Original Article

Carbon-Nitrogen Metabolic Responses and Adaptive Strategies to Low-Nitrogen Stress in *Glycine soja*

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Abstract

Nitrogen (N) is an essential mineral nutrient for plant growth and development. Wild soybean (*Glycine soja*), which has many superior traits, is an important germplasm resource and is also an excellent experimental material for researching the mechanisms of low-N tolerance. In this study, the physiological differences between common wild soybean (W1) and low-N tolerant wild soybean (W2) among growth characteristics, photosynthetic carbon (C) metabolism, N metabolism and C-N metabolic-coupling relationship were investigated, and the mechanism of low-N tolerance of wild soybean was explained at three different levels of low-N stress. Both W1 and W2 showed some resistance to low-level N stress. However, W2 could withstand the damage by increasing the root length and root–shoot ratio under high-level stress conditions. Moreover, when resisting low-N stress, W2 maintained a stable photosynthetic rate and coordinated ion balance to maintain required nutrient levels. W2 also tolerated low N by coordinating the C-N metabolic balance through the accumulation of soluble sugars to provide energy and C skeletons for N metabolism and through enhanced N metabolic enzyme activities and soluble protein accumulation levels to supply the enzyme proteins and photosynthetic pigments for C metabolism. The current results provide a physiological methodology and theoretical basis for protecting wild soybean germplasm resources and improving cultivated soybean.

Keywords: carbon-nitrogen mechanism; *Glycine soja*; low-nitrogen; physiology; stress

Introduction

Carbon (C) and nitrogen (N) are essential for plants to perform their routine and fundamental cellular activities during development (Sun *et al.*, 2013); therefore, adequate supplies are critical for plant growth and stress responses. C and N metabolism are basic processes of plant physiology. The intensity and dynamic changes directly affect the formation of photosynthetic products, transformation, mineral nutrient absorption and protein synthesis, which significantly impacts the growth and development of plants (Yu *et al.*, 2015). N, as the primary nutrient factor and a main component of plant cells, is a major limiting factor affecting crop C and N metabolism (Huang *et al.*, 2015).

The amount of N required by *Glycine soja* is high, and the N provided by the symbiotic rhizobia is insufficient to support the plant's demands for N (Ciocco *et al.*, 2011). Low N stress can affect the normal physiological and metabolic activities, limit the growth of seedlings, and reduce the biomass accumulation of soybean plants (Shah, 2017). Wild soybean can oppose the damage caused by saline-alkali stress by adjusting the ion content distribution using high tolerance- and low absorption-related mechanisms (Jiao *et al.*, 2018). The photosynthetic physiological characteristics of cultivated and wild soybean are affected significantly under low N stress. Wild soybean can increase the accumulation of carotenoids (*Car*s) to resist coercive damage (Li *et al.*, 2018). However, there are few reports on the C-N metabolism pathways and adaptive mechanisms of N tolerance among different types of wild soybeans.

Wild soybean is a leguminous soybean species that is an annual herb (Hao *et al.*, 2016). It is the wild relative of cultivated soybean and has a high protein content, strong stress tolerance and high propagative coefficient (Phang *et al.*, 2008). Wild soybean is the most effective resource for broadening the genetic basis of cultivated soybean breeding (Farag *et al.*, 2012; Xue *et al.*, 2014). In this study, we used common and low-N-tolerant wild soybean as experimental materials. We compared the effects of three different levels of low-N stress on growth, photosynthetic parameters and

enzyme activities related to N metabolism between the two wild soybean varieties in a sand culture experiment. By analyzing the response and adaptation processes of C-N metabolic-coupling relationship with low-N stress, the physiological mechanism of N tolerance in wild soybean was revealed. This study provides physiological references for the conservation and utilization of wild soybean germplasm resources by adapting the basic metabolism and physiology of wild soybean during environmental selection.

Materials and Methods

Plant materials and growth conditions

The seeds of wild soybean ('Huinan 06116', W1) and low N- tolerant wild soybean ('Tongyu 06311', W2), were provided by the Jilin Changchun Crop Germplasm Introduction and Breeding Center. The seedlings were grown in sand culture using cleaned and sieved river sand. Soybean seeds were arranged in 14-cm diameter pots with a bottom hole (2-cm diameter), with three seeds of a single line per pot. During the experiment, temperatures were 18.5 ± 1.5 °C and 26 ± 2 °C during the night and day, respectively, and the relative humidity was 60 ± 5%.

Stress treatments

The low N treatment was initiated after the plants grew their third leaves. In the low-N-treated group, W1 and W2 seeds were placed in three types of stress solutions: one half-strength (N1), one fourth-strength (N2) and one eighth-strength (N3) modified Hoagland's solution (Table 1). Calcium (Ca) and potassium (K) were supplied by $CaCl_2 \cdot 2H_2O$ and KCl, respectively, at equivalent concentrations in low-N Hoagland's solution for 2 weeks. In the check control (CK), both types of wild soybean seedlings were cultivated under normal conditions (1× Hoagland's solution). W1 and W2 were each divided into four groups: control, and N1, N2 and N3 treated. Each group consisted of eight pots: four pots for measuring growth parameters and photosynthesis, and four pots for ion content and enzyme activity analyses.

Measurement of growth indices

After the wild soybean plants were harvested, plant heights, root lengths, aboveground fresh weights (Up FWs), underground FWs (Root FWs), aboveground dry weights (Up DWs) and underground dry weights (Root DWs) were measured (Shao et al., 2016).

Measurement of ion contents

Dry 0.05 g samples were treated with 4 mL of deionized water at 100 °C for 40 min and then centrifuged at 3,000 g for 15 min. The supernatants were collected and the methods were repeated twice, with extracts queued up to 15 mL. Unified supernatants were used to determine SO_4^{2-}, nitrate (NO_3^-), $H_2PO_4^-$ and $C_2O_4^-$ concentrations using ion chromatography (DX-300 ion chromatographic system, AS4A-SC chromatographic column, CDM-II electrical conductivity detector, mobile phase: $Na_2CO_3/NaHCO_3$ = 1.7/1.8 mM; Dionex, Sunnyvale, CA, USA). An atomic absorption spectrophotometer (Super 990F, Beijing Purkinje General Instrument Co. Ltd. Beijing, China) was used to determine the concentrations of Zn^{2+}, Mn^{2+}, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, B^{3+} and Cu^{2+}.

Measurement of photosynthetic pigments

Two weeks after the stress treatment, the photosynthetic gas exchange parameters were determined using the fully expanded functional blade at the third upper node in four plants per plot receiving the same treatment. The gas exchange parameters, including leaf net photosynthetic rate (P_N), stomatal conductance (g_s), ratio of sub-stomatal to atmospheric CO_2 concentrations and transpiration rate (E), were determined using a LI-6400 portable openflow gas-exchange system (LI-COR, USA) at 11:00 AM. P_N, g_s, E and the ratio of sub-stomatal to atmospheric CO_2 concentrations are presented in μmol CO_2 m^{-2} s^{-1}, mol m^{-2} s^{-1}, μmol H_2O m^{-2} s^{-1} and cm^3 m^{-3}. Water use efficiency was calculated as the ratio of P_N/E. The photosynthetically active radiation was 1,200 ± 50 μmol m^{-2} s^{-1}, CO_2 concentration was 380 ± 5 cm^3 m^{-3}, and the air temperature and relative humidity were 24 °C and 50%. Gas exchange parameters were measured in fully expanded leaves. There were five replications for each measurement, using three leaves per pot, and three data points were recorded per leaf, for a total of 45 data points per treatment.

To extract the photosynthetic pigments, dried leaf samples (30 mg) were dipped into 10 mL of an 80% acetone:anhydrous ethanol mixture (1:1) in darkness at room temperature until the leaves became white. Five pots were used to measure the photosynthetic pigment content, and the measurement was repeated three times per pot for a total of 15 data points per treatment. Spectrophotometric (SpectrUV-754, Shanghai Accurate Scientific Instrument Co.) determinations at 440, 645 and 663 nm for each sample were performed three times. The photosynthetic pigment content (mg g^{-1}), including chlorophyll a (*Chl* a), chlorophyll b (*Chl* b), chlorophyll a + chlorophyll b [*Chl* (a + b)] and *Car*, was calculated (Holm, 1954; Jiao et al., 2018).

Determination of enzyme activity associated with N metabolism

Nitrate reductase (NR) activity was determined according to the method described by Robin (1979). An extract of 0.1 mL was incubated in a reaction mixture containing 0.5 mL of 0.1-M potassium phosphate buffer (pH 7.4), 0.1 mL 100 mM EDTA, 0.1 mL of 0.15-mM NADH and 0.1 mL of 0.1-M KNO_3 at 30 °C for 30 min. The reaction was stopped by adding 0.1 mL of 1-M zinc acetate. The absorbance of the supernatant was determined at 540 nm after diazotation with 1 mL of 5.8-mM sulfanilamide in 1.5-N HCl and 1 mL of 0.8-mM N-naphthyl-ethylenediamine dichloride. The glutamine synthase (GS) activity was measured according to Yu and Zhang (2012). Fresh plant leaves were cut into pieces and homogenized in 50-mM Tris-HCl (pH 8.0) containing 2-mM $MgSO_4$, 2-mM DTT and 0.4-M sucrose in a pre-cooled mortar in an ice bath. The homogenate was centrifuged at 12,000 g at 48 °C for 20 min. Next, 1.0 mL crude enzyme solution was added to 1.6 mL reaction mixture (0.6 mL 0.25-M imidazole-HCl buffer, 0.4 mL 0.3-M sodium hydrogen glutamate, 0.4 mL 0.03-M ATP-Na

and 0.2 mL 0.5-M $MgSO_4$). The mixture was incubated at 258 °C for 5 min, 0.2 mL hydroxylamine hydrochloride was subsequently added, and the mixture was incubated for 15 min. Next, 0.8 mL $FeCl_3$ solution (0.37-M $FeCl_3$, 0.2-M trichloroacetic acid and 0.6-M HCl) was added to terminate the reaction. After centrifugation at 4,000 g at 48 °C for 10 min, the absorbance of the supernatant was recorded at 540 nm. GDH activity was analyzed using the method described by Gupta (2012). The extraction buffer (pH 7.9) consisted of 0.05-M imidazole and 5-mM DTT. Leaves (1 g) were ground in liquid N_2 in a chilled mortar and pestle and were centrifuged at 12,000 g for 40 min at 4 °C. The supernatant was collected and stored at -20 °C. The assays were carried out using the continuous spectrophotometric rate determination method. The GOT and GPT activities were determined according to the method of Zhang and Qu (2004). Fresh leaves (0.5 g) were cut into pieces and homogenized in 50-mM Tris-HCl (pH 7.2) in a precooled mortar in an ice bath. The homogenate was centrifuged at 12,000 g at 48 °C for 20 min. The supernatant was added to a GOT reaction solution (3 mg

mL^{-1} of NADH, 0.2-M L-aspartate, 2,000 U malate dehydrogenase and 50-mM a-ketoglutaric acid), and the absorbance of the mixture at 340 nm was recorded to measure the GOT activity. The supernatant was added to a GPT reaction solution (3 mg mL^{-1} of NADH, 0.2-M L-alanine, 2,000 U lactate dehydrogenase and 50-mM a-ketoglutaric acid), and the absorbance of the mixture at 340 nm was recorded to measure the GPT activity. The soluble protein contents of leaves were estimated at 595 nm using the Coomassie brilliant blue reagent by following the procedure described by Lowry (1951) and was expressed in $mg\,g^{-1}$ FW.

Statistical analyses
Statistical analyses of the data were performed using the statistical program SPSS 13.0 (SPSS, Chicago, IL, USA). All the data are presented as averages of five biological replicates with standard errors (SEs). Curves in figures were determined using the regression curve-fitting function of Sigma Plot 12.0 (Jandel, Erkrath, Germany) and Visio 2016 (Visio for Windows).

Table 1. Formulation of the nutrient- and stress-treatment solutions for plant growth

Reagent name	CK (mmol L^{-1})	N1 (mmol L^{-1})	N2 (mmol L^{-1})	N3 (mmol L^{-1})
$Ca(NO_3)_2\cdot4H_2O$	34.776	17.388	8.695	4.347
$MgSO_4\cdot7H_2O$	25.049	25.049	25.049	25.049
KH_2PO_4	20.001	20.001	20.001	20.001
KNO_3	50.009	25.005	12.502	6.251
Na-EDTA	2.366	2.366	2.366	2.366
$FeSO_4\cdot7H_2O$	1.379	1.379	1.379	1.379
H_3BO_3	4.628	4.628	4.628	4.628
$MnSO_4$	0.663	0.663	0.663	0.663
$CuSO_4\cdot5H_2O$	0.032	0.032	0.032	0.032
$ZnSO_4\cdot7H_2O$	0.077	0.077	0.077	0.077
H_2MoO_4	0.056	0.056	0.056	0.056
$CaCl_2\cdot2H_2O$	0	13.034	26.068	52.136
KCl	0	18.753	37.505	75.01

Results

Changes in plant growth parameters under low-N stress
There were differences in growth performances between W1 and W2 under different low-N stress conditions (Fig. 1). Compared with the CK, the low-N stress level resulted in decreased Plant heights, Up FWs, Root FWs, Up DWs and Root DWs in W1. The decreases were more prominent under the most severe low-N stress compared with the low and medium levels of low-N stress. However, there was no significant effect on the growth parameters of W2 under low-N stress conditions, just a slight decrease in the parameters under the most severe low-N stress level. In addition, the root length of W2 increased significantly as the stress became more severe, while there was no change in these parameters in W1.

Ion accumulation
With an increase in the intensity of the low-N stress, there were obvious differences between the ion contents of the roots and leaves of the two wild soybean varieties (Table 2). Under the lowest low-N stress level, the K^+, Fe^{2+}, B^{3+},

$C_2O_4^-$, $H_2PO_4^-$ and Mg^{2+} contents in W2 roots increased, but there were no significant changes in W1 roots compared with the CK. Under the medium stress level, the NO_3^-, Cu^{2+}, Zn^{2+}, Mn^{2+}, SO_4^{2-} and Ca^{2+} contents in the W2 roots remained stable, while the levels in the W1 roots decreased more at the medium and high levels compared with the lowest stress level. Under the high-level stress, the ion content in W1 roots decreased significantly, while it remained high in W2 roots. Under low-N stress, the $H_2PO_4^-$, Mg^{2+}, K^+, Fe^{2+}, B^{3+}, $C_2O_4^-$, NO_3^-, Cu^{2+} and Zn^{2+} contents decreased in the leaves of both varieties, with greater reductions in W1 than in W2.

Photosynthetic parameters
Under low-N stress, the photosynthetic physiological parameters of both genotypes were affected, and the P_N and *Chl* (a + b) content decreased significantly (Fig. 2). With an increase in the intensity of low-N stress, the P_N, g_s and water use efficiency values decreased more in W1 than in W2. *E* decreased in W2 but increased in W1. Compared with the CK, the photosynthetic pigment contents decreased significantly in W1 but the decrease in W2 was not obvious
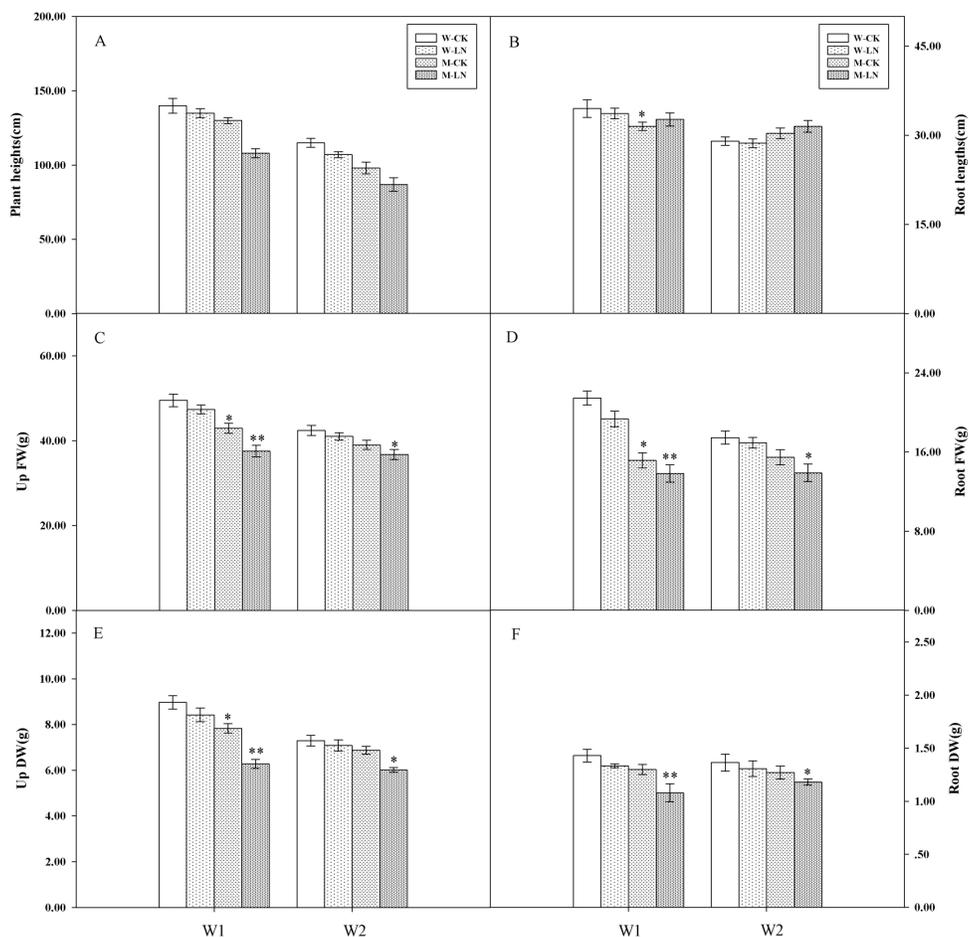
Fig. 1. The changes in plant growth parameters in the two wild soybean under control and LN-stress conditions
Note: (A) Plant heights; (B) Root lengths; (C) aboveground FW (Up FW); (D) underground fresh weight (Root FW); (E) aboveground dry weight (Up DW:); (F) underground dry weight (Root DW). * and ** indicate significant (P < 0.05) and highly significant (P < 0.01) differences, respectively

Table 2. Ion contents in roots of two wild soybean seedlings under control and low nitrogen stress conditions

Samples	Relative concentration								Fold changes $\log_2$(N1/CK)		Fold changes $\log_2$(N2/CK)		Fold changes $\log_2$(N3/CK)	
	W1				W2									
	CK	N1	N2	N3	CK	N1	N2	N3	W1	W2	W1	W2	W1	W2
Mn^{2+}	0.03	0.02	0.05	0.03	0.03	0.03	0.04	0.05	-0.26	0.05	0.94*	0.27*	0.19*	0.59*
B^{3+}	0.07	0.11	0.14	0.12	0.03	0.09	0.11	0.12	0.74	1.43*	1.02*	1.66	0.26*	0.54*
Cu^{2+}	0.44	0.34	0.32	0.28	0.34	0.33	0.33	0.31	-0.38	-0.02	-0.47	-0.06	-0.20	-0.05
Fe^{2+}	0.06	0.07	0.08	0.06	0.04	0.06	0.07	0.07	0.28	0.46*	0.50	0.73	0.01	0.24*
Zn^{2+}	0.02	0.02	0.01	0.00	0.01	0.01	0.01	0.01	-0.43*	-0.21	-0.74	-0.49	-0.78	-0.42
K^+	233.48	221.00	294.92	254.35	178.90	234.25	270.69	281.98	-0.08	0.39*	0.34	0.60	0.04	0.20*
Ca^{2+}	21.00	15.14	13.85	15.75	10.34	19.10	17.99	23.05	-0.47	0.89*	-0.60	0.80*	-0.12	0.35*
Mg^{2+}	32.13	21.38	21.61	23.53	15.19	21.68	24.26	26.66	-0.59	0.51*	-0.57	0.67*	-0.14	0.24*
Cl^-	3.39	8.81	7.82	8.48	2.26	4.73	5.02	8.20	1.38*	1.06*	1.21*	1.15*	1.32*	1.86*
NO_3^-	3.75	1.03	0.24	0.36	3.41	2.22	1.01	1.38	-1.86*	-0.62	-3.99*	-1.75*	-3.38*	-1.30*
$H_2PO_4^-$	9.79	13.11	15.41	14.36	8.20	10.09	11.15	12.10	0.42	0.30	0.66	0.44	0.55	0.56
SO_4^{2-}	6.84	13.12	16.08	13.50	4.66	10.96	13.29	11.42	0.94	1.23	1.23	1.51	0.98	1.29
$C_2O_4^-$	1.58	1.74	1.83	1.78	1.59	1.66	1.82	1.97	0.14	0.07	0.21	0.20	0.17	0.31*

Note: The ion contents are the mean of data from four biological replicates; the fold changes were calculated using the formula log2(nitrogen/control); W1, common wild soybean; W2, low nitrogen-tolerant wild soybean; CK, control treatment; N1, low-intensity low nitrogen stress stress; N2, medium-intensity low nitrogen stress; N3, high-intensity low nitrogen stress;
*significant (P < 0.05) differences, respectively.

under the low-level stress. With the increase in stress intensity, the *Chl* a, *Chl* b and *Chl* (a+b) contents in the leaves of W1 seedlings decreased more than in W2. In particular at the N3 level, the contents in W1 decreased by 53.32%, 55.53% and 50.08%, respectively, while those in W2 decreased by 39.41%, 45.82% and 28.51%, respectively, compared with the CK. It is worth noting that the *Car* and soluble sugar contents showed an opposite trend, increasing under low-N stress in W2 but decreasing in W1.

Enzyme activities of N metabolism
There were significant differences in the activity levels of

N metabolism-related enzymes and the soluble protein content between W1 and W2 leaves at different levels of low-N stress (Table 3). Compared with the CK, NR and GDH showed no significant changes in either W1and W2 under the low-level stress, but they decreased under the medium-level stress, especially in W1. With an increase in the intensity of the low-N stress, GS decreased in W1 but increased in W2. Meanwhile, the soluble sugar content increased in W2 but did not significantly change in W1. Glutamine oxoglutarate aminotransferase (GOGAT) and GPT decreased in W1 but remained high in W2 as the intensity of the low-N stress increased.
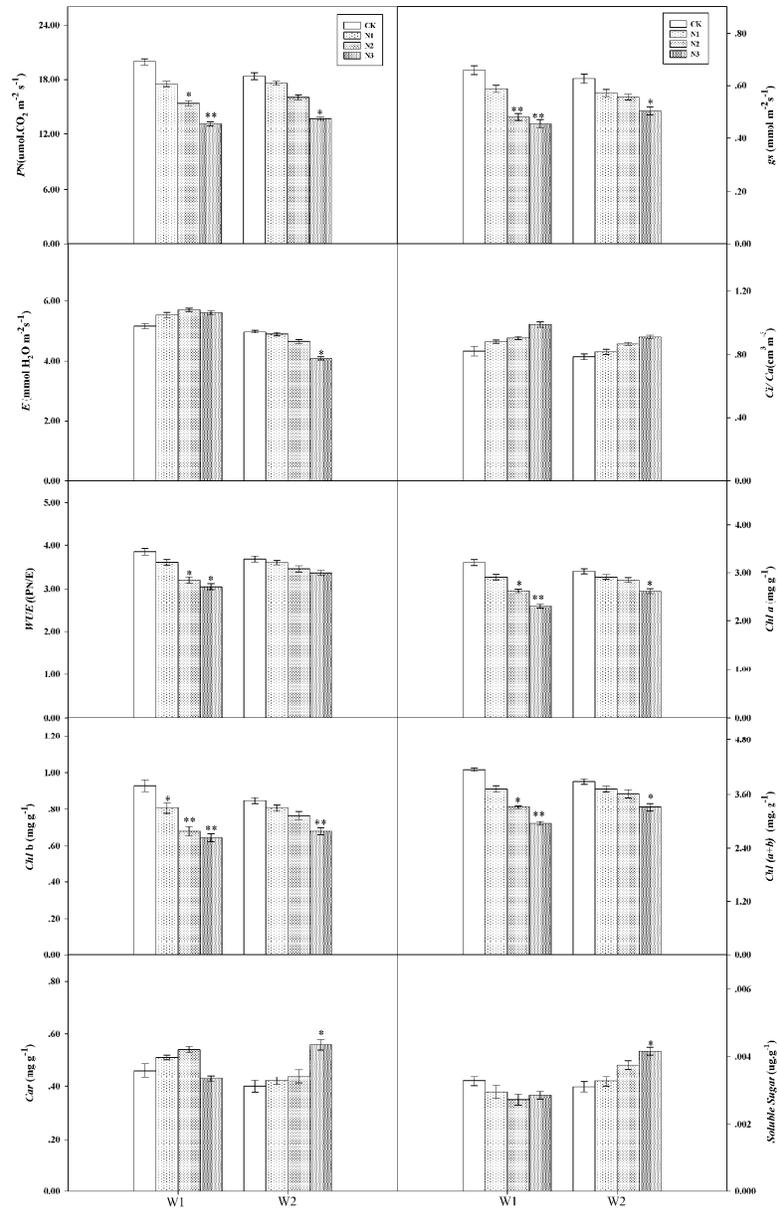

Fig. 2. The changes in photosynthetic characteristics of the two wild soybean under CK and LN stress
Note: (A) Net photosynthetic rate (p_N); (B) stomatal conductance (g_s); (C) transpiration rate (E); (D) ratio of sub-stomatal to atmospheric CO_2 concentrations (C_i/C_a); (E) ratio of p_N / E (*WUE*); (F) chlorophyll a (*Chl* a); (G) chlorophyll b (*Chl* b); (H) chlorophyll a+b (*Chl* (a + b)); (I) carotenoid (*Car*); (J) Soluble Sugar. * and ** indicate significant (P < 0.05) and highly significant (P < 0.01) differences, respectively

Table 3. The changes in enzyme activities of nitrogen metabolism in the two wild soybean under control and LN-stress conditions

Samples	Relative concentration								Fold changes $\log_2$(N1/CK)		Fold changes $\log_2$(N2/CK)		Fold changes $\log_2$(N3/CK)	
	W1				W2									
	CK	N1	N2	N3	CK	N1	N2	N3	W1	W2	W1	W2	W1	W2
NR	2.72	2.61	2.27	2.11	3.53	3.15	2.94	2.88	-0.06	-0.26	-0.37*	-0.16	-0.26*	-0.29*
GS	1.56	1.52	1.44	1.31	1.48	1.51	1.58	1.60	-0.04	-0.12	-0.26*	0.03	0.09**	0.12
GOGAT	12.17	11.07	9.10	8.67	13.23	12.20	11.70	10.67	-0.14	-0.42	-0.49*	-0.12	-0.18*	-0.31*
GPT	0.10	0.09	0.08	0.04	0.11	0.09	0.08	0.07	-0.14	-0.23	-1.18	-0.24	-0.31*	-0.59
GOT	0.05	0.06	0.05	0.04	0.04	0.07	0.07	0.06	0.29**	0.09**	-0.03	0.58**	0.70	0.57*
GDH	144.83	131.00	105.83	91.00	100.50	95.13	90.83	86.67	-0.14	-0.45	-0.67*	-0.08*	-0.15*	-0.21*
Soluble protein	33.70	31.29	28.47	27.64	42.67	43.55	44.81	46.39	-0.11	-0.24	-0.29*	0.03*	0.07**	0.12*

Note: NR, nitrate reductase; GS, glutamine synthetase; GOGAT, glutamate synthase; GPT, glutamic-pyruvic transaminase; GOT, glutamic-oxaloacetic transaminase; GDH, glutamate dehydrogenase ; Soluble protein.* and ** indicate significant ($P < 0.05$) and highly significant ($P < 0.01$) differences, respectively.
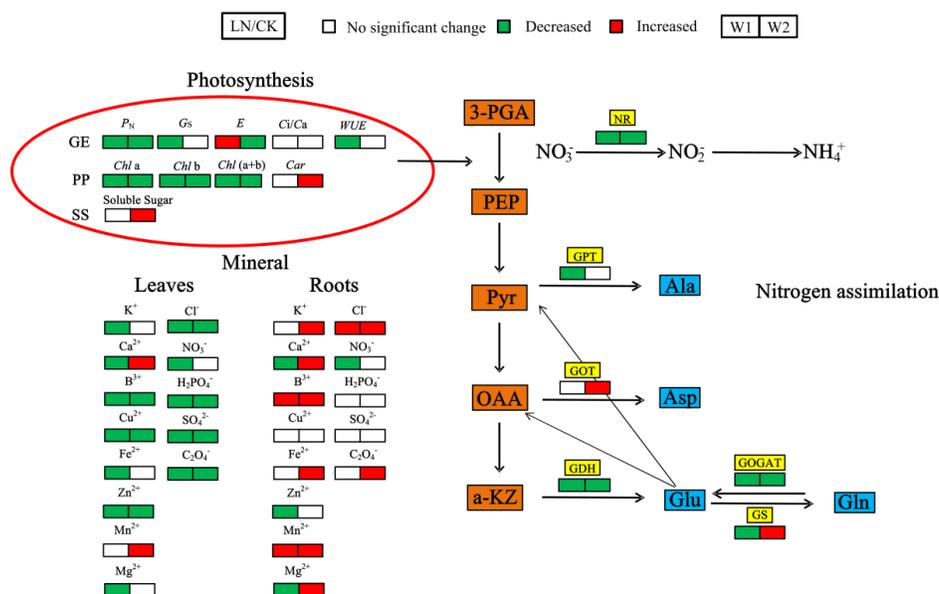

Fig. 3. Simplified integrative model for carbon and nitrogen metabolic pathways
Note: This scheme summarizes the main interaction points between carbon and nitrogen metabolism. a-KZ, a-ketoglutaric acid; Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Gln, glutamine; OAA, oxaloacetate; PEP, phosphoenolpyruvate; Pry, pyruvic acid

Discussion

N is an essential element for plant growth and development. An N deficiency can rapidly inhibit plant growth and biomass accumulation, especially plant height and dry matter, respectively (Osborne, 2006). There were significant differences in growth and biomass accumulation between two different types of wild soybean under different low-N stress levels. The growth inhibition was clearly greater in W1 than in W2. Roots are a vital organ system of plants owing to their involvement in water and nutrient acquisition, storage functions, and metabolite synthesis and accumulation. In addition, roots have formed a series of mechanisms to resist adverse environmental conditions (Quain, 2015). W2 was better able to adapt to the low-N stress, as reflected by the significant increase of root length and R/S (Fig. 1).

The absorption of nutrients by plants can be inhibited by low-N stress. The lack of any nutrient element changes plant metabolism (Amiour *et al.*, 2012; Takehisa, 2013). Maintaining ion nutrient homeostasis under low-N stress is an important low-N tolerance strategy for plants (Prasanna, 2013). P is an important substrate for energy metabolism and biofilm biosynthesis, and it participates in regulating electron transport and the oxidative phosphorylation of mitochondria (Oosterhuis, 2012). In this study, $H_2PO_4^-$ gradually accumulated in the W2 root system as the intensity of the low-N stress increased, which allowed chloroplast energy transfer and photosynthetic phosphorylation in W2, which promoted the following metabolic pathway: $ADP + Pi + NADP^+ + H_2O \rightarrow APT + NADPH + O_2$.

Meanwhile, APT and NADPH provide more energy for C assimilation in W2 (Ng'etich, 2013). Mg^{2+} can activate RuBP carboxylase to promote CO_2 assimilation and cause GS to contribute to the transformation of ammonium

(NH_4^+) to amino acid. Iron can stimulate the participation of carbohydrates in photosynthesis and N fixation, leading to the absorption of iron by plant roots in the form of Fe^{2+} (Mohammed, 2011; Akay, 2012). K^+ has a positive effect on enzyme activation and protein synthesis in plants. B is an essential component and is related to the stability of plant cell wall structures, carbohydrate transport and NR activity, which contributes to an enhancement of the N fixation capacity (Alemán, 2011). One low-N tolerance strategy maintained by W2 was a high absorptive ability for ions, including Mg^{2+}, Fe^{2+}, B^{3+} and K^+ under N1 and N2 conditions. Simultaneously, there was no significant change under N3 conditions, indicating that W2 could adjust the mineral ion nutrient balance to reduce the damage caused by low-N stress. Accumulations of SO_4^{2-}, Ca^{2+} and Mn^{2+} were maintained in W2 leaves to ensure a sufficient nutrient balance at all low-N stress levels, which may be an effective way of adapting to soil nutrient deficiencies.

N metabolic processes and regulation are crucial for plant-stress resistance. The key enzyme activity of N metabolism is an important index in determining the relationship between stress and N assimilation, which connects C and N metabolism in a plant and influences the whole N metabolic process (Chen, 2017). Plants can promote N assimilation to reduce the damage from low-N stress by self-regulating the activities of N-metabolic enzymes and increasing the soluble protein content (Zhao, 2018). The enzymes of N metabolism are synergetic. NR is a NO_3^--inducible and rate-limiting enzyme in the process of NO_3^- assimilation (Kaiser and Huber, 2001). NO_3^- is reduced to NO_2^- by NR, and then to NH_4^+ by nitrite reductase (Ren, 2014). NH_4^+ is synthesized to glutamine (Gln) by GS after entering the N-assimilation pathway. Then, Gln and a-oxoglutarate (a-KZ) were transformed into two molecules of glutamate (Glu) by GOGAT. One could be used as the substrate of GS, while the other could be converted to synthesize proteins, nucleic acids and other nitrogenous compounds. This pathway is the GS/GOGAT cycle in which GS and GOGAT work at the same time assimilating NH_4^+ (Chang, 2017). a-KZ is reduced and ammoniated to Glu by GDH. GOT and GPT are transaminases. The former catalyzes the transamination between Glu and pyruvate (Pyr), and the latter between Glu and oxaloacetate (OAA), which play important roles in the redistribution of C-N metabolism among plant cell cytoplasm and other compartments (Pang, 2015; Jia-Ling, 2015). In our study, N-deficiency reduced the activities of NR, GOGAT and GPT, but they remained essentially constant in W2 at the N3 level. GS accumulated gradually as the intensity of the low-N stress increased. With high NR, GS/GOGAT and GDH activities, W2 could effectively hasten the conversion of NH_4^+ and generate more Glu as the substrate for transamination reactions. High GOT and GPT activities occurred in W2 under low-N stress in this work, which increased the flow of N from amino and amide groups, both of which are essential for plant growth. Soluble protein accumulation increased in response to low-N stress (Fig. 3), which played a role in reducing water potential to maintain a water and nutrient balance. It also provides enzyme proteins that participate in various metabolic pathways for the rapid recovery from stress, ultimately improving plant growth and development (Ya-Wei, 2017).

Stress tolerance, which helps plant to survive under stressed conditions, inevitably depends on the endogenous C-N metabolism (Liu *et al.*, 2014). C metabolism provides a C source and energy for N metabolism. Under low-N conditions, W2 can adjust leaf g_s and E to reduce water evaporation and avoid affecting photosynthesis. Additionally, W2 could adjust photosynthetic metabolism through compensatory mechanisms that allowed the plants to adapt to low-N stress. Soluble sugar, a main product of C metabolism in plants, reflects the carbohydrate and energy levels in plants. The low-N stress noticeably affected the accumulation of soluble sugar (Fig. 2). With the increased intensity of low-N stress, the soluble sugar content increased in W2 but decreased in W1. The accumulation of soluble sugar could enhance the osmotic potential and provide availability of energy and C skeletons for the N metabolism in W2 (Nunes-Nesi *et al.*, 2010). Under low-N stress, W2 had a stable and sufficient photosynthetic assimilation ability to directly provide NADPH, Fdred and ATP for NO_3^-, NO_2^- and NH_4^+ assimilation. C metabolism ensures that there is enough C diverted to produce C skeletons that are needed during the assimilation of N.

In turn, N metabolic enzymes maintained high activity levels, which provided enzyme proteins and photosynthetic pigments for C metabolism. As shown in Fig. 3, phosphoenolpyruvate, generated from glycolysis, is converted to Pyr and OAA, respectively. OAA and Pyr enter the TCA cycle, producing energy and key C intermediates, including a-KZ. NR and nitrite reductase reduce cellular NO_3^- into NH_4^+, which is then incorporated into a-KZ to form Gln by the GS/GOGAT pathway or which directly interacts with a-KZ to form Glu by GDH. Glu and Gln further exchange amine to form other amino acids and amides. W2 can maintain a high total C level, adjust the ion and nutrient relationship and increase the N-assimilation rate, to regulate the C-N balance and avoid the harm caused by low-N stress.

Conclusions

Both W1 and W2 are wild soybeans with similar visual structures, but they adapt to adverse conditions differently by adjusting physiological characteristics and metabolic pathways. We demonstrated that both W1 and W2 showed certain resistance capabilities to a low intensity of low-N stress. As the intensity of low-N stress increased, W2's ability to survive was greater than that of W1 owing to physiological and metabolic plasticity strategies, including increasing root lengths that it employed. W2 had a less pronounced decrease in its photosynthetic rate and accumulated more *Car* and soluble sugar than W1. It also promoted C assimilation to provide more energy and C skeletons for N metabolism under low-N stress conditions. Additionally, W2 maintained greater N-metabolic enzyme activity levels to ensure a photosynthetic pigment and enzyme protein supply for C metabolism, which maintained a C–N assimilation balance that could alleviate the inhibition of plant growth caused by low-N stress. W2 also had strong ion absorption ability and could regulate the ion balance in root system to resist low-N stress. Our results provide an important theoretical basis for the protection, exploitation and utilization of wild soybean resources.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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