Tables

Table 1 Effects of nitrogen regimes on the leaf morphology, anatomy and biomass in a shade-tolerant plant *Panax notoginseng*.

Variables	Nitrogen level		
	LN	MN	HN
Upper epidermis (mm)	11.209 ± 0.024 c	17.694 ± 1.927 a	14.738 ± 0.269 b
Lower epidermis (mm)	10.590 ± 1.027 c	13.177 ± 2.186 a	11.420 ± 0.918 b
Spongy tissue (mm)	42.551 ± 2.194 c	70.378 ± 0.182 a	56.518 ± 0.189 b
Palisade tissue (mm)	17.069 ± 1.283 c	32.867 ± 0.173 a	24.490 ± 1.825 b
Palisade/spongy	0.401 ± 0.002	0.467 ± 0.016	0.433 ± 0.016
Leaf length (cm)	6.484 ± 1.980 c	7.515 ± 1.068 a	6.795 ± 1.238 b
Max width (cm)	2.777 ± 0.698 c	3.114 ± 0.621 ab	3.273 ± 0.519 a
Leaf length/max width	2.341 ± 1.339	2.413 ± 0.8445	2.076 ± 0.879
Leaf dry weight (g $plant^{-1}$)	0.413 ± 0.040 c	0.545 ± 0.025 a	0.496 ± 0.064 b
Total dry weight (g $plant^{-1}$)	1.068 ± 0.294 c	1.649 ± 0.181 a	1.524 ± 0.088 b

Values are means ± SD. (*n*=7). Different letters among nitrogen regimes indicate significant difference (*P*≤0.05).

Table 2 Effects of N regimes on leaf photosynthesis in *Panax notoginseng*.

Variables	LN	MN
g_s (mol CO_2 m^{-2}·s^{-1})	0.05 ± 0.02 a	0.03± 0.02 ab
g_m(mol CO_2 m^{-2}·s^{-1})	0.09 ± 0.01 c	0.26 ± 0.04 b
R_d(mmol CO_2 m^{-2}·s^{-1})	1.0 ± 0.04 a	0.52 ± 0.02 b
g_{lip}(mol CO_2 m^{-2}·s^{-1})	2.61 ± 0.08 b	5.52 ± 0.03 a
C_C(mmol CO_2 m^{-2}·s^{-1})	199.53 ± 8.27 b	265.45 ± 7.31ab
S(mol mol^{-1})	844.15 ± 7.56 b	1057.25 ± 5.41 a
S*(mol mol^{-1})	739.39 ± 95.61b	983.75± 67.32 a
Rubisco activitynmol/min/g	0.643 ± 0.24 c	40.51 ± 5.39 a
Rubisco content (mg/g^{-1})	6.931 ± 0.36 c	10.057 ± 0.67 b
S_c(m^2 m^{-2})	8.42 ± 1.25 b	12.01 ± 1.65 a
C_{lip}(mol CO_2 m^{-2}·s^{-1})	0.31 ± 0.06 ab	0.46 ± 0.02 a
g_i (mol CO_2 m^{-2}·s^{-1})	0.13 ± 0.01 b	0.35 ± 0.04 a
g_m/Rubisco content	12.99 ± 1.23 b	25.81 ± 1.90 a

Values are means ± SD. (*n*=7). Different letters among nitrogen regimes indicate significant difference (*P*≤0.05).

Table 3 Steady-state photosynthetic-related traits in *Panax notoginseng* under different levels of nitrogen.

Variables	Nitrogen level		
	LN	MN	HN
A_{max} (μmol·m^{-2}·s^{-1})	2.378 ± 0.261c	3.437 ± 0.241a	2.600 ± 0.165 b
CEmol·mol^{-1}	0.017 ± 0.002 ab	0.022 ± 0.003 a	0.018 ± 0.005 ab
Γ*μmol·mol^{-1}	124.399 ± 8.014 a	99.259 ± 10.957 b	122.121 ± 21.084 ab
J_{max}μmol·mol^{-1}	66.558 ± 6.123 b	74.518 ± 15.599 a	63.334 ± 23.251b
V_{cmax}μmol·mol^{-1}	16.480 ± 1.821b	20.771 ± 2.939 a	16.830 ± 5.058 b
J_{max}/V_{cmax}	4.059 ± 0.127 ab	3.527 ± 0.337 b	4.329 ± 0.106 a
SLN (g m^{-2})	0.890 ± 0.130 c	1.245 ± 0.006 b	2.178 ± 0.348 a

Values are means ± SD. (*n*=7). Different letter among nitrogen treatments represents a significant level (P≤0.05).

Table 4 Photosynthetic-related pigment in a shade-tolerant plant *Panax notoginseng* grown under different levels of nitrogen, means ± SD were given (*n*=7).

Variables	Nitrogen Level		
	LN	MN	HN
Nmg·cm^{-2}	0.362 ± 0.129 c	0.865±0.265 b	1.643 ± 0.332 a
Vmg·cm^{-2}	0.913 ± 0.124 a	0.267±0.195c	0.493 ± 0.458b
Amg·cm^{-2}	0.213 ± 0.019 a	0.043±0.072c	0.153 ± 0.079b
Lmg·cm^{-2}	1.284 ± 0.352 c	3.018±0.970 b	5.852 ± 0.926a
Zmg·cm^{-2}	0.194 ± 0.023 a	0.032 ± 0.048 c	0.073 ± 0.201b
Chlg·cm^{-2}	12.270 ± 1.783 c	31.618 ± 2.356 b	60.101 ± 2.455 a
β-Carg·cm^{-2}	4.08 ± 2.14 a	1.59 ± 0.69 c	2.95 ± 0.69 b
V+A+Z g·cm^{-2}	1.314 ± 0.023 a	0.332 ± 0.035 c	0.712 ± 0.043 b
(A+Z)/(V+A+Z)	0.309 ± 0.015 ab	0.226 ± 0.017 b	0.317 ± 0.037 a
(V+A+Z)/Chl	0.107 ± 0.018 a	0.011 ± 0.027 b	0.012 ± 0.028 b

Different letter among nitrogen treatments represents a significant level (*P*≤0.05).

Figures

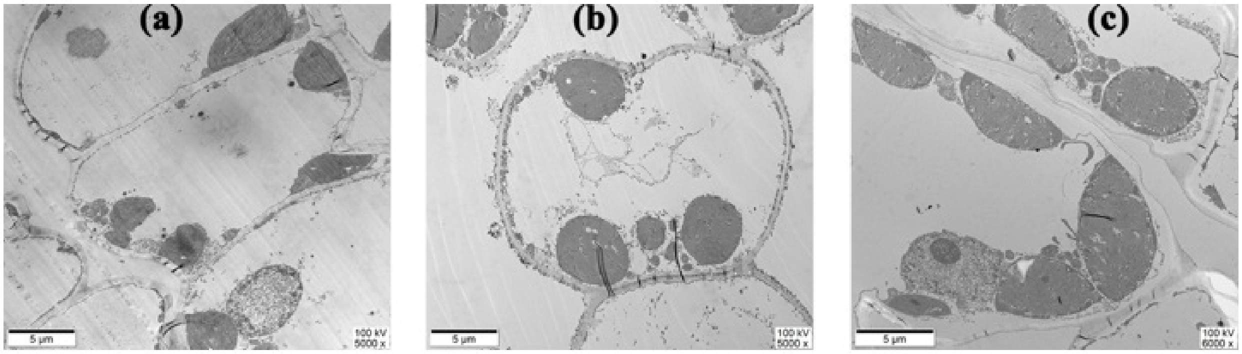


Figure 1

Electron micrograph of chloroplast with low(a), moderate(b) and high(c) nitrogen level were taken at 5000, 5000 and 6000 times, respectively.

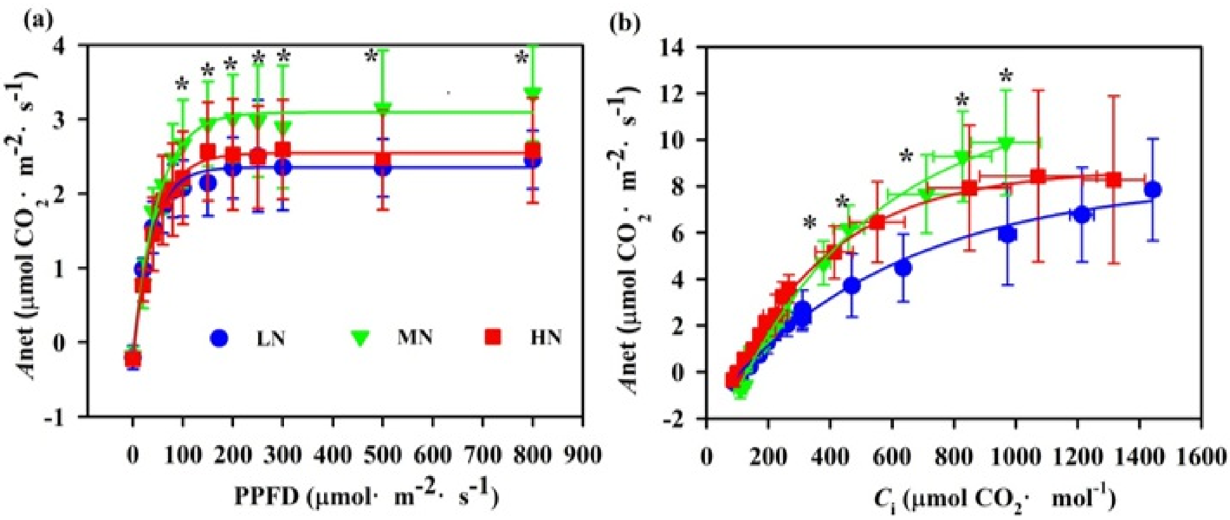


Figure 2

(a) Response of net photosynthetic rate (Anet) to photosynthetic photon flux density (PPFD) in Panax notoginseng grown under low nitrogen (LN), moderate nitrogen (MN), high nitrogen (HN). (b) The change of net photosynthetic assimilation (Anet) with intercellular CO2 concentration (Ci) in Panax notoginseng grown under different nitrogen levels. Values for each point were means ± SD (n=7). Significant differences are indicated by asterisks (ANOVA; P values ≤ 0.05).

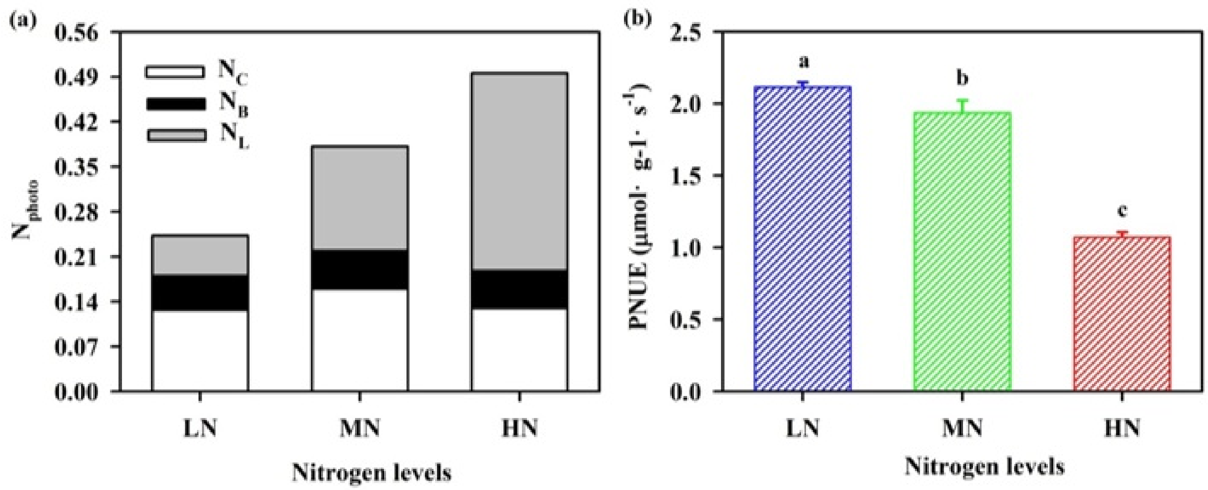


Figure 3

Effects of different nitrogen levels on nitrogen distribution (n=7) and photosynthetic nitrogen use efficiency (n=7) in Panax notoginseng leaves. Nphoto: Photosynthetic apparatus; NC: Carboxylation system; NB: Bioenergetics; NL: Light harvesting system; PNUE: Photosynthetic nitrogen use efficiency. Data are mean with bars depicting standard deviation (± SD). Significant differences are indicated by letters (ANOVA; P values ≤ 0.05).

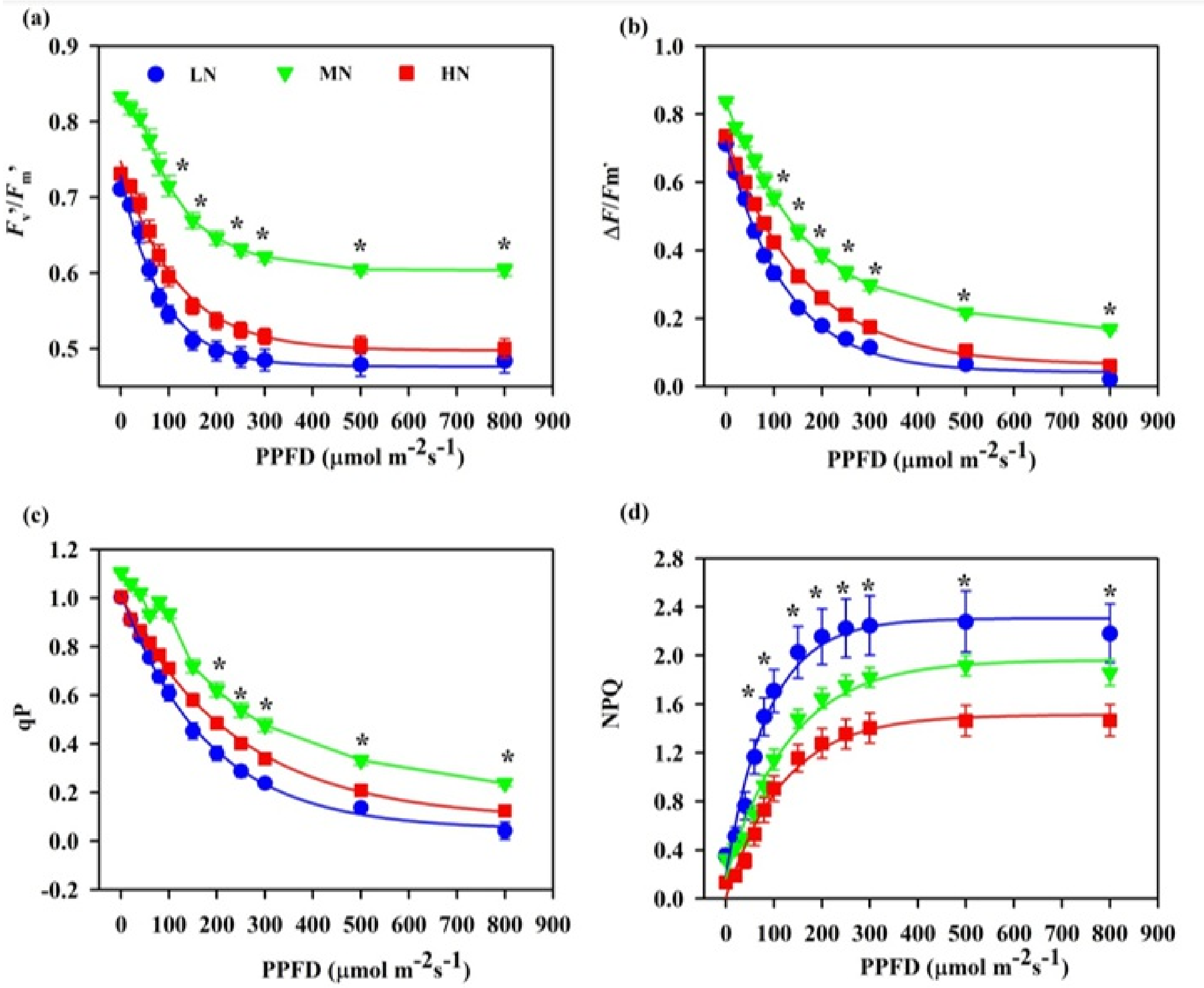


Figure 4

Responses of PSII maximum quantum efficiency (Fv\`/Fm\`, a), PSII photochemical quantum yield (ΔF/Fm\`, b), photochemical quenching (qP, c), non-photochemical quenching (NPQ, d) to photosynthetic photon flux density (PPFD) in Panax notoginseng grown under different levels of nitrogen. Values for each point were means ± SD (n=7). Significant differences are indicated by asterisks (ANOVA; P values ≤ 0.05).

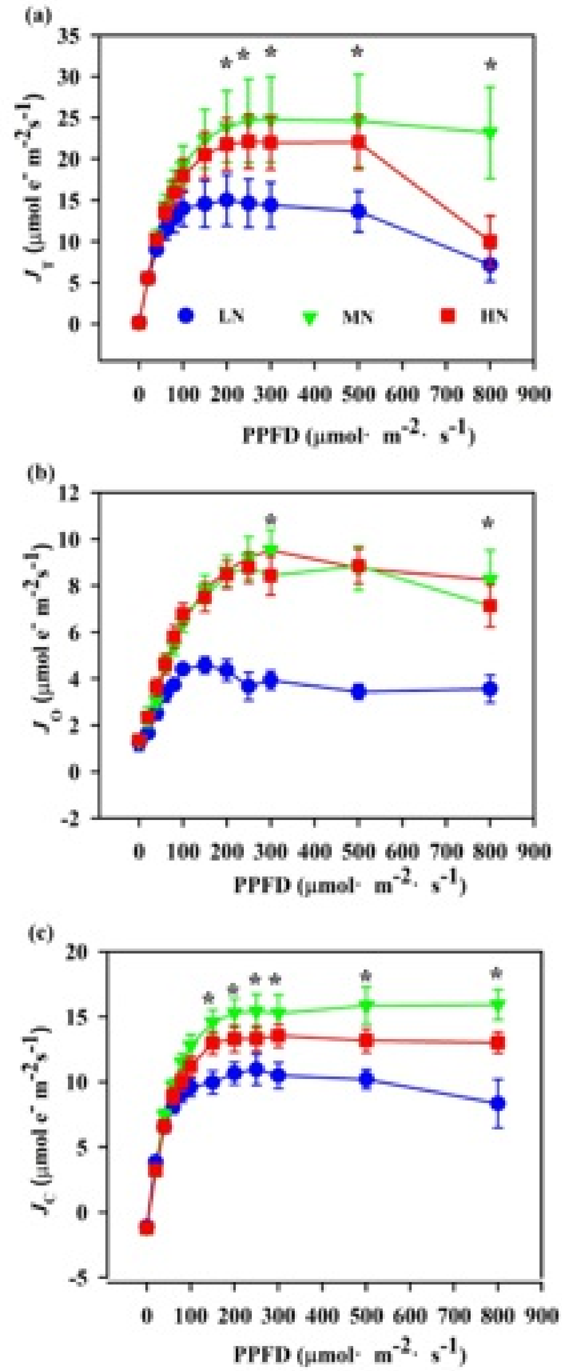


Figure 5

Responses of PSII total electron transport rate (JT, a), rate of electron transport for oxidation reaction (JO, b) and carboxylation reaction (JC, c) to photosynthetic photon flux density (PPFD) in Panax notoginseng grown under different levels of nitrogen. Values for each point were means ± SD (n=7). Significant differences are indicated by asterisks (ANOVA; P values ≤ 0.05).

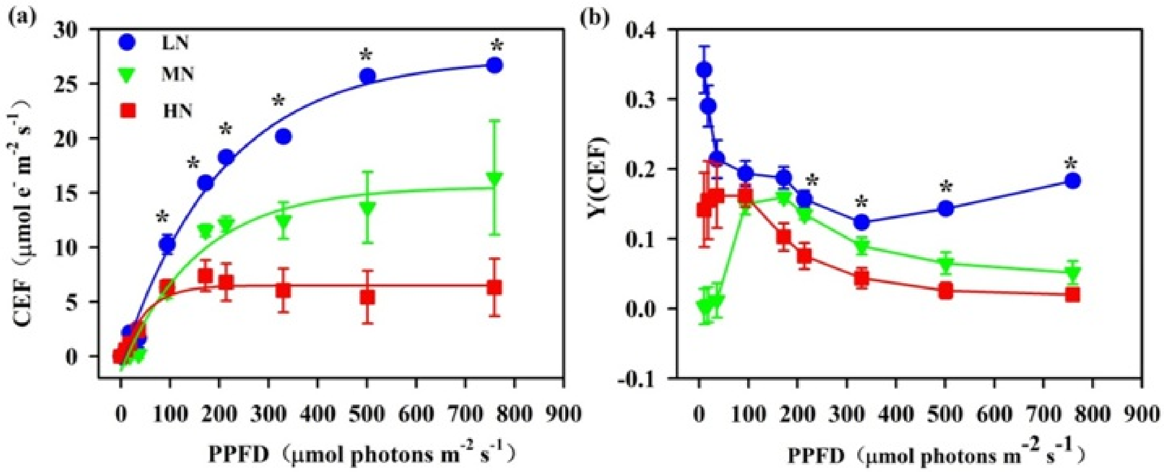


Figure 6

Light response changes in CEF (n=7) and Y(CEF) (n=7) for leaves of Panax. notoginseng grown under nitrogen regimes. Data are mean with bars depicting standard deviation (± SD). Significant differences are indicated by asterisks (ANOVA; P values ≤ 0.05).

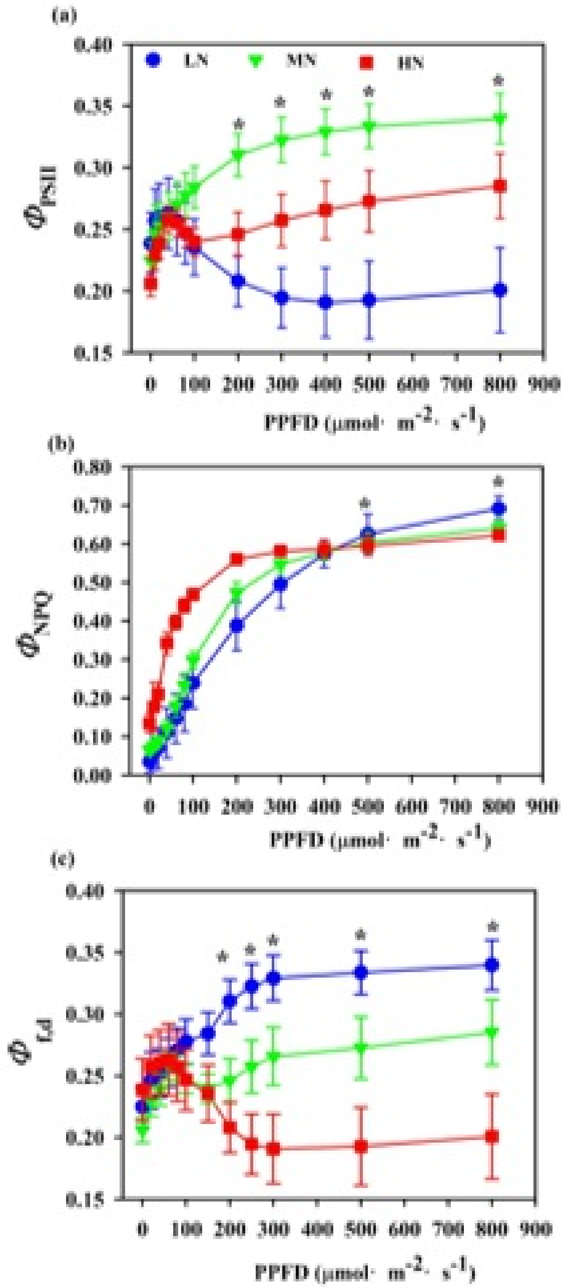


Figure 7

The allocation characteristics of photons absorbed by the PSII antennae to the photochemistry dissipation (ΦPSII, a), non-photochemistry dissipation (ΦNPQ, b) and thermal dissipation (Φf,d, c) in the fluorescence-light response processes in Panax notoginseng grown under different levels of nitrogen. Values for each point were means ± SD (n=7). Significant differences are indicated by asterisks (ANOVA; P values ≤ 0.05).

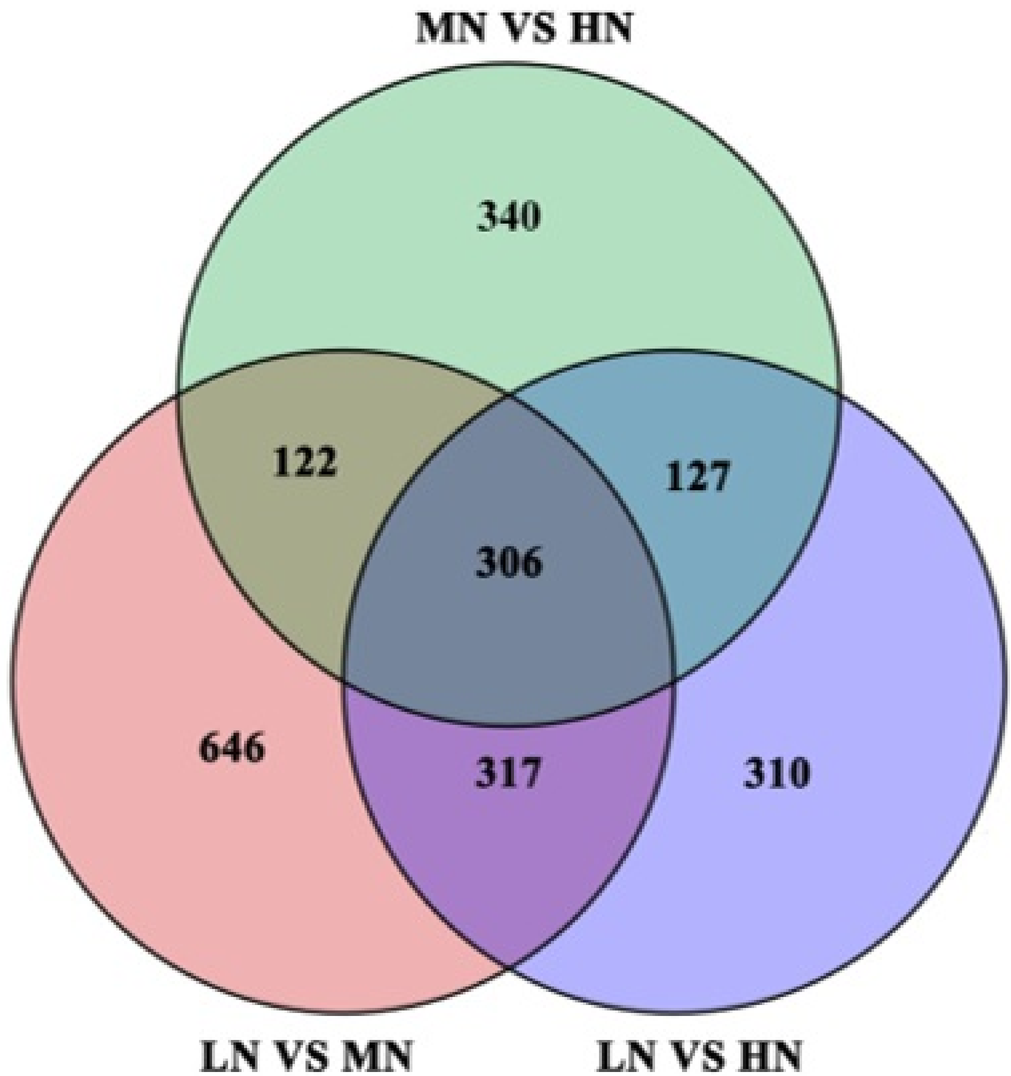


Figure 8

Venn diagrams of differentially expressed genes (DEGs) in response to varied nitrogen level.

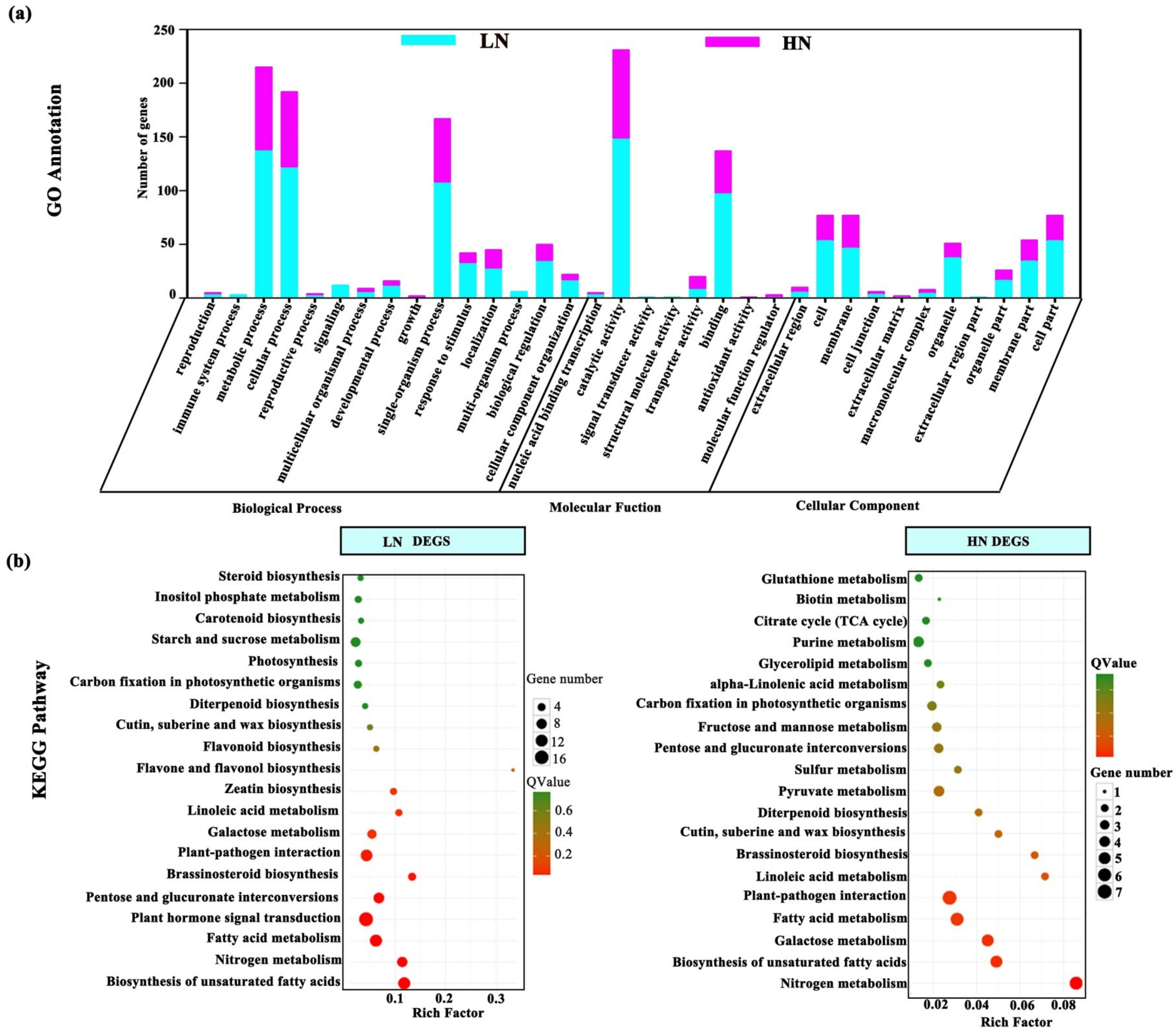
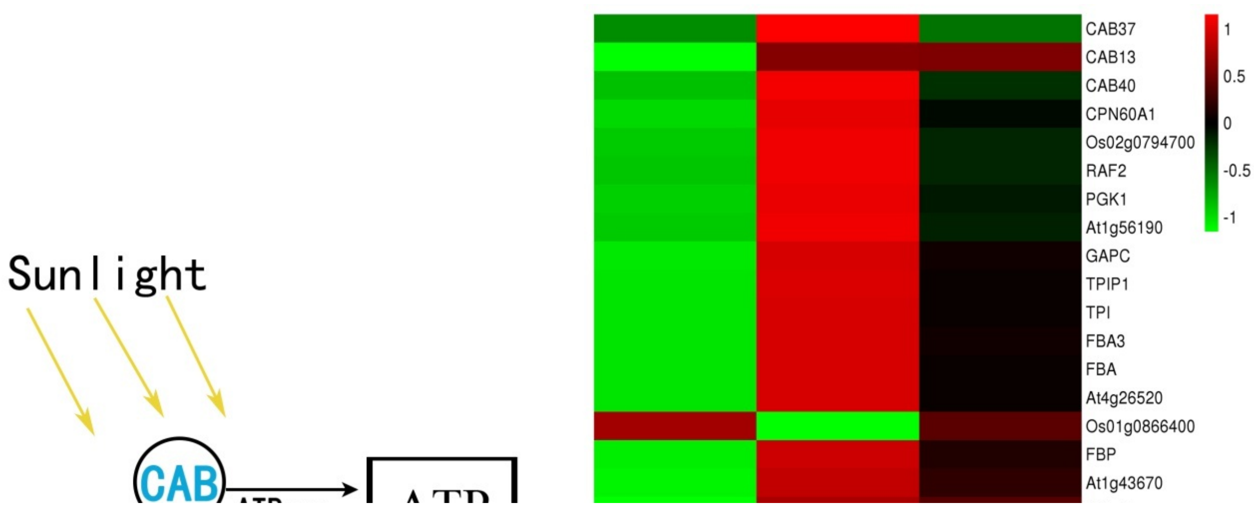


Figure 9

Functional annotation and enrichment analysis of differentially expressed genes (DEGs) responsive to low nitrogen and high nitrogen. (a) Gene annotation of DEGs. (b) KEGG enrichment analysis for DEGs



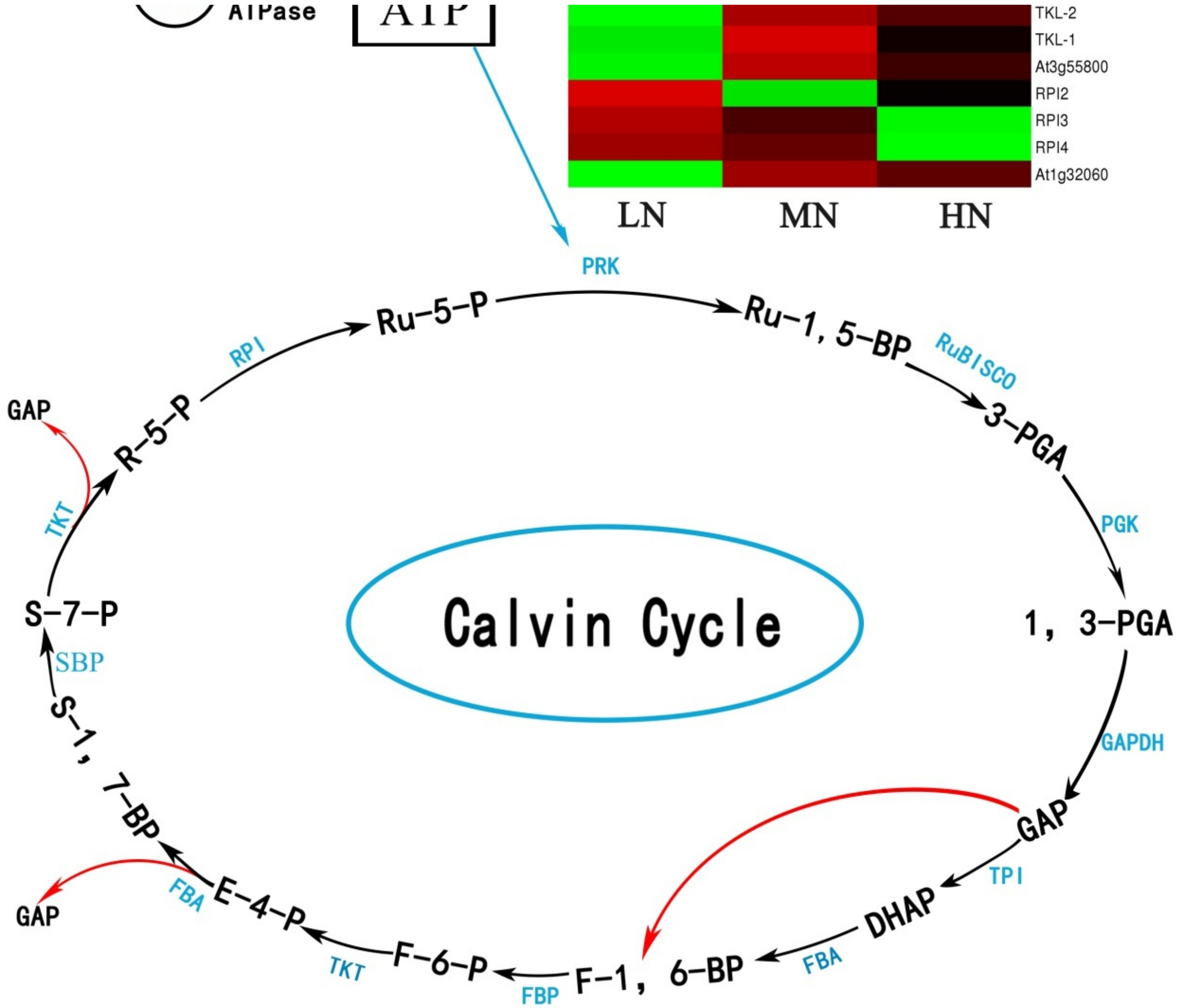


Figure 10

Calvin cycle pathways of Panax notoginseng and hierarchical cluster analysis of genes that were differentially expressed under different nitrogen level. Red indicates that the gene has a high expression in the nitrogen level; green indicates that the gene has a lower expression in the nitrogen level.

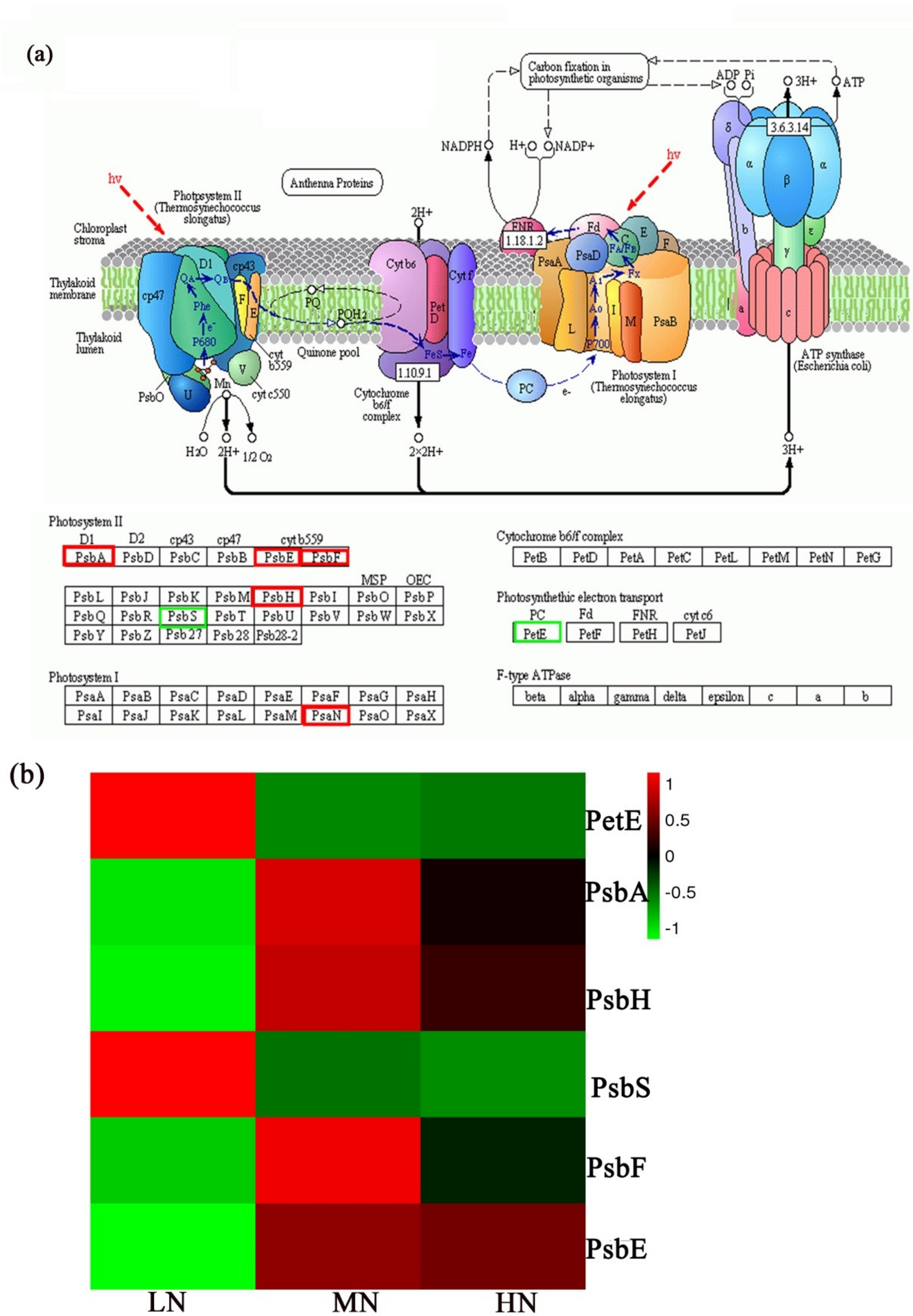

(a)
Carbon fixation in photosynthetic organisms
ADP Pi
3H+
ATP
3.6.3.14
NADPH
H+
NADP+
hv
Anthenna Proteins
Photpsystem II (Thermosynechococcus slongatus)
Chloroplast stroma
Thylakoid membrane
Thylakoid lumen
cp47
D1
cp43
Phe
P680
PQ
PQH2
Quinone pool
cyt b559
V
PsbO
U
Mn
cyt c550
H2O
2H+
1/2 O2
2H+
Cyt b6
Pet D
Cyt f
FeS
Fe
1.10.9.1
Cytochrome b6/f complex
2×2H+
FNR
1.18.1.2
Fd
PsaD
PsaA
PsaB
P700
PC
e-
Photosystem I (Thermosynechococcus elongatus)
ATP synthase (Escherichia coli)
3H+
Photosystem II
D1 D2 cp43 cp47 cyt b559
PsbA PsbD PsbC PsbB PsbE PsbF
MSP OEC
PsbL PsbJ PsbK PsbM PsbH PsbI PsbO PsbP
PsbQ PsbR PsbS PsbT PsbU PsbV PsbW PsbX
PsbY PsbZ Psb27 Psb28 Psb28-2
Photosystem I
PsaA PsaB PsaC PsaD PsaE PsaF PsaG PsaH
PsaI PsaJ PsaK PsaL PsaM PsaN PsaO PsaX
Cytochrome b6/f complex
PetB PetD PetA PetC PetL PetM PetN PetG
Photosynthethic electron transport
PC Fd FNR cyt c6
PetE PetF PetH PetJ
F-type ATPase
beta alpha gamma delta epsilon c a b
(b)
PetE
PsbA
PsbH
PsbS
PsbF
PsbE
LN
MN
HN
1
0.5
0
-0.5
-1

Figure 11

Differentially expressed genes (DEGs) participating in light reaction under varied nitrogen level. (a) MN vs LN and MN vs HN differential gene of photosynthesis pathway for samples of control group, the red box labeled for raising genes, green box labeled as the blue box labeled as there are raised and lowered genes at the same time, the box numbers for the number of the enzyme, suggests that the corresponding gene is associated with the enzyme, and the whole passage is there are many different forms through complex biochemical reactions, an enzyme that differences in genes associated with this pathway are marked by different color box. (b) The expression pattern of DEGs involved in photosynthesis pathway. Red indicates that the gene has a high expression in the nitrogen level; green indicates that the gene has a lower expression in the nitrogen level.

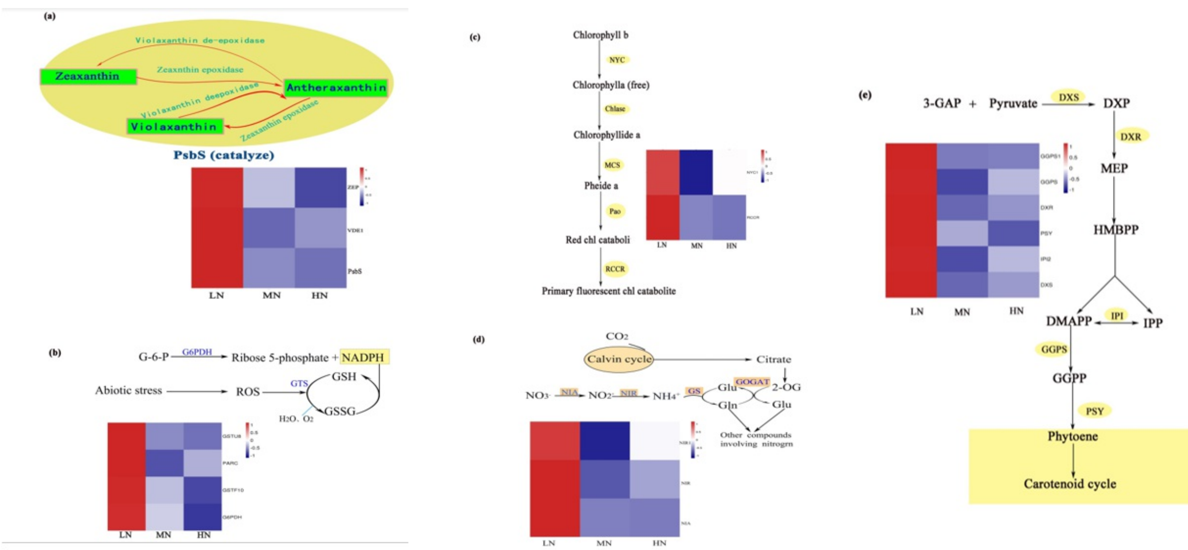


Figure 12

The pathway and genes encoding for the photoprotection. In heat map, firebrick indicates that the gene has a high expression in the nitrogen level; navy indicates that the gene has a lower expression in the nitrogen level. (a) The expression pattern of DEGs involved in Lutein cycle. (b) The expression pattern of DEGs involved in Antioxidant pathway. (c) The expression pattern of DEGs involved in Chlorophyll degradation pathway. (d) The expression pattern of DEGs involved in nitrate assimilation. (e) The expression pattern of DEGs involved in Carotenoid metabolism

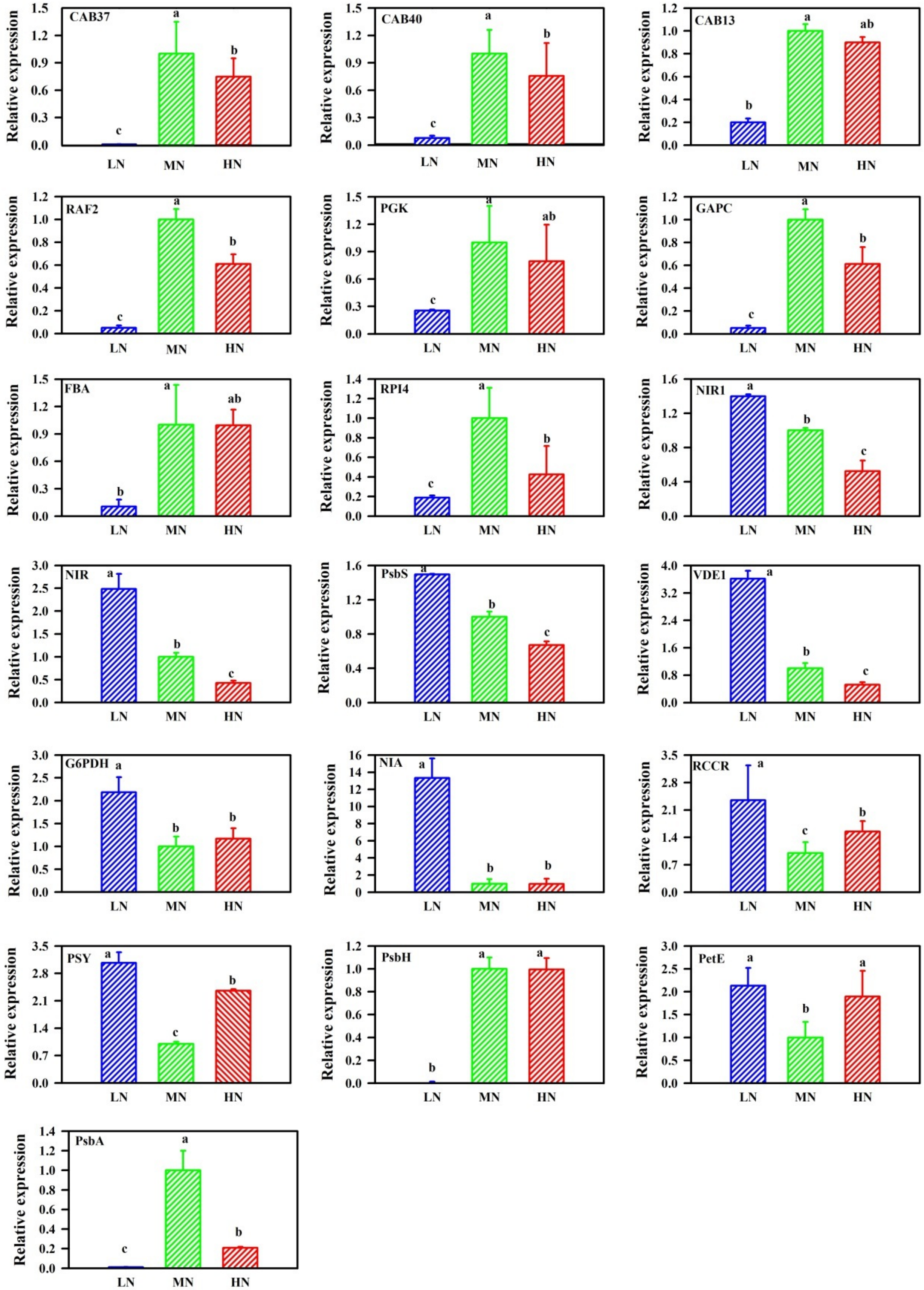

CAB37
CAB40
CAB13
RAF2
PGK
GAPC
FBA
RPI4
NIR1
NIR
PsbS
VDE1
G6PDH
NIA
RCCR
PSY
PsbH
PetE
PsbA
Relative expression
LN
MN
HN

Figure 13

Quantitative real-time PCR validation of 19 differentially expressed genes (DEGs) (n=5). Data are mean with bars depicting standard deviation (± SD). Significant differences are indicated by letters (ANOVA; P values ≤ 0.05). The columns represent relative expression obtained by RT-qPCR.

Supplementary Files

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