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Full Length Article

Comparative Differences in Carbon and Nitrogen Metabolism of Young and Old Leaves from Wild and Cultivated Soybean Under Low Nitrogen Conditions

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Abstract

Low nitrogen (LN) stress is the main restrictive factor in agricultural production, and the excessive application of nitrogen (N) fertilizer results in serious production and environmental problems. The carbon and nitrogen metabolism of young and old leaves of two soybean genotypes under different LN stress conditions were measured, which provided theoretical support for the improvement of LN-tolerant cultivated soybeans. Under LN stress, the growth of wild soybean was better than cultivated soybean, especially root growth. The growth of wild soybean increased by 0.14, 0.34 and 0.74-fold respectively, while of cultivated soybean decreased by 0.08, 0.02 and 0.30-fold respectively. Under three different intensities LN stress, wild soybean absorbed more nitrogen by increasing root length and the young and old leaves of wild soybean maintained stable photosynthetic gas exchange parameters, photosynthetic pigment contents, and anion and cation content balances. Wild soybean also maintained stable levels of the key enzymes in N metabolism, nitrate reductase, glutamine synthetase, aspartate aminotransferase, alanine aminotransferase, glutamate dehydrogenase and glutamate synthase. As wild soybean maintained a stable balance of carbon and nitrogen metabolism relative to cultivated one under LN stress conditions, showing tolerance of wild soybean tolerance to LN stress. This study provides new information and a reliable theoretical basis for utilizing wild soybean, improving cultivated soybean, and studying the LN tolerance mechanisms of other plants. © 2022 Friends Science Publishers

Keywords: Low nitrogen; Carbon and nitrogen metabolism; Young leaves; Old leaves; Cultivated soybean; Wild soybean

Introduction

Nitrogen (N) is a key mineral for plant growth as well key factor limiting crop yield (Liu *et al*. 2017). Under nitrogen deficiency, plants will cause the lower leaves to chlorosis, limit the growth of buds and reduce the growth of plants (Zhao *et al*. 2020; Mu and Chen 2021). In the field, a large amount of nitrogen fertilizer is applied to increase the yield. In the past decade, the amount of N fertilizer applied in China has increased, with the application amount accounting for more than 35% of the world's total, making China the largest N consumer in the world (Wu *et al*. 2017). However, crops have limited N fertilizer absorption efficiencies, leaving large amounts of N fertilizers to pollute the atmosphere in the form of nitric oxide and nitrogen dioxide. Additionally, the excess decreases water quality and pollutes rivers as nitrate nitrogen and ammonia nitrogen (Wang *et al*. 2013). This not only causes environmental pollution, but also increases the production costs of crops. Therefore, cultivating low nitrogen (LN)-tolerant varieties, improving N use efficiency and reducing N use are effective strategies for sustainable agriculture (Zhao *et al*. 2019).

Soybean (*Glycine max* L.) is an important food and oil crop worldwide, providing 69% of the world's dietary protein and 30% of the edible oil. Wild soybean (W) is the ancestor of the cultivated soybean (M), it has the benefits of high protein content, wide distribution, and strong stress resistance (Kong *et al*. 2017). Moreover, there is no inter-specific hybridization barrier between them, allowing wild soybean to play a key role in the genetic improvement and the selection of elite varieties of cultivated soybean (Liu *et al*. 2019). Previous studies have shown that wild soybean has a stronger tolerance to LN stress than cultivated soybean (Li *et al*. 2017). Therefore, it is necessary to systematically study the mechanism of wild soybean resistance to LN stress from the perspective of carbon (C) and N metabolism.

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The main plant organs for the absorption, transformation and transmission of energy are the leaves, and their functional characteristics to directly reflect plant hereditary characteristics and the effective utilization of resources (Guo *et al*. 2016). The functional characteristics of the leaves vary with location, which affects the exchange of substances and energy between the plant and the surrounding environment (Wang *et al*. 2012a), as well as the plant's survival strategy, which is formed to adapt to changes in the environment. The function, structure and activity of young and old leaves are different, old leaves are sacrificed to ensure the normal growth and development of young leaves (Shen *et al*. 2021). Previous studies have found that under low nitrogen stress, nitrogen in old leaves can be transported to new leaves for growth (Feller and Fischer 1994); chlorophyll binding protein can be induced in new leaves, which is conducive to the recovery and reuse of nitrogen in old leaves (Avice and Etienne 2014). Therefore, studying the photosynthetic characteristics, C and N metabolism of wild soybean and cultivated soybean under LN-stress conditions in young and old leaves can provide new information to improve soybean production.

In this study, wild soybean and cultivated soybean were selected as experimental materials and treated with an artificial simulation of LN stress. Biomass, ion accumulation, photosynthetic parameters and key N metabolism-related enzymatic activities were investigated in wild soybean and cultivated soybean under LN stress. The main purposes of this experiment were to study the physiological adaptation mechanisms, differences between wild and cultivated soybean under LN stress. Additionally, we investigated how to use wild soybean to improve cultivated soybean to cultivate LN-tolerant crop varieties by measuring photosynthetic pigments and its gas exchange traits, key enzymes of nitrogen metabolism, thereby revealing the dynamic response processes of the C–N coupling relationship.

Materials and Methods

Plant materials

The seed of wild ('Huinan06116') and cultivated soybean ('Jinong24') were provided by the Jilin Center of Germplasm Introduction and Breeding of Crops, Changchun City, China.

Plant growth conditions and stress treatments

The wild and cultivated soybean seeds were planted in a 14 cm diameter plastic pot containing 2.5 kg of washed sand and germinated in water. All test materials are rainproof. After seedling emergence, they were watered with 1×Hoagland's solution every morning. The plants were grown in the outdoor experimental fields, and the night and day temperatures was 17.0–20.0°C and 24.0–28.0°C, respectively, and a relative humidity of 55–65% at Northeast Normal University, Changchun City, Jilin Province, China.

The LN treatment was initiated when the third compound leaf emerged. Seeds of wild and cultivated soybean from the LN treatment group were grown in the modified Hoagland solution of 1/2 intensity (N1), 1/3 intensity (N2) and 1/4 intensity (N3) for 2 weeks, respectively. CK culture under normal conditions (1 × Hoagland solution). Wild and cultivated soybean were divided into 4 groups: control (cK) and treatments N1, N2 and N3. Each group of 8 pots: 4 pots were used to measure growth and photosynthesis parameters, 4 pots were used to analyze ion content and enzyme activity level (Table 1).

Measurement of growth, Total C and N contents

After two weeks of stress treatment, soybean plants were harvested. The plant heights, root lengths, above-ground fresh weights (Up FWs), below-ground FWs (Under FWs), above-ground dry weights (Up DWs), and below-ground DWs (Under DWs) were measured (Shi *et al*. 2015). A stable isotope mass analyzer (isprime Element Analyzer, isprime Ltd., Japan) was used to evaluate the total nitrogen and carbon content (%) of the seedling leaves through a 1 mg dry powder sample. SigmaPlot 10.0 (Systat Software Inc.) and SPSS 16.0 (SPSS Inc.) software were used to analyze the experimental data (Leticia *et al*. 2019).

Photosynthetic indices measurements

After two weeks of treatment, the first two leaves at the top and the first two leaves at the bottom were selected from the four pots to represent enough new and old leaves. The LI-6400 portable open gas exchange system (LI-COR, USA) was used to measure the photosynthesis rate (P_N), stoma conductivity (g_s) and leaf transpiration rate (E) at 11:00 in the morning. Atmospheric CO_2 concentration, effective photosynthetic radiation, temperature and air humidity were 375~385 cm^3 m^{-1}, 1150~1250 μmol m^{-2} s^{-1}, 24°C and 50% (Li *et al*. 2018).

Dried leaf samples (30 mg) were immersed in 10 mL of a mixture of 80% acetone: absolute ethanol (1: 1) and photosynthetic pigments were extracted in the dark at room temperature until the leaves turned white. Three spectrophotometric measurements (Spectrov-754, Shanghai Accurate Scientific Instruments) were performed on each sample at 440, 645 and 663 nm using Holm's (1954) formula. The content of photosynthetic pigments were calculated in mg g^{-1}, photosynthetic pigments included chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), chlorophyll a + chlorophyll b[Chl(a+b)] and carotenoids (auto) (Jiao *et al*. 2018).

Ion content measurements

To 50-mg dry samples, 4 mL of deionized water was added. Samples were boiled in a water bath for 40 min, cooled and

centrifuged at 3,000 rpm for 15 min. Then, the liquid supernatants were collected. This extraction procedure was repeated twice, finally the supernatants were combined and raised to a set volume of 10 mL. Determination of NO_3^-, $H_2PO_4^-$, SO_4^{2-}, $C_2O_4^{2-}$ and Cl^- in the supernatant by means of ion chromatography (DX-300ion chromatographic system, AS4A-SC chromatographic column, CDM-II electrical conductivity detector, mobile phase: $Na_2CO_3/NaHCO_3$ = 1.7/1.8 m*M*, Dionex, Sunnyvale, CA, USA). The concentrations of Na^+, Mg^{2+}, Ca^{2+}, K^+, P^{5+}, Fe^{3+}, B^{3+}, Zn^{2+}, $Cu^{2+}$and Mn^{2+} were measured with an atomic absorption spectrophotometer (Super 990F, Beijing Purkinje General Instrument Co. Ltd., Beijing, China). Each sample was measured in triplicate (Yang *et al*. 2017).

Determination of key enzymatic activity levels

Determination of the nitrate reductase (NR) activity level: The sulfamate colorimetric method was used to determine the activity level of NR (Li *et al*. 2019). Add a reaction mixture containing 200 m*M* KNO3, 5 m*M* EDTA and 0.15 m*M* NADH to 100 m*M* phosphate buffer pH 7.5, incubate at 30°C for 1 h, then add 2 mL sulfate and 2 mL α-naphthylamine. Measure the absorbance at 540 nm. Enzyme activity is expressed in μmol·min^{-1}.

Determination of the glutamine synthetase (GS) activity level: The GS enzyme extract was prepared as follows: 1g sample was weighed, placed in liquid nitrogen and ground. Then, 8 mL of extractive solution (100 m*M* Tris-HCl, 0.5 m*M* EDTA and 5 m*M* β-Mercaptoethanol, pH 7.5) was added, and the sample was filtered through two layers of gauze, followed by centrifugation at 4°C for 15,000 r·min^{-1} for 20 min. The supernatant was used for enzymatic activity determination.

Enzyme activity determination: 1.6 mL reaction solution, which contains 50 m*M* hydrochloric acid buffer solution pH (7.0), sodium glutamate solution 0.3 *M* pH (7.0), 0.3 *M* $MgSO_4$ and 30 m*M* ATP Solution-Na (pH 7.0), then It is 0.6 mL enzyme extract solution (CK immediately accepts 1.0 mL $FeCl_3$ reagent). Keep the reaction solution in a water bath at 25°C for 5 min, add 0.2 mL of hydroxylamine reagent to start the reaction, and continue in a water bath at 25°C for 15 min. Then add 1 mL of $FeCl_3$ reagent to stop the reaction. The mixed solution was centrifuged at 15,000 rpm for 10 min and the optical density of the supernatant was measured at 540 nm (Rhodes *et al*. 1975).

Determination of the aspartate aminotransferase (GOT) activity level: To determine the GOT level, two test tubes were used, one as the sample tube, to which 0.1 mL of crude enzyme preparation and 0.5 mL of GOT substrate solution was added and the other was the CK tube, to which only 0.1 mL of crude enzyme preparation was added. Both tubes were placed in a 37°C water bath at the same time. Take it out after 30 min, add 0.5 mL of 2,4-dinitrophenylhydrazine solution to each tube to stop the reaction and add 0.5 mL of GOT substrate solution to the CK tube. The two tubes were then placed in a 37°C water bath for 20 min and 5.0 mL of 0.4 mol·L^{-1}NaOH was added to each tube and mixed. After 10 min, a spectrophotometer was used to measure the absorbance at 500nm after distilled water was adjusted to read zero (Wu *et al*. 1998).

Determination of the alanine aminotransferase (GPT) activity level: The determination of GPT activity was assessed by determining the reduction in absorbance owing to the consumption of NADH at 340 nm. The reaction buffer was composed of 15 m*M* a-oxoglutarate, 0.15 m*M* NADH, 0.5 *M* 1-alanine, and 5-unit lactate dehydrogenase in 50 m*M* Tris HCl buffer (pH 7.5). Then, 100 μL enzyme was added to initiate the extraction reaction (Ullah *et al*. 2019).

Determination of glutamate dehydrogenase (GDH) activity: A reaction mixture of 23.1 m*M* α-ketoglutarate, 231 m*M* NH_4Cl, 30 m*M* $CaCl_2$ and 6 m*M* NADH was used to determine the activity of NADH-GDH in 100 m*M* Tris-HCl buffer (pH 8.0). The reaction starts with the addition of the enzyme extract. The NADH-GDH reaction mixture consists of 100 m*M* 1-glutamic acid, 1 m*M* NAD in 100 m*M* Tris-HCl buffer (pH 8.8) and enzyme extract. The oxidizing activity of NADH (NADH-GDH) and the reducing activity of NADH (NADH-GDH) were measured by spectrophotometry. Enzymatic activity was expressed in units of μmol·g^{-1} protein (Loulakakis and Roubelakis-Angelakis 1991).

Determination of glutamate synthase (GOGAT) activity: The extraction solution was prepared in the same way as GS. Determination of enzymatic activity: the total volume of the reaction system was 3 mL (containing 0.4 mL 20 m*M* L-glutamine, 0.05 mL 0.1*M* α-ketoglutarate, 0.1 mL 10 m*M* KCl, 0.1 mL 3 m*M* NADH and 0.3 mL enzyme solution), an insufficient volume was made up with 25 m*M* Tris-HCl at pH 7.6. The reaction is initiated by L-glutamine and 10 successive absorbance values are measured at 340 nm every 30 sec. The segment with a stable decrease in optical density was used to measure enzymatic activity (Singh and Srivastava 1986).

Data analysis

The data were organized using Microsoft Excel 2007. The data values are presented as means ± SE. The data were analyzed statistically using a two-way analysis of variance in SPSS (Version 13.0, SPSS, Chicago, IL, USA) and significant differences among treatment means were detected at $P < 0.05$ in accordance with Duncan's method. SigmaPlot 10.0 was used to construct the graphics.

Results

Changes in plant growth and total C and N contents

Under LN-stress conditions, the plant heights, Up and Under FWs and Up and Under DWs of cultivated soybean

Table 1: Chemical composition of CK, N1, N2 & N3

Characteristics	Chemical reagents	10 L (1/2)	10 L (1/3)	10 L (1/4)	Total mass (g)
		Weighed mass (g)	Weighed mass (g)	Weighed mass (g)	
1	$Ca(NO_3)_2.4H_2O$	41.035	27.360	20.520	88.910
2	$MgSO_4.7H_2O$	61.620	61.620	61.620	184.860
3	KH_2PO_4	27.220	27.220	27.220	81.660
4	KNO_3	25.280	16.850	12.640	54.770
5	Na-EDTA	7.450	7.450	7.450	22.350
6	$FeSO_4.7H_2O$	5.570	5.570	5.570	16.710
	H_3BO_3	2.860	2.860	2.860	8.580
	$MnSO_4$	1.015	1.015	1.015	3.045
	$CuSO_4.5H_2O$	0.079	0.079	0.079	0.237
	$ZnSO_4.7H_2O$	0.220	0.220	0.220	0.660
Substitute reagent	H_2MoO_4	0.090	0.090	0.090	0.270
$Ca(NO_3)_2.4H_2O$					
KNO_3	$CaCl_2.2H_2O$	25.550	34.060	38.320	97.930

Preparation of low nitrogen stress solution (10 times solution)

Common Hoagland Nutrient Solution Formula

Number	Name of drug	100 times mother liquor ($g \cdot L^{-1}$)	1 times the amount ($mL \cdot L^{-1}$)	100 times mother liquor ($g \cdot L^{-1}$)	1 times the amount ($mL \cdot L^{-1}$)
1	$Ca(NO_3)_2$	82.07 g	10	410.35g	2
2	$MgSO_4 \cdot 7H_2O$	61.62 g	10	308.10g	2
3	KH_2PO_4	27.22 g	10	136.10g	2
	KNO_3	50.56 g	10	252.80g	2
4	Fe-EDTA	Na-EDTA 7.45 g; $FeSO_4 \cdot 7H_2O$ 5.57 g			1
5	Trace elements	H_3BO_3 2.860 g; $MnSO_4$ 1.015 g; $CuSO_4 \cdot 5H_2O$ 0.079 g; $ZnSO_4 \cdot 7H_2O$ 0.220 g; H_2MoO_4 0.090 g			1

Table 2: Biomass changes of wild soybean and cultivated soybean under control and three different intensities of low nitrogen stress

Soybeans	Growth parameters	Treatments						
		CK	N1	N2	N3	$\log_2^{(N1/CK)}$	$\log_2^{(N2/CK)}$	$\log_2^{(N3/Ck)}$
	Shoot height (cm)	120.00 ± 10.00	135.00 ± 20.21	130.33 ± 4.67	115.00 ± 43.00	0.17	0.12	-0.15
	Root length (cm)	30.50 ± 2.50	33.67 ± 0.88	38.67 ± 4.70	51.00 ± 1.50	0.14	0.34	0.74*
W	Fresh weight of shoots (g)	57.50 ± 1.90	55.33 ± 4.05	51.93 ± 3.20	50.55 ± 11.35	-0.06	-0.15	-0.54
	Dry weight of shoots (g)	9.98 ± 0.40	9.24 ± 0.70	8.98 ± 0.71	7.28 ± 0.20	-0.11	-0.15	-1.22**
	Fresh weight of roots (g)	13.80 ± 1.50	13.33 ± 1.62	15.83 ± 1.33	12.15 ± 1.95	-0.05	0.20	-0.18
	Dry weight of roots (g)	1.37 ± 0.26	1.13 ± 0.12	1.25 ± 0.15	1.07 ± 0.19	-0.27	-0.14	-0.36
	Shoot height (cm)	59.50 ± 1.50	54.30 ± 2.30	61.70 ± 4.90	51.00 ± 5.00	-0.13	0.05	-0.17
	Root length (cm)	34.50 ± 0.50	32.67 ± 3.93	34.00 ± 5.57	28.00 ± 3.00	-0.08	-0.02	-0.30
M	Fresh weight of shoots (g)	65.30 ± 13.60	69.93 ± 3.17	75.27 ± 5.78	56.60 ± 6.90	0.10	0.21	-0.21
	Dry weight of shoots (g)	11.53 ± 2.76	11.55 ± 0.97	13.05 ± 1.22	6.50 ± 1.57	0.002	0.18	-0.14
	Fresh weight of roots (g)	23.55 ± 4.85	21.00 ± 1.15	21.33 ± 1.42	18.00 ± 1.30	-0.17	-0.14	-0.09
	Dry weight of roots (g)	3.43 ± 0.74	2.62 ± 0.11	2.79 ± 0.34	2.80 ± 0.41	-0.39	-0.30	-0.29

decreased, that correlated with the increase in the LN stress' intensity (Table 2). Under high-intensity stress conditions, decrease was by 10.92, 9.00, 13.26, 6.14 and 10.83%, respectively; however, the decreasing trends in plant heights, Up and Under FWs, and Up and Under DWs of wild soybean did not reach a significant level ($P > 0.05$) and as the LN-stress intensity increased, there were no significant differences among the treated tissues. The root length of wild soybean increased significantly ($P < 0.05$) and the root-shoot ratio showed an increasing trend, but there were no such increasing trends in cultivated soybean. Compared to CK, there was a slight increase in the nodule weights in the two soybean genotype groups, but the difference was not significant ($P > 0.05$). Under stress, the contents of C and N in the young and old leaves of the two genotypes of soybean were lower than CK (Table 3). In addition, as the LN stress intensity increased, the C and N contents decreased gradually. Under high-intensity stress conditions, the C contents of wild soybean young and old leaves decreased by 6.06 and 18.93%, respectively, and the N contents decreased by 54.4 and 31.2%, respectively. The C contents of cultivated soybean young and old leaves decreased by 39.53 and 23.84%, respectively, and the N contents decreased by 55.5 and 39.6%, respectively. Compared with the old leaves, the decrease in the N content of the young leaves was greater. As the LN-stress intensity increased, the degrees of C and N decreases in wild soybean was less than in cultivated soybean.

Changes in photosynthetic traits

As the stress intensity increased, P_N and Cr values in young and old leaves of cultivated soybean showed decreasing trends (Fig. 1). Under high-intensity stress conditions, compared with the CK, the P_N values of cultivated soybean young and old leaves decreased significantly ($P < 0.05$), while in wild soybean young and old leaves also decreased, but not significantly ($P > 0.05$). Among the three different

Table 3: Carbon and nitrogen contents in young and old leaves of two soybean varieties under control and three different intensities of low nitrogen stress

Leaf age		Total content (%)								Fold changes Log_2 (N1/CK)		Fold changes Log_2(N2/CK)		Fold changes Log_2(N3/CK)	
		M				W									
		CK	N1	N2	N3	CK	N1	N2	N3	M	W	M	W	M	W
YL	Nitrogen	4.49 ± 0.11	3.13±0.09	4.67±0.06	1.99±0.16	4.74±0.08	2.47±0.17	4.19±0.06	2.08±0.07	-0.52**	-0.94**	0.06	-0.18**	-1.17**	-1.19**
	Carbon	41.61±0.81	36.18±0.33	44.39±0.25	39.09±1.38	41.28±0.58	22.83±1.72	40.74±0.34	24.96±0.56	-0.20**	-0.85**	0.10*	-0.02	-0.09	-0.73**
	C/N	9.27	11.57	9.500	19.58	8.71	9.22	9.72	11.99	0.32	0.08	0.04	0.16	1.08	0.38
OL	Nitrogen	3.82±0.04	2.01±0.25	3.65±0.11	2.31±0.11	3.44±0.07	2.54±0.05	3.76±0.05	2.37±0.06	-0.93**	-0.44**	-0.07*	0.13*	-0.73**	-0.54**
	Carbon	42.80±0.03	39.01±1.83	42.24±0.24	34.84±0.19	40.62±0.40	32.19±0.62	41.52±0.11	30.93±0.67	-0.13	-0.34**	-0.02	0.03	-0.30**	-0.40**
	C/N	11.19	19.40	11.59	15.08	11.81	12.69	11.05	13.08	0.80	0.10	0.05	-0.10	0.43	0.15

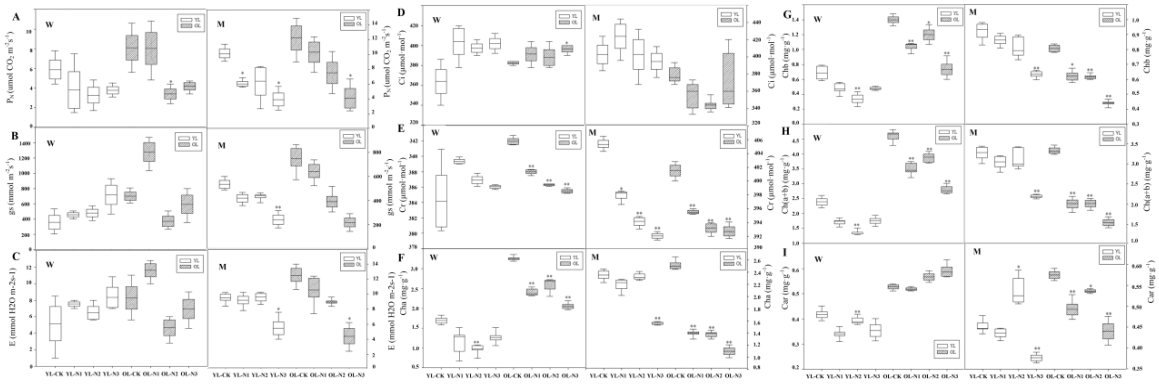

Fig. 1: The changes in photosynthetic characteristics in young and old leaves from wild and cultivated soybean under control and N deficiency

(A) pN: net photosynthetic rate, (B) gs: stomatal conductance, (C) E: transpiration rate, (D) Ci: CO_2 concentration under stomata, (E) Cr: atmospheric CO_2 concentrations, (F) Chl *a*: chlorophyll *a*, (G) Chl *b*: Chlorophyll *b*, (H) Chl a+b: chlorophyll a + chlorophyll b, (I) Car: carotenoid, *and ** indicate significant ($P < 0.05$) and highly significant ($P < 0.01$) differences

intensity LN-stress treatments, Cr values in cultivated soybean young and old leaves decreased significantly ($P < 0.05$), but not in wild soybean young leaves ($P > 0.05$). Gs and E values increased in wild soybean young leaves, but decreased in wild soybean old leaves. The Gs and E values of the young leaves and old leaves of the cultivated soybean plant showed a downward trend. With the increase of stress intensity, the Ci of wild soybean young and old leaves increased, while the Ci of cultivated soybean leaves decreased. As the LN-stress intensity increased, the Chl *a*, Chl *b*, and Chl (a+b) contents in the young and old leaves of the two soybean genotypes showed gradual decreases compared with the CK. Under high-intensity stress conditions, the Chl *a*, Chl *b* and Chl (a+b) contents in wild soybean young leaves decreased by 25, 32.4 and 27.5%, respectively, while in the old leaves they decreased by 36.8, 47.1 and 39.9%, respectively, compared with the CK. Correspondingly, their contents in cultivated soybean young leaves decreased by 33.72, 31.34 and 33.05%, respectively, while in the old leaves they decreased by 56.10, 45.32 and 53.47%, respectively. Compared with the CK, the Chl *a*, Chl *b* and Chl (a+b) contents decreased more in the old leaves than in the young leaves. As the LN-stress intensity increased, the Car content showed decreased in both young and old leaves of cultivated soybean and under high-intensity stress, the Car contents of cultivated soybean young and old leaves decreased significantly ($P < 0.05$).

Ion content changes

With the increase in the LN-stress intensity, the Na^+ content of the young cultivated soybean leaves decreased significantly ($P < 0.05$) and the Na^+ content of the young wild soybean leaves and old leaves increased significantly (Table 4). Under high-intensity stress, the Na^+ content in cultivated soybean young leaves significantly ($P < 0.01$) decreased compared with the CK group, but wild soybean is not like that. The K^+ contents decreased significantly in wild soybean young and old leaves under high-intensity stress conditions ($P < 0.05$). The SO_4^{2-} content decreased in wild soybean young leaves, increased in wild soybean old leaves, but increased in both cultivated soybean young and old leaves. Compared with the CK, the P^{5+}, PO_4^{2-} and B^{3+}contents increased and the Mg^{2+} and the NO_3^- content in the young and old leaves of the two genotypes of soybeans showed a downward trend. P^{5+} content in wild soybean young leaves increased significantly under high-intensity LN stress ($P < 0.05$), but there was no such phenomenon in cultivated soybean. The growth of young leaves of B^{3+} is much higher than that of cultivated soybean. The content of NO_3^- wild soybean in young leaves and old leaves was lower than cultivated soybean, and young leaves were lower than old leaves. The decrease in Mg^{2+} content in young wild soybean leaves was less than that in cultivated soybean young leaves. As the stress intensity increased, the $C_2O_4^-$contents in wild soybean and cultivated soybean

young leaves increased, and under high-intensity stress conditions, the $C_2O_4^-$ content in wild soybean young leaves showed a significant increase compared with the CK. With the increase of stress intensity, the content of Fe^{3+}, Mn^{2+} and Zn^{2+} in wild soybean leaves was significantly higher than that in cultivated soybean leaves. More useful ions are accumulated in new leaves than in old leaves. As the stress intensity increased, the extent of the ion content decrease in cultivated soybean was greater than in wild soybean.

Changes in the key N metabolism-related enzymatic activities

As the LN-stress intensity increased, the NR and GOT activities increased in wild soybean young leaves, but decreased in cultivated soybean young leaves, compared with CK (Fig. 2). NR and GPT activities increased in wild soybean young leaves, but decreased in old leaves. The NR, GS and GOT activities decreased in cultivated soybean young and old leaves. The GPT activity increased in cultivated soybean young leaves, but there was no significant change in old leaves ($P > 0.05$). The level of increase in GPT activity was greater in wild soybean than in cultivated soybean young leaves. The GDH activities decreased in the young and old leaves of the two soybean genotypes, but the changes were not significant ($P > 0.05$). GOGAT activity in wild soybean young leaves decreased, while GOGAT activity increased in wild soybean old leaves and GOGAT activity increased in cultivated soybean young leaves and old leaves. In the two soybean genotypes, the NR activities in the young leaves were significantly higher than in the old leaves. The activities of NR and GS in old leaves of two soybean genotypes were significantly reduced under high-intensity stress ($P < 0.01$). The GOT activity of cultivated soybean young leaves is significantly reduced ($P < 0.05$). The GPT activity significantly increased in wild soybean young leaves ($P < 0.05$), but not in cultivated soybean young leaves. As the stress intensity increased, the key enzymes maintained higher activities in the young leaves compared with the old leaves. As the LN stress intensity increased, the activities of these key enzymes were higher in wild soybean than that in cultivated soybean.

Discussion

N is an essential nutrient for crop growth and development, and LN stress can inhibit plant development (Boussadia *et al*. 2010). This experiment showed that the growth rates and biomasses of two soybean genotypes were severely inhibited under different degrees of LN stress, especially in cultivated soybean. The root length of wild soybean increased, indicating that wild soybean can resist the LN stress. The number of nodules in the two genotypes of soybean seedlings is very limited and the nodule weight does not change significantly under the conditions of CK and LN, which is in agreement with previous studies (Li *et al*. 2018). The total C content (%) and total N content (%) showed that cultivated soybean young and old leaves had a worse nitrogen and N deficiencies than wild soybean, confirming that the latter had a stronger tolerance to LN stress than cultivated soybean. These results confirmed that wild soybean was more tolerant to LN stress than cultivated soybean.

The photosynthetic capacity of a leaf is related to the N content, mainly because the proteins and thylakoids in the Calvin cycle form the majority of N present in leaves (Evans 1989). However, the photosynthesis of crops can be reduced under LN-stress conditions (Cechin and Fumis 2004). Here, under different degrees of LN stress, P_N decreased in the two soybean genotypes, especially under high-intensity stress. The P_N values decreased in the young and old leaves of cultivated soybean, indicating that its inhibition in cultivated soybean is greater under LN-stress conditions. Both g_s and Ci are related to plant transpiration intensity, stomatal opening, and the ability of mesophyll cells to assimilate CO_2 (Mohamed *et al*. 2017). In this experiment, the change trends of Ci and g_s were consistent, with both decreasing in cultivated soybean young and old leaves. The P_N in cultivated soybean decreased as a result of stomatal limitation. The change trends of g_s and Ci in wild soybean old leaves were inconsistent, with g_s decreasing and Ci increasing, which indicated that the decrease in wild soybean's P_N was the result of nonstomatal factors. The increase of E in wild soybean young leaves promoted the transport of water and inorganic salts, as well as the migration and transportation of ions (Li *et al*. 2019). The Chl *a*, Chl *b*, and Chl (a+b) contents decreased as the LN-stress intensity increased. The Chl contents of the two soybean genotypes decreased under different degrees of LN stress, and the Chl contents in wild soybean young and old leaves decreased less than in cultivated soybean. A decrease in the photosynthetic pigment content can cause a decrease in P_N, which indicates that the inhibition of wild soybean's P_N was weaker under LN-stress conditions. Between young and old leaves, the extent of the Chl content's decrease was greater in the latter, indicating that LN stress inhibited the P_N levels of old leaves to a greater extent than young leaves. Wild soybean can increase Car accumulation to resist abiotic stress (Li *et al*. 2018). Here, the Car contents decreased significantly in cultivated soybean young and old leaves ($P < 0.05$) and the contents also decreased in wild soybean young and old leaves, but not significantly ($P > 0.05$), which corroborated previous results. Thus, the inhibitory effect of LN stress on the light contracting function was stronger in cultivated soybean than wild soybean.

An insufficient N supply leads to a reduction in amino acids, proteins and other N-containing compounds, disrupting multiple energy and substances' metabolic pathways. This results in changes in plant energy supply and multiple nutrient transporter activities, ultimately leading to changes in the absorption and distribution of nutrient

Table 4: Ion contents in young and old leaves of two soybean genotypes under control and three different intensities of low nitrogen stress

Young leaves	concentration (mmol. L^{-1})								Fold changes					
	M				W				$Log_2^{(N1/CK)}$		$Log_2^{(N2/CK)}$		$Log_2^{(N3/CK)}$	
	CK	N1	N2	N3	CK	N1	N2	N3	M	W	M	W	M	W
NO_3^-	2.78 ± 0.00	3.62 ± 0.09	3.26 ± 0.08	1.29 ± 0.00	2.26 ± 0.11	5.94 ± 0.29	3.16 ± 0.08	1.40 ± 0.01	0.38**	1.40**	0.23**	0.49**	-1.10**	-0.69**
$H_2PO_4^-$	1.62 ± 1.53	1.53 ± 0.06	1.90 ± 0.02	2.46 ± 0.30	1.86 ± 0.26	1.95 ± 0.11	1.82 ± 0.04	3.20 ± 0.97	-0.09**	0.07**	0.23**	-0.03**	0.60	0.78**
SO_4^{2-}	1.73 ± 0.42	2.15 ± 0.08	1.20 ± 0.03	1.81 ± 2.25	2.61 ± 0.20	2.54 ± 0.00	1.81 ± 0.04	2.36 ± 0.70	0.31**	-0.04**	-0.52**	-0.53**	0.07	-0.15
$C_2O_4^{2-}$	1.37 ± 0.02	0.73 ± 0.00	0.43 ± 0.00	1.47 ± 0.03	1.03 ± 0.06	0.57 ± 0.02	0.54 ± 0.00	1.72 ± 0.02	-0.91**	-0.85**	-1.66**	-0.92**	0.09	0.74**
Cl^-	1.28 ± 0.01	0.88 ± 0.02	1.82 ± 0.05	1.84 ± 0.02	2.43 ± 0.13	1.73 ± 0.34	2.03 ± 0.02	4.47 ± 0.08	-0.54	-0.49	0.51	-0.25	0.52	0.88
Na^+	2.79 ± 0.01	2.12 ± 0.04	2.27 ± 1.13	1.52 ± 0.06	2.15 ± 0.15	2.19 ± 0.03	2.26 ± 0.00	2.05 ± 0.05	-0.39**	0.03	-0.30	0.08	-0.87**	0.06
Mg^{2+}	24.63 ± 1.29	19.89 ± 0.97	19.72 ± 7.24	20.39 ± 0.31	28.30 ± 0.79	23.42 ± 0.08	23.56 ± 0.10	26.48 ± 0.13	-0.31	-0.27	-0.32	-0.26	-0.27	-0.10
Mn^{2+}	0.14 ± 0.01	0.15 ± 0.01	0.23 ± 0.00	0.12 ± 0.00	0.09 ± 0.00	0.13 ± 0.01	0.14 ± 0.00	0.13 ± 0.00	0.18	0.52	0.76	0.63	-0.15	0.59**
B^{3+}	0.33 ± 0.00	0.54 ± 0.03	0.88 ± 0.11	0.69 ± 0.01	0.35 ± 0.01	1.68 ± 0.02	1.39 ± 0.01	1.62 ± 0.00	0.71**	2.15	1.41**	1.87	1.05**	2.22**
Fe^{3+}	0.06 ± 0.00	0.06 ± 0.01	0.13 ± 0.01	0.08 ± 0.00	0.05 ± 0.00	0.08 ± 0.00	0.03 ± 0.00	0.12 ± 0.00	1.00	0.49	1.12	0.58	0.43*	1.17**
Ca^{2+}	11.66 ± 0.39	10.06 ± 0.44	13.80 ± 0.83	15.41 ± 0.14	14.60 ± 0.41	11.92 ± 0.02	12.79 ± 0.10	14.00 ± 0.05	-0.21	-0.29	0.24*	-0.19	0.40**	-0.06
K^+	144.34 ± 4.49	134.37 ± 4.34	146.62 ±9.79	152.64±2.08	191.66±5.18	190.22±1.11	168.46±1.45	169.57±0.30	-0.10	-0.01	0.02	-0.19	0.08	-0.18*
P^{5+}	35.01 ± 1.78	31.37 ± 0.19	35.97 ± 2.30	39.01 ± 0.38	41.81 ± 0.68	48.61 ± 0.23	49.90 ± 0.07	57.06 ± 0.13	-0.16	0.22	0.04	0.26	0.16	0.45**
Zn^{2+}	0.22 ± 0.21	0.23 ± 0.24	0.23 ± 0.28	0.24 ± 0.29	0.14 ± 0.01	0.28 ± 0.00	0.23 ± 0.00	0.24 ± 0.00	0.06	1.06	0.06	0.78	0.13	0.78
NO_3^-	2.78 ± 0.00	3.62 ± 0.09	3.26 ± 0.08	1.29 ± 0.00	2.26 ± 0.11	5.94 ± 0.29	3.16 ± 0.08	1.40 ± 0.01	0.38**	1.40**	0.23**	0.49**	-1.10**	-0.69**
$H_2PO_4^-$	1.62 ± 1.53	1.53 ± 0.06	1.90 ± 0.02	2.46 ± 0.30	1.86 ± 0.26	1.95 ± 0.11	1.82 ± 0.04	3.20 ± 0.97	-0.09**	0.07**	0.23**	-0.03**	0.60	0.78**
SO_4^{2-}	1.73 ± 0.42	2.15 ± 0.08	1.20 ± 0.03	1.81 ± 2.25	2.61 ± 0.20	2.54 ± 0.00	1.81 ± 0.04	2.36 ± 0.70	0.31**	-0.04**	-0.52**	-0.53**	0.07	-0.15

Old leaves	concentration (mmol. L^{-1})								Fold changes					
	M				W				$Log_2^{(N1/CK)}$		$Log_2^{(N2/CK)}$		$Log_2^{(N3/CK)}$	
	CK	N1	N2	N3	CK	N1	N2	N3	M	W	M	W	M	W
NO_3^-	4.94 ± 0.04	6.72 ± 0.14	2.93 ± 0.03	1.08 ± 0.04	3.96 ± 0.04	2.21 ± 0.14	1.25 ± 0.00	0.92 ± 0.04	0.44**	-0.84**	-0.75**	-1.66**	-2.19**	-2.10**
$H_2PO_4^-$	2.00 ± 0.14	1.49 ± 0.02	1.70 ± 0.00	2.73 ± 0.66	2.06 ± 0.04	1.90 ± 0.15	2.56 ± 0.08	4.60±0.66**	-0.43**	-0.11**	-0.24**	0.32**	0.45**	1.16**
SO_4^{2-}	1.23 ± 0.15	3.03 ± 0.03	2.33 ± 0.02	2.80 ± 0.11	2.16 ± 0.01	3.33 ± 0.27	2.35 ± 0.04	2.79 ± 0.11	1.30**	0.63*	0.93**	0.13*	1.19**	0.37*
$C_2O_4^{2-}$	1.05 ±0.04	0.51 ± 0.05	0.44 ± 0.11	1.14 ± 0.04	1.33 ± 0.02	0.46 ± 0.01	0.77 ± 0.00	1.34 ± 0.04	-1.03**	-1.52**	-1.24**	-0.79**	0.12	0.01
Cl^-	2.25 ± 0.01	2.31 ± 0.02	3.06 ± 0.09	3.10 ± 0.04	2.49 ± 0.21	3.21 ± 0.22	3.43 ± 0.05	7.77 ± 0.28	0.04	0.37	0.44	0.46	0.46	1.64
Na^+	1.03 ± 0.03	1.56 ± 0.16	1.62 ± 0.01	2.40 ± 0.06	1.01 ± 0.05	2.05 ± 0.07	3.88 ± 0.75	1.82 ± 0.06	0.60*	1.02	0.66**	1.94	1.22	0.85
Mg^{2+}	45.05 ± 0.75	40.76 ± 0.75	34.88 ± 1.54	38.95 ± 0.18	46.4 ± 0.12	37.90 ± 0.20	30.04 ± 0.68	33.28 ± 0.18	-0.14*	-0.29	-0.37**	-0.63	-0.21*	-0.48
Mn^{2+}	0.17 ± 0.01	0.23 ± 0.00	0.22 ± 0.01	0.19 ± 0.04	0.12 ± 0.00	0.21 ± 0.01	0.20 ± 0.01	0.16 ± 0.04	0.47**	0.83	0.41**	0.77	0.21	0.46
B^{3+}	0.86 ± 0.02	3.31 ± 0.07	4.15 ± 0.16	5.21 ± 0.05	1.09 ± 0.03	5.74 ± 0.10	4.79 ± 0.06	4.86 ± 0.05	1.94**	2.40	2.27**	2.14	2.60**	2.16
Fe^{3+}	0.10 ± 0.03	0.07 ± 0.00	0.07 ± 0.01	0.09 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.09 ± 0.00	-0.52	0.02	-0.53	0.34	-0.15	0.98
Ca^{2+}	59.14 ± 0.55	59.24 ± 0.40	44.15 ± 1.13	48.22 ± 0.07	48.96 ± 0.14	43.87 ± 0.47	27.27 ± 0.90	31.32 ± 0.07	0.01	-0.16	-0.42**	-0.84	-0.29**	-0.64
K^+	152.58 ± 2.48	149.48 ± 3.00	150.37 ± 7.24	139.95 ± 0.70	163.44 ± 0.07	170.07±0.09	162.14±5.60	172.59±0.07	-0.03	0.06	-0.02	-0.01	-0.12	0.08
P^{5+}	27.36 ± 0.05	26.85 ± 0.14	33.38 ± 0.83	47.0 8 ±0.04	32.87 ± 0.12	36.64 ± 0.88	52.92 ± 2.30	70.81 ± 0.04	-0.03*	0.16	0.29**	0.69	0.78**	1.11
Zn^{2+}	0.18 ± 0.20	0.19 ± 0.20	0.22 ± 0.24	0.24 ± 0.22	0.09 ± 0.00	0.26 ± 0.00	0.26 ± 0.01	0.35 ± 0.00	0.08	1.48	0.29	1.51	0.42	1.93
NO_3^-	2.78 ± 0.00	3.62 ± 0.09	3.26 ± 0.08	1.29 ± 0.00	2.26 ± 0.11	5.94 ± 0.29	3.16 ± 0.08	1.40 ± 0.01	0.38**	1.40**	0.23**	0.49**	-1.10**	-0.69**
$H_2PO_4^-$	1.62 ± 1.53	1.53 ± 0.06	1.90 ± 0.02	2.46 ± 0.30	1.86 ± 0.26	1.95 ± 0.11	1.82 ± 0.04	3.20 ± 0.97	-0.09**	0.07**	0.23**	-0.03**	0.60	0.78**
SO_4^{2-}	1.73 ± 0.42	2.15 ± 0.08	1.20 ± 0.03	1.81 ± 2.25	2.61 ± 0.20	2.54 ± 0.00	1.81 ± 0.04	2.36 ± 0.70	0.31**	-0.04**	-0.52**	-0.53**	0.07	-0.15

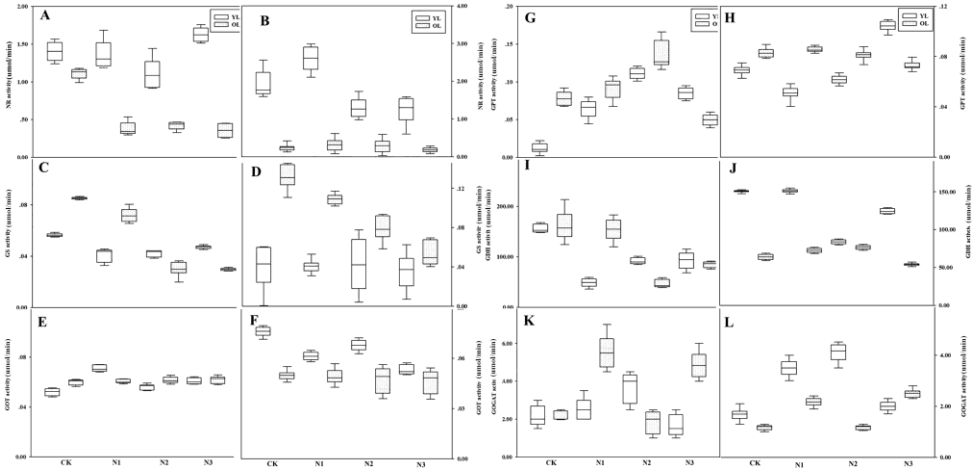

Fig. 2: The changes in nitrogen metabolism enzyme activities in young and old leaves from wild and cultivated soybean under control and N deficiency

(**A**) NR: nitrate reductase, (**C**) GS: glutamine synthetase, (**E**) GOT: aspartate aminotransferase, (**B**) NR: nitrate reductase, (**D**) GS: glutamine synthetase, (**F**) GOT: aspartate aminotransferase, (**G**) GPT: alanine aminotransferase, (**I**) GDH: glutamate dehydrogenase, (**K**) GOGAT: glutamate synthase, (**H**) GPT: alanine aminotransferase, (**J**) GDH: glutamate dehydrogenase, (**L**) GOGAT: glutamate synthase. *and ** indicate significant ($P < 0.05$) and highly significant ($P < 0.01$) differences, respectively

elements (Quan *et al*. 2016). In this study, under LN-stress conditions, especially high-intensity stress, the P^{5+} content increased in the young and old leaves of the two soybean genotypes, the Na^+ content increased in wild soybean young leaves, but decreased significantly in cultivated soybean young leaves, the K^+ content decreased significantly in

young and old leaves of wild soybean but increased in young leaves of cultivated soybean and the Mg^{2+} content of young soybean leaves of the two genotypes all showed a downward trend and the Mg^{2+} content of young soybean leaves of cultivated soybean type soybean decreased more obviously. Compared with the control, the content of Fe^{3+}, Mn^{2+}, B^{3+} and Zn^{2+} in wild soybean young leaves was significantly higher than that in cultivated soybean young leaves. K^+ participates in very important metabolic activities in plants and plays important roles in regulating ion balance and in maintaining cell turgor, ribosome function, and protein synthesis (Fan *et al*. 2009). Na^+ can inhibit K^+ absorption (Li *et al.* 2019). In this experiment, as the LN-stress intensity increased, Na^+ accumulated, but the K^+ content decreased in wild soybean young and old leaves. However, the K^+ content was much greater than the Na^+ content, indicating that wild soybean maintained a high K^+ /Na^+ value to resist abiotic stress, which was consistent with a previous study (Li *et al*. 2019). Here, the K^+ content in cultivated soybean young leaves increased, but it decreased in the old leaves, which indicated that K^+ can be transferred from the cultivated soybean old leaves to young leaves, thereby regulating their ion balance and maintaining the growth and development of young leaves. Wild soybean maintained a certain Mg^{2+} content to produce more photosynthetic pigments, which reduces the damage to photosynthesis (Han *et al*. 2013). Fe^{3+} is a carrier in the electron transport of photosynthesis, and it participates in photosynthesis and N fixation, protecting the photosynthetic apparatus and maintaining normal growth (Cakmak 2000). Mn^{2+} as an enzyme activator, participates in many metabolic processes by regulating enzymatic activities, such as glycolysis and TCA, and the Mn^{2+}content in wild soybean young leaves was significantly higher than in cultivated soybean, probably because the former can maintain responses to life processes by increasing enzymatic activities in the absence of N (He and Liu 2010). Zn^{2+} is an important component of Chl synthesis, and it contributes to the ability of plants to tolerate environmental stress factors (Koshiba 2009). In this study, the NO_3^- content of the young and old leaves of the two soybean genotypes were significantly reduced under stress conditions, but the former decreased to a lesser extent. This corroborates previous results in which NO_3^- was transferred from old leaves to young leaves, and this may be closely related to LN tolerance (Aoyama *et al*. 2014). Our research shows that wild soybean can remain relatively stable levels of beneficial ion transport and migration, as well as a stable nutritional balance, especially in young leaves, which plays an important role in maintaining the growth and development of plants under LN-stress conditions.

N metabolism regulation includes N uptake, N assimilation, ammonia assimilation, and amino acid metabolism (Wang *et al*. 2019). Key enzymes of N metabolism are important regulatory factors of plant ammonia assimilation and amino acid synthesis (Wang *et al*. 2012b). Here, as the LN-stress intensity increased, especially under high-intensity stress, NR and GOT activities increased in wild soybean young leaves, however, compared with CK, the number of young cultivated soybean leaves is reduced. The opposite was true for the GOGAT activity. The extent of the increase in GPT in wild soybean young leaves was greater than in cultivated soybean. The activity levels of GS and GDH in wild soybean young leaves decreased more than cultivated soybean young leaves. Young leaves have increased NR and GPT activities, while old leaves have decreased NR and GPT activities. In the two soybean genotypes, the NR activity of young leaves was significantly higher than that of old leaves. Under LN-stress conditions, plants may synthesize more secondary metabolites to resist the oxidation of reactive oxygen species and alleviate the environmental stress (He *et al*. 2010). Additionally, as the enzymatic activity of assimilated $N0_3^-$increases, the key enzymatic activity of synthetic amino acids decreases, and most of the NH_4^+ assimilated by the GS/GOGAT pathway does not combine with the C chain produced by photosynthesis to form primary metabolite amino acids, but is used to synthesize secondary metabolites, such as non-protein N. The remaining C chains are used to synthesize secondary metabolites, such as polyols and the unfavorable growth conditions lead to polyol accumulation. Here, the NR activity increased in wild soybean young leaves, which is consistent with results of previous studies; however, whether NR can alleviate the LN stress remains to be determined (Li *et al.* 2018). The GPT activity increased in wild soybean young leaves, but decreased in old leaves and the GPT enzyme could regulate young leaves to control old leaves, which affected amino acid metabolism and nutrient transport. The decrease in the GDH activity of wild soybean young leaves was greater than that of cultivated soybean, showing less glutamate synthesis in wild soybean and lower amino acid metabolism and metabolic energy consumption levels in wild soybean, which allows other sources of energy to be used for the survival and growth of wild soybean. This may be a strategy to increase the LN tolerance of wild soybean. The regulation of GDH plays a unique physiological role in the processes of plant stress and aging (Cai *et al*. 2016). Our experiments indicated that wild soybean could resist LN stress by regulating the activities of key enzymes involved in amino acid synthesis, producing more non-protein N and reducing the energy consumption of amino acid metabolism. The enzymatic activity in young leaves was higher than in old leaves, especially under low-intensity stress, and the young leaves could maintain higher N assimilation levels, which is beneficial to N reuse.

C and N metabolism are tightly coupled in the plant life-related processes (Wei *et al*. 2019) (Fig. 3). The reasonable regulation of C and N metabolism by plants has significance in the integrated processes of regulating plant growth, development, and stress (Zhu *et al*. 2015). The results showed that under high-intensity stress, compared with the CK, the NR activities in the old leaves of wild

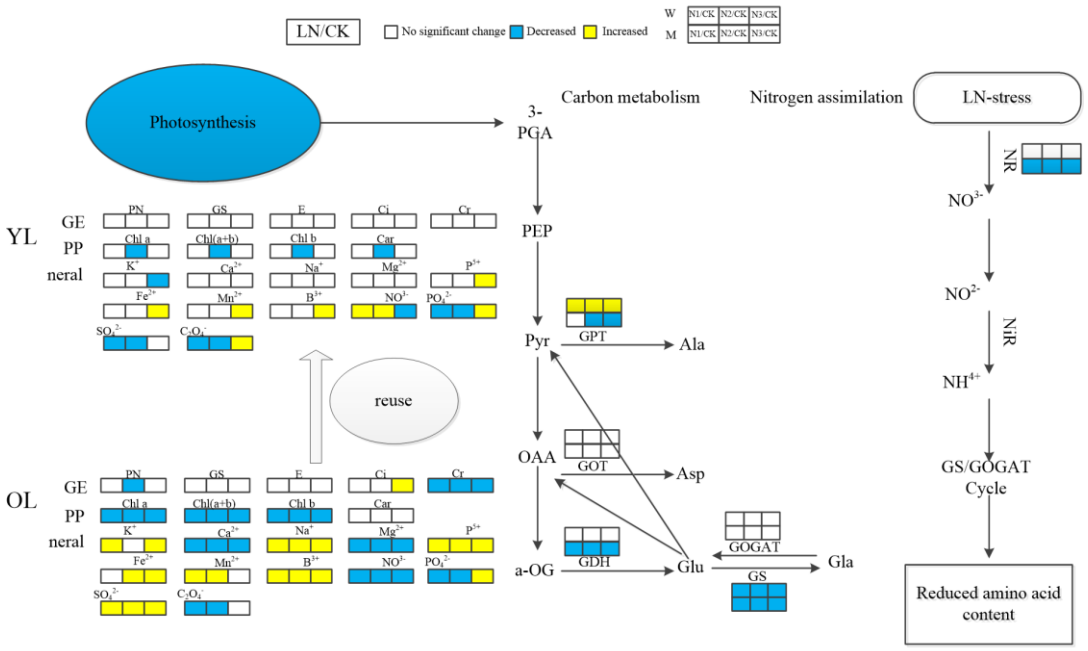

Fig. 3: Comprehensive simplified model of carbon and nitrogen metabolism
Note: This program summarizes the most important points of interaction between carbon and nitrogen metabolism. a-KZ, a-ketoglutaric acid; Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Gln, glutamine; OAA, oxaloacetate; PEP, phosphoenolpyruvate; Pry, pyruvic acid

soybean and cultivated soybean decreased significantly. This corroborated previous results in which the cyclic electron transport was enhanced under environmental stress conditions and the generation of NADPH was reduced, which leads to less of NR electron donors and inhibition. The important organic acids in N metabolism, α-OG, OAA, and pyr are derived from important C metabolism cycles, such as the Calvin cycle and TCA (Singh and Srivastava 1986). The synthesis of photosynthetic pigments and the photosynthetic C metabolism cycle depend on glutamic acid and various enzymes provided by N metabolism, and C and N metabolism also require common reduction sources, NADPH, ATP and C skeletons, which compete with each other (Jiang *et al*. 2019). C and N metabolism are highly correlated in the young and old leaves of plants (Zhu *et al*. 2015). Under abiotic stress conditions, young leaves maintain relatively stable C metabolism by maintaining stable pigment accumulation, increasing photosynthesis processes, and energy consumption, while C metabolism is highly reduced in old leaves (Hu *et al*. 2019). Additionally, young leaves have greater abilities to maintain C and N metabolism than old leaves (Hajlaoui *et al*. 2010). Improving the metabolism of polyols in leaves and the transport strategy of polyols from old leaves to young leaves, effectively increases the LN tolerance of wild soybean (Li *et al*. 2018). Under abiotic stress conditions, the effects on N metabolism in old leaves was greater than in young leaves (Wang *et al*. 2012a, b). Our study indicated that under LN-stress conditions, wild soybean young and old leaves maintained relatively stable balances of C and N metabolism, especially in the young leaves. A stable C and N metabolic balance maintains the stable production of C and N metabolites, which are transported from the old leaves to the young leaves to support the growth and development of young leaves and improve the LN tolerance of the leaves, thereby increasing plant resistance to LN stress.

Conclusion

The survival and growth of plants under LN environment depend on their physiological adjustments and metabolic changes, as well as the interactions of C and N metabolism at the cellular level. Under three different intensities of LN stress, the ability of wild soybean to resist LN stress was significantly greater than cultivated soybean, and this was closely correlated with its adaptation to a barren natural environment. This was determined based on the following: (1) The root to shoot ratio increased by increasing the root length; (2) The young and old leaves of wild soybean maintained a relatively stable P_N; (3) The young and old leaves of wild soybean maintained a stable nutrient balance, and Na^+, Fe^{3+}, Mn^{2+}, B^{3+} and Zn^{2+} in the old leaves were transported to the young leaves; and (4) The young leaves of wild soybean have increased the activity of GPT and decreased the activity of GDH, thus reduced energy consumption during amino acid metabolism. The young and old leaves of wild soybean maintained stable dynamic balances of C and N metabolism. This study provides a

theoretical basis for the utilization of wild soybean, the improvement of cultivated soybean, and the study of plant resistance to abiotic stresses. The results can be used to help improve sustainable agriculture.

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Author Contributions

YNH, XYL, and MXL planned and designed the research. YNH, XYL, SJG, YJH and JXG performed the experiments. YNH analyzed the data, and XYL, MXL, SJG, and LXS wrote the manuscript. YNH, XYL, and MXL contributed equally. All authors reviewed the manuscript.

Conflicts of Interest

The authors declare no confict of interest.

Data Availability

Data supporting the fndings of this study are available in the supplementary material of this article.

Ethics Approval

This article does not contain any studies with human participants or animals. The collection materials of the plants, complies the relevant institutional, national, and international guidelines and legislation.

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