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Photosynthetic characteristics and metabolic analyses of two soybean genotypes revealed adaptive strategies to low-nitrogen stress

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ABSTRACT

Nitrogen is an essential macronutrient for plants and the common limiting factor for crop productivity worldwide. An effective approach to combat N deficiency and overuse is to understand the mechanism of low-nitrogen tolerance in plants and develop low-nitrogen-tolerant crop cultivars. Wild soybean has a high tolerance to poor environmental conditions, but, until now, no study has illustrated the mechanism of low-nitrogen tolerance at a metabolomic level. In this study, the photosynthetic characteristics and metabolomics of wild and cultivated soybean seedlings were analyzed, and the mechanism of wild soybean's low-nitrogen tolerance was explained using a sand culture experiment. Wild soybean was less affected by low-nitrogen stress than cultivated soybean as assessed by plant growth parameters and photosynthesis. The root length of wild soybean increased, and a high root-shoot ratio was maintained under low-nitrogen stress. Carotenoids accumulated, which contributed to its higher low-nitrogen tolerance. A total of 48 and 60 differentially accumulated metabolites were identified in leaves and roots, respectively, between the low-nitrogen stress and control groups. The ability of wild soybean to tolerate low nitrogen also resulted from its capability to enhance the TCA cycle, synthesize key amino acids, accumulate metabolites, such as soluble sugars and organic acids, and synthesize favorable secondary metabolites under low-nitrogen stress. The current results reveal the mechanism underlying wild soybean's high low-nitrogen tolerance and provide the methodology and theoretical basis for utilizing wild soybean, improving cultivated soybean, and studying the low-nitrogen tolerance of other plants.

1. Introduction

Nitrogen (N) is an important component of plant cells and is also the most needed mineral element in plants (Huang et al., 2015). N can increase the protein content in seeds, as it is an important component of protein (Worku et al., 2007; Ghassemigolezani et al., 2015). To increase crop yield, which is related to the amount of N accumulated in plants, excessive amounts of N fertilizer have been applied in agricultural production. However, due to leaching, surface oxidation, microbial consumption, denitrification, and volatilization, the utilization rate of N fertilizer is reduced and a large amount of N fertilizer is lost (Ju et al., 2009). On average, nitrogen use efficiency (NUE) is only about 30% in China, about 50–60% in western developed countries, and about 59% in the world (Raun and Johnson, 1999; Ju, 2014). The loss of N fertilizer causes severe damage to the surrounding ecosystems and serious environmental degradation, including global warming, acid rain, soil acidification, contaminated rivers and lakes, and air pollution, all of which affect human health (Williamson, 2011). Cultivating low-nitrogen tolerant crop varieties is an effective way to reduce the amount of N fertilizer, thus avoiding the excessive waste associated with it (Zhang et al., 2016a).

Soybean is an important cash crop, with 69% of the world's edible protein and 30% of its edible oil coming from cultivated soybean (Zhang et al., 2016b). N is a major participant in the main physiological processes of soybean, and soybean requires a large amount of N fertilizer (Ciocco et al., 2011). LN stress affects the normal physiological and metabolic activities of soybean, including inhibiting the increase in soybean leaf area, lowering photosynthesis, and changing the volume and total length of the root system (Sun et al., 2008). Furthermore, the expression levels of many plant genes are regulated by N limitation (Bi

Abbreviations: Car, carotenoid; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; *Chl* (a + b), chlorophyll a + chlorophyll b; C_i/C_a, ratio of sub-stomatal to atmospheric CO2 concentrations; CK, control group; *E*, transpiration rate; GS, glutamine synthetase; g_s, stomatal conductance; LN, low nitrogen; M, cultivated soybean; PC1, the first principal component; PC2, the second principal component; P_N, photosynthetic rate; TCA, tricarboxylic acid cycle; W, wild soybean; *WUE*, water use efficiency

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et al., 2007). Recently, Wang et al. (2013) found that microRNAs play important roles in the soybean response to low N. Protein content is an important index to measure soybean quality. The protein content and the N-fertilizer utilization rate in the seeds of wild soybean are significantly higher than those of cultivated soybean (Han et al., 2014). Wild soybean has the advantages of high protein content and strong adaptability to adverse environment, including resistance to drought and salt stresses (Chen et al., 2006; Lu et al., 2013). Improving the quality of cultivated soybean and providing genetic resources and theoretical guidance for the cultivation of soybean with LN tolerance (Yang et al., 2017) is important. To improve the LN tolerance of soybean, it is necessary to systematically study the effects of LN on the physiological metabolism of soybean.

Metabolomics is defined as the comprehensive analysis of all the small molecular metabolites of a biological system (Hillenmeyer et al., 2010). Since Fiehn (2002) introduced metabolomics to botany, the study of plant stress, including salt, drought, and LN, has increased. Quan et al. (2016) studied the metabolic changes of barley under LN stress and explained the mechanism of LN tolerance in wild barley. Metabolomics can clearly explain the growth pattern and adaptation mechanism of plants in adverse environments. Nevertheless, there are no similar studies in soybean.

Our study used wild and cultivated soybean as experimental materials. An open-flow gas-exchange system was used to analyze the photosynthetic characteristics of seedling leaves under normal and LN conditions. Gas chromatography–mass spectrometry (GC–MS) was used to analyze the metabolomics of seedling leaves and roots. By comparing the stress reaction of wild soybean and cultivated soybean with their CK respectively, the physiological basis of the soybean response to LN was clarified, and the mechanism of LN tolerance in wild soybean was determined. This study will help improve the N-use efficiency of soybean and cultivate new varieties with LN tolerance.

2. Materials and methods

2.1. Plant materials

Seeds of wild soybean (W; ‘Huinan06116′) and cultivated soybean (M; ‘Jinong24′) from the same latitude in northeastern China were kindly provided by the Jilin Academy of Agriculture Science, China.

2.2. Plant growth conditions

Seeds of W and M were sown in 14-cm diameter plastic pots containing 2.5 kg of washed sand and were germinated by irrigation with water. Plants were grown in an outdoor experimental field with day/night temperatures of 26.0 ± 2.0 /18.5 ± 1.5 °C at Northeast Normal University, Changchun, Jilin.

The LN treatment was initiated after the plants grew their third leaves. In the LN-treated group, W and M seeds were placed in one-fourth-strength modified Hoagland solution (Table 1). Calcium (Ca) and potassium (K) were supplied by $CaCl_2{\cdot}2H_2O$ and KCl, respectively, at equivalent concentrations in LN Hoagland solution for two weeks. In the CK, soybean was cultivated under normal conditions (1 × Hoagland solution). W and M were both divided into two groups: control and LN-treated. Each group consisted of eight pots: four pots for measuring growth parameters and photosynthesis, and four pots for metabolomic analyses.

2.3. Photosynthetic indices measurements

Two weeks after the stress treatment, the photosynthetic gas exchange parameters were determined using the fully expanded functional blade at the third node of the upper number in four pots receiving the same treatment in each pot. The gas exchange parameters, including leaf net photosynthetic rate (P_N), stomatal conductance (g_s), ratio of sub-stomatal to atmospheric CO_2 concentrations (C_i /Ca), and transpiration rate (E), were determined using a LI-6400 portable open-flow gas-exchange system (LI−COR, USA) at 11:00 AM. P_N, g_s, E and C_i/C_a unit are 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 (*WUE*) was calculated as the ratio of P_N /E. The photosynthetically active radiation (PAR) was 1200 ± 50 μmol m^{-2} s^{-1}; CO_2 concentration was 380 ± 5 cm^3 m^{-3}; air temperature and relative humidity were 24 °C and 50%. Gas exchange parameters were measured in fully expanded leaves. Four pots were used to measure the photosynthetic gas exchange parameters, three leaves were selected in each pot, and three data points were recorded per leaf, for a total of 36 data points per treatment.

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

Reagent name	CK (mmol L^{-1})	LN (mmol L^{-1})
$Ca(NO_3)_2{\cdot}4H_2O$	34.776	8.695
$MgSO_4{\cdot}7H_2O$	25.049	25.049
KH_2PO_4	20.001	20.001
KNO_3	50.009	12.502
Na-EDTA	2.366	2.366
$FeSO_4{\cdot}7H_2O$	1.379	1.379
H_3BO_3	4.628	4.628
$MnSO_4$	0.663	0.663
$CuSO_4{\cdot}5H_2O$	0.032	0.032
$ZnSO_4{\cdot}7H_2O$	0.077	0.077
H_2MoO_4	0.056	0.056
$CaCl_2{\cdot}2H_2O$	0	26.068
KCl	0	37.505

Dry leaf samples (30 mg) were dipped into 10 ml of 80% acetone: anhydrous ethanol mixture (1:1) to extract the photosynthetic pigments in darkness at room temperature until the leaves became white. Four pots were used to measure the photosynthetic pigment content, and the measurement was repeated three times per pot for a total of 12 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. Photosynthetic pigment content (mg g^{-1}), including chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), chlorophyll *a* + chlorophyll b (Chl (*a* + *b*)), and carotenoid (*Car*) was calculated (Holm, 1954; Jiao et al., 2018).

2.4. Growth indices measurements

After the soybean plants were harvested, plant heights, root lengths, aboveground fresh weight (Up FW), underground FW (Under FW), aboveground dry weight (Up DW), and underground dry weight (Under FW) were measured (Shao et al., 2016).

2.5. Metabolite profiling analysis

For the metabolite profiling analysis, metabolites were extracted from wild and cultivated soybean leaves and roots (100 ± 5 mg of plant material) and a GC–MS analysis was performed using a one-dimensional Agilent 7890 gas chromatograph system coupled with a Pegasus HT time-of-flight mass spectrometer. The data was acquired and pre-processed using the manufacturer’s ChromaTOF software (versions 2.12, 2.22, 3.34; LECO, St. Joseph, MI, USA). Data analysis was performed by SIMCA-P 13.0 software package (Umetrics, Umea, Sweden), as described by Li et al. (2017).

3. Results

3.1. Changes in plants’ growth parameters under LN stress

Compared with the CK, LN stress significantly reduced the plant height and root length of M by 33.33% and 30.43% respectively. The