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Research article

Identifying the metabolomics and physiological differences among *Soja* in the early flowering stage

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ABSTRACT

Wild soybean (*Glycine soja*) and cultivated soybean (*Glycine max*) belong to the subgenus *Soja*. We investigated the photosynthetic activity, mineral nutrition and metabolomics of the salt-tolerant wild soybean (W2), salt-sensitive wild soybean (W1) and cultivated soybean (C) in the early flowering stage, with a focus on the physiological and cellular metabolism-related differences among *Soja* to reveal the adaptive mechanisms. The photosynthetic activity of W2 was greater than that of W1 and the Mg, Zn, Mo, Mn and B contents showed the same trend. Carbohydrate, polyol, organic acid and fatty acid contents, as well as the secondary metabolism, were greater in W2 than W1, while the amino acid metabolism was lower in W2 than W1. These levels could minimize damage and maximize survival and growth, which might be the mechanisms that W2 adapts under adverse environmental conditions. The photosynthetic activity of C was greater than that of W1 and C also contained more K, Zn and B. The metabolomics study indicated that carbohydrate and organic acid metabolism were relatively greater, while the amino acid content and secondary metabolism level were lower in C than W1. These were presumably the result of long-term breeding and domestication. This comparative study among *Soja* will help in increasing the understanding and protection of wild soybean resources, as well as the improvement and utilization of cultivated soybean.

1. Introduction

Both wild soybean (*Glycine soja*) and cultivated soybean (*Glycine max*) belong to the subgenus *Soja*, which are widely distributed plants that originated in China (Wang et al., 2008; Wang and Li, 2012). *Glycine soja* is a slender and sprawling annual herb found in wild environments, such as fields and roadsides (Broich and Palmer, 1981). In accordance with its different environments, wild soybean has evolved a variety of adaptations, including salt tolerance and drought tolerance (Yan et al., 2014; Shao et al., 2016). During natural evolution, wild soybean represents the fittest survivors, struggling for life and undergoing genetic variation (Wang et al., 2010). *Glycine max* is an erect-stem annual crop grown extensively. It is an economically important crop, as well as an oil crop, and a rich source of protein and vegetable oil (Fehr et al., 2015). Artificial breeding retains useful characteristics and eliminates adverse variations to improve the desired biological traits of soybean and provide new varieties to serve human needs (Lam et al., 2010b). Significant differences in plant metabolism occur in response to environmental adaption and to long-term artificial acclimation.

Several tools have been employed to investigate soybean' genetic diversity, such as plant morphology, seed protein allozymes, random amplified polymorphic DNA, restriction fragment length polymorphisms, DNA sequence analysis, chloroplast DNA and microsatellite markers (Malik et al., 2009; Lam et al., 2010a; Zhao et al., 2018a).

Abbreviations: C, cultivated soybean; *Car*, carotenoid; *Chl* a, chlorophyll a; *Chl* b, chlorophyll b; *Chl* t, chlorophyll total; C_i/C_a, ratio of sub-stomatal to atmospheric CO_2 concentrations; *E*, transpiration rate; g_s, stomatal conductance; PC1, the first principal component; PC2, the second principal component; P_N, photosynthetic rate; W1, salt-sensitive wild soybean; W2, salt-tolerant wild soybean; *WUE*, water-use efficiency

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Metabolomics is an important component of systems biology that operates downstream of genomics and proteomics. Currently, the metabolome is thought of as the link between genotype and phenotype (Fiehn, 2002). Metabolites often change in response to artificial and environmental stresses; therefore, these changes not only reflect the plants' morphological structure, physiology and growth characteristics but also have vital economic values to society (Harrigan et al., 2007). In current metabolomics studies, gas chromatography combined with mass spectrometry (GC–MS) is most commonly used to detect metabolites (Aliferis and Chrysayi-Tokousbalides, 2010).

Photosynthesis and mineral nutrition are important chemical reactions in plants that are directly related to the plants' growth and biological activities (Okunlola and Adelusi, 2014; Shi et al., 2015). Metabolomics has been used in the study of soybean. Lu et al. (2013) revealed the salt-tolerance mechanism of wild soybean leaves using GC − MS and LC − FT/MS. Das et al. (2017) studied the metabolomics profiles of soybean leaves under drought and heat stress. Additionally, Ribeiro et al. (2014) discriminated gamma-irradiated soybean seeds using ^{1}H NMR-based metabonomics. The mechanisms of salt tolerance and low nitrogen tolerance in wild soybeans were also studied using metabonomics (Zhang et al., 2016; Yang et al., 2017; Li et al., 2017; Jiao et al., 2018). These studies were controlled experiments using seedling-stage plants. However, research conducted under normal field management conditions is more meaningful and could provide a more direct and valuable theoretical basis for soybean agricultural production (Jha et al., 2018). The early flowering stage is the key link between the vegetative growth period and the reproductive growth period that marks a critical event in the life cycles of plants. Additionally, soybean pod number and yield are determined during the period that begins around flowering (Nico et al., 2006; Zhao et al., 2018b). Nevertheless, there have been few investigations regarding the differences in photosynthetic activity, nutrient elements and metabolic profiles among *Soja* under natural field management conditions, as well as their correlations. Based on the new perspective provided by a combination of basic physiology and metabolomics, we revealed the diversity of different soybean genotypes in the early flowering stage under natural field management conditions. Because it was conducted under field management conditions, this study provides a more direct and meaningful basis for soybean agricultural production.

Our study used salt-sensitive wild soybean (W1), salt-tolerant wild soybean (W2) and cultivated soybean (C) as experiment materials. An open-flow gas-exchange system, an inductively coupled plasma emission spectrometer, ion chromatography and gas chromatography–mass spectrometry (GC–MS) were used to analyze the photosynthetic characteristics, the cation and anion contents and the metabolomics, respectively, of soybean leaves in the early flowering stage. The main objective of this study was to reveal the adaptive mechanisms based on the differences in photosynthetic activity, mineral nutrition and metabolites among *Soja* during the early flowering stage. This study will be helpful to the understanding and utilization of wild soybean resources, as well as the improvement and utilization of cultivated soybean.

2. Materials and methods

2.1. Plant materials

The salt-sensitive wild soybean (W1, *Glycine soja* var. Huinan06116), salt-tolerant wild soybean (W2, *G. soja* var. Tongyu06311) and cultivated soybean (C, *Glycine max* 'JN24') were kindly provided by the Jilin Center of Germplasm Introduction and Breeding of Crops. The seeds were sown on 7 May 2013 and terminated in the Jilin Province New Variety Introduction and Breeding Center Experimental Field. The field management practices were the same as for the General Experimental Field. The gas exchange parameters were determined in the early flowering stage. Fully expanded functional leaves of fresh samples were picked in the early flowering stage on 7 July 2013 and immediately plunged into liquid nitrogen to store for further analyses. The fresh samples were kept at 100 °C for 10 min and then dried to constant weights at 80 °C for the determination of nutrition element and chlorophyll contents.

2.2. Determination of photosynthetic activity

The gas exchange parameters were determined using a LI-6400 (LI-COR, Lincoln, NE, USA) portable open flow gas exchange system (LI-COR) at 11:00 a.m. The leaf net photosynthetic rate (p_N), stomatal conductance (g_s), transpiration rate (E) and ratio of sub-stomatal to atmospheric CO_2 concentrations (C_i/C_a) are presented as μmol CO_2 $m^{-2}\,s^{-1}$, mol $m^{-2}\,s^{-1}$, μmol H_2O $m^{-2}\,s^{-1}$ and $cm^3\,m^{-3}$, respectively. Water use efficiency (*WUE*) was calculated as the ratio of p_N/E. The photosynthetically active radiation was 1200 ± 50 μmol $m^{-2}\,s^{-1}$, the CO_2 concentration was 380 ± 5 $cm^3\,m^{-3}$, and the air temperature and relative humidity were 24 °C and 50%, respectively. Gas exchange parameters were measured in fully expanded leaves. Three plants per group were used to measure the photosynthetic gas exchange parameters, three leaves were selected per plant, and three data points were recorded per leaf, resulting in 27 data points per treatment.

The photosynthetic pigments of leaf dry samples (30 mg) were extracted with an 80% acetone/anhydrous ethanol mixture (1:1) until the leaf turned white. Three plants per group were used to measure the photosynthetic pigment content, and the measurement was repeated three times per plant, resulting in nine data points. The optical densities at 440, 645 and 663 nm were determined by spectrophotometry (SpectrUV-754, Shanghai Accurate Scientific Instrument Co.). According to the method of Holm (1954), we calculated the chlorophyll a (*Chl* a), chlorophyll b (*Chl* b), total chlorophyll (*Chl* t) and carotenoid (*Car*) contents. The experimental data were analyzed using SigmaPlot version 10.0 (Systat Software Inc., USA) and SPSS 16.0 (SPSS Inc., Chicago, IL, USA) (Zhang et al., 2011; Chaskda et al., 2013).

2.3. Determination of nutritional element contents

Leaf dry samples (30 mg) were steeped in 65% (v/v) HNO_3 for 6 h, digested three times with 65% (v/v) HNO_3 at 120 °C, and then, the extracts were used to determine the K, Ca, Mg, Fe, Mn, Zn, Cu, B and Mo contents using an inductively coupled plasma atomic emission spectrometer (Prodigy, Leeman, USA). Leaf dry samples (30 mg) were transferred into 4 mL of deionized water in a centrifuge tube and placed in a boiling water bath for 40 min. The tubes were centrifuged at 4000 rpm for 10 min, and the supernatants were collected and this cycle was repeated two more times. The sample volume was made up to 14 mL. The supernatant was used to determine the NO_3^-, SO_4^{2-} and $H_2PO_4^-$ contents 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) (Shi et al., 2009). Each determination was performed three times. The experimental data were analyzed using the SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) (Chaskda et al., 2013).

2.4. Metabolomics of leaves

2.4.1. Leaf extraction preparation

Six plants per group were used for the metabolomics analysis. Approximately 100 mg of each frozen soybean leaf sample was transferred into a 2-mL centrifuge tube, and then 60 μL of water containing ribitol was added to each tube as an internal standard. The mixtures were vortexed, and 0.3 mL of methanol and 0.1 mL of chloroform were added. After mixtures were vortexed, a 70-Hz grinding mill system (Jinxin Biotech Ltd., Shanghai, China) was used to grind the samples for 5 min, followed by incubating at 70 °C for 10 min. Subsequently, the tubes were centrifuged at 12,000 rpm at 4 °C for 10 min. Then, 0.35 mL

of the supernatant was decanted into a 2-mL screw-top glass tube, and samples were dried in a vacuum concentrator at 30 °C for 2 h. Afterward, each sample was dissolved in 80 μL of methoxamine hydrochloride (20 mg/mL in pyridine) and incubated at 37 °C for 2 h. Samples were further derivatized with N, O-bis (trimethylsilyl)-trifluoroacetamid containing 1% trimethylchlorosilane (100 μL) at 70 °C for 1 h. Finally, the derived samples were cooled to room temperature before injection.

2.4.2. GC–TOF/MS analysis

The GC–TOF/MS analysis was performed using a one-dimensional Agilent 7890 gas chromatograph system coupled with a Pegasus 4D time-of-flight mass spectrometer (LECO, St. Joseph, MI, USA). The system utilized a DB-5MS capillary column coated with 5% diphenyl cross-linked with 95% dimethylpolysiloxane (30 m × 250-μm inner diameter, 0.25-μm film thickness; J&W Scientific, Folsom, CA, USA). A 1-μL aliquot of the analyte was injected in splitless mode. Helium was used as the carrier gas, the front inlet purge flow was 3 mL min^{-1}, and the gas flow rate through the column was 1 mL min^{-1}. The initial temperature was maintained at 90 °C for 15 s, raised to 180 °C at a rate of 10 °C·min^{-1}, then raised to 240 °C at a rate of 5 °C·min^{-1} and finally to 285 °C at a rate of 20 °C·min^{-1} for 11.5 min. The injection, transfer line and ion source temperatures were 280, 270 and 220 °C, respectively. The energy was −70 eV in electron impact mode. The MS data were acquired in full-scan mode with the *m/z* range of 20–600 at a rate of 100 spectra per s after a solvent delay of 492 s.

2.4.3. Data processing and MVDA

The data were acquired and pre-processed using the manufacturer's ChromaTOF software (versions 2.12, 2.22 and 3.34; LECO) (Allwood et al., 2009). Metabolites were identified by searching the commercial EI-MS library, FiehnLib (GC–TOF) (Kind et al., 2009). Afterward, features with at least 80% missing values were removed. The missing values were replaced with a small value, which was half of the minimum positive value in the original data. Then, the data were filtered using an interquantile range. In addition, an internal standard normalization method was employed in this data analysis. The resulting three-dimensional data involving the peak number, sample name and normalized peak area were inputted into the SIMCA-P 13.0 software package (Umetrics, Umea, Sweden) for principal component analysis (PCA), partial least square-discriminant analysis (PLS-DA) and orthogonal partial least square-discriminant analysis (OPLS-DA). Subsequently, non-commercial databases, including KEGG (http://www.genome.jp/kegg/), were utilized to search for metabolite pathways.

3. Results

3.1. Growth performance

The seeds of C were large, without a mud film, but the seeds of W1 and W2 were smaller and had a mud film. Observations revealed that there were significant differences in the morphology and structure between the C and both the W1 and W2. The stems of C were erect and stout. The leaves were the largest and were lanceolate. The apex was acuminate. On the contrary, the stems of W1 and W2 were slender and trailing, and the leaves were smaller. The apex was sharp and blunt rounded, while the proximal part was rounded. There were no significant differences in the morphology between W1 and W2.

3.2. Photosynthetic activity

In the early flowering stage, there were significant differences in photosynthetic characteristics among the different genotypes (Fig. 1). The gas exchange parameters and photosynthetic pigment contents indicated that the photosynthetic ability of W2 was significantly greater than that of W1 ($p < 0.05$). The P_N, g_s, *E* and *WUE* values, as well as the *Chl* a, *Chl* b, *Chl* t and *Car* contents in W2 were greater by 57.07%, 55.24%, 22.83%, 28.46%, 34.45%, 37.68%, 35.37% and 31.90%, respectively, than in W1. The photosynthetic ability increased significantly from W1 to C. The P_N, g_s, *E* and *WUE* values, as well as the *Chl* a, *Chl* b, *Chl* t and *Car* contents in C were greater by 63.54%, 71.35%, 24.87%, 31.81%, 28.83%, 33.20%, 30.07% and 21.73%, respectively, than in W1.

3.3. Mineral nutritional status

There were significant differences in the mineral element contents in leaves among the different soybean genotypes in the early flowering stage (Table 1). The SO_4^{2-}, Mg, Zn, Mo, Mn and B contents were 9.84%, 19.46%, 50.59%, 11.69%, 1.17% and 24.92% greater, respectively, in W2 than in W1 in the early flowering stage. The NO_3^-, $H_2PO_4^-$, Ca, K, Fe and Cu contents were 54.36%, 18.75%, 11.81%, 13.52%, 13.18% and 3.06%, lower, respectively. The difference between W1 and C in mineral nutrition physiology was obvious. The NO_3^-, SO_4^{2-}, Ca, Mg, Fe, Cu, Mo and Mn contents in C were 55.35%, 20.48%, 28.07%, 13.26%, 36.31%, 51.01%, 12.33% and 17.88% lower, respectively, than in W1, and the $H_2PO_4^-$, K, Zn and B contents were 12.85%, 1.81%, 44.13% and 71.77% greater in C, respectively, than in W1 in the early flowering stage (see Table 2).

3.4. Metabolomics

3.4.1. Metabolic profiles

The total ions in the chromatogram of the soybean leaf extracts were obtained by a GC–TOF/MS analysis. Spectra were aligned, normalized and subjected to an MVDA to detect metabolites using ChromaTOF software. Ribitol was added to the samples to monitor the stability of the GC–TOF/MS (Gika et al., 2007; Lv et al., 2010). The 0.038 standard deviation value for the retention time (min) indicated a robust system that produced reliable data (Table 1).

A total of 409 peaks were detected and 306 metabolites were identified after pre-processing. The pre-processed data were then inputted into SIMCA-P 13.0 software for the MVDA using the Pareto scaling method (scaled to the square root of the standard deviation). Each pretreatment scaling method has advantages and disadvantages (Jeong et al., 2013). Based on the characteristics of the metabolomics data, using the Pareto scaling method before clustering can produce more reliable and intuitive results, because this method can not only eliminate variability but also avoid amplifying systemic deviation. A similarity value for compound identification accuracy was obtained by searching FiehnLib. If the similarity was > 700, then the identification of the metabolite is reliable; if the similarity was within 200–700, then the compound was putatively annotated; and if similarity was < 200, then the compound was considered inaccurate and qualitative (Kind et al., 2009). We screened 168 metabolites having similarities > 700. To conform to a normal distribution, data was log-transformed before further analysis. Initially, a PCA was employed for unsupervised data analysis to preview the data clustering and trends. The samples classified into three main groups conformed to the different genetic types (Fig. 2; Table S1). The first principal component (PC1) explained 34.8% of the variance (Fig. 2A) and predominantly reflected the difference between the wild soybean groups and the cultivated soybean group. The second principal component (PC2) differed between W1 and C samples and explained 20.6% of the total variation (Fig. 2A), indicating that large metabolic changes occurred in the plants. Sarcosine, fructose, putrescine, valine, citric acid, citraconic acid, myo-inositol and glycine contributed greatly to PC1. Ferulic acid, myo-inositol, succinic acid, arachidonic acid, putrescine, citric acid, citraconic acid, methionine and glucose contributed greatly to PC2 (Fig. 2B). Then, the PC1 of the variable importance projection (VIP) was obtained using an OPLS-DA. Those with VIP values > 1.0 were selected as the altered metabolites. The remaining variables were then assessed using Student's *t*-test (*t*-test)

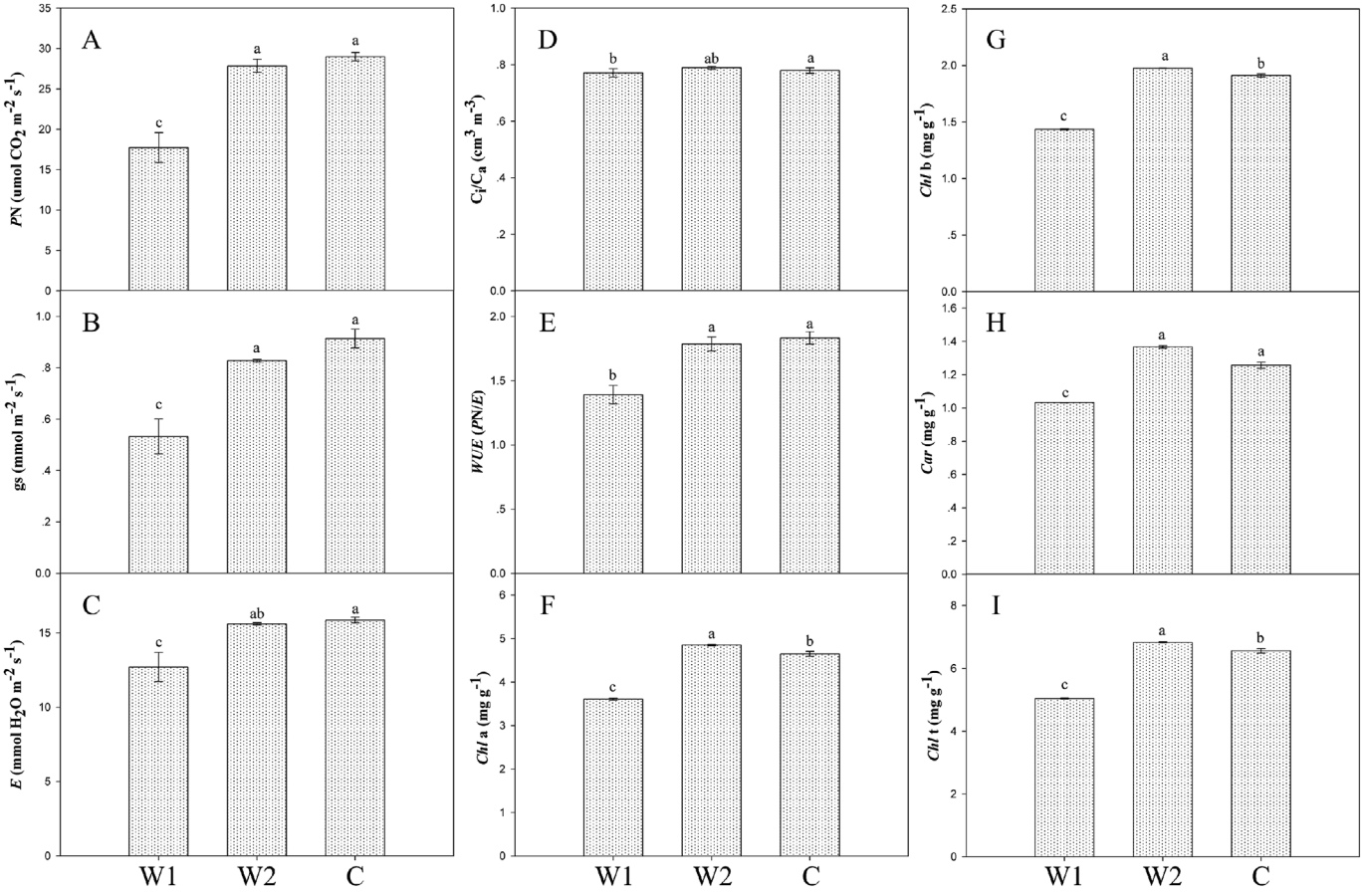

Fig. 1. The differences in photosynthetic activities among Soja in the early flowering stage. (A) Net photosynthetic rate (PN); (B) Stomatal conductance (gs); (C) Transpiration rate (E); (D) Ratio of sub-stomatal to atmospheric CO2 concentrations (Ci/Ca); (E) The ratio of PN/E (WUE); (F) Chlorophyll a (Chl a); (G) Chlorophyll b (Chl b); (H) Carotenoid (Car); (I) chlorophyll total (Chl t). Different letters indicate significant differences ($P < 0.05$).

Table 1
Retention time (R.T.; min) of ribitol as an internal standard.

Samples	R.T. (minutes)
W1-1	14.764
W1-2	14.763
W1-3	14.760
W1-4	14.764
W1-5	14.769
W1-6	14.777
W2-1	14.770
W2-2	14.592
W2-3	14.769
W2-4	14.766
W2-5	14.737
W2-6	14.764
C-1	14.780
C-2	14.780
C-3	14.782
C-4	14.781
C-5	14.782
C-6	14.775
Average	14.763
STD.	0.038

with SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA) between the two comparison groups and were discarded if $P > 0.05$. Briefly, based on the contributions of the metabolites to PC1 and PC2 (Fig. 2B) and if the VIP was > 1, P was < 0.05 and similarity was > 700, then the metabolites were selected as potential markers. The comparisons W2 VS

Table 2
Comparison of the mineral nutrient contents of *Soja* in the early flowering stage.

	W1	W2	C
NO_3^-	2.46 ± 0.00a	1.12 ± 0.05b	1.10 ± 0.08b
$H_2PO_4^-$	6.11 ± 0.04a	4.96 ± 0.31b	6.89 ± 0.25a
SO_4^{2-}	9.98 ± 0.38a	10.93 ± 1.30a	7.94 ± 0.32a
Ca	3.21 ± 0.10b	4.71 ± 0.05a	3.03 ± 0.04c
K	5.91 ± 0.13a	5.11 ± 0.11b	6.02 ± 0.06a
Mg	2.40 ± 0.04b	2.86 ± 0.03a	2.08 ± 0.01c
Fe	0.01 ± 0.00a	0.01 ± 0.00b	0.01 ± 0.00c
Cu	0.01 ± 0.00b	0.02 ± 0.00a	0.01 ± 0.00b
Zn	0.01 ± 0.00b	0.01 ± 0.00a	0.01 ± 0.00a
Mo	0.02 ± 0.00a	0.02 ± 0.00a	0.01 ± 0.00b
Mn	0.05 ± 0.00a	0.05 ± 0.00a	0.04 ± 0.00b
B	0.01 ± 0.00b	0.02 ± 0.00a	0.01 ± 0.00c

Different letters indicate significant differences ($P < 0.05$).

W1 and W1 VS C produced different metabolomics. Then, different metabolites were searched using the KEGG database to obtain the specific KEGG codes. In addition, Cytoscape 3.0 software was used to establish the networks by loading CytoKEGG modules as performed by Skogerson et al. (2011). The species was set as specific for soybean; therefore, the established networks were the exact biochemical pathways that exist in soybean. According to the direct and indirect correlations in the networks, provisional pathway graphics were drawn for W1 and W2 (Fig. 3), and for W1 and C (Fig. 4). The relative compounds and putative pathways might reveal differences among *Soja*.

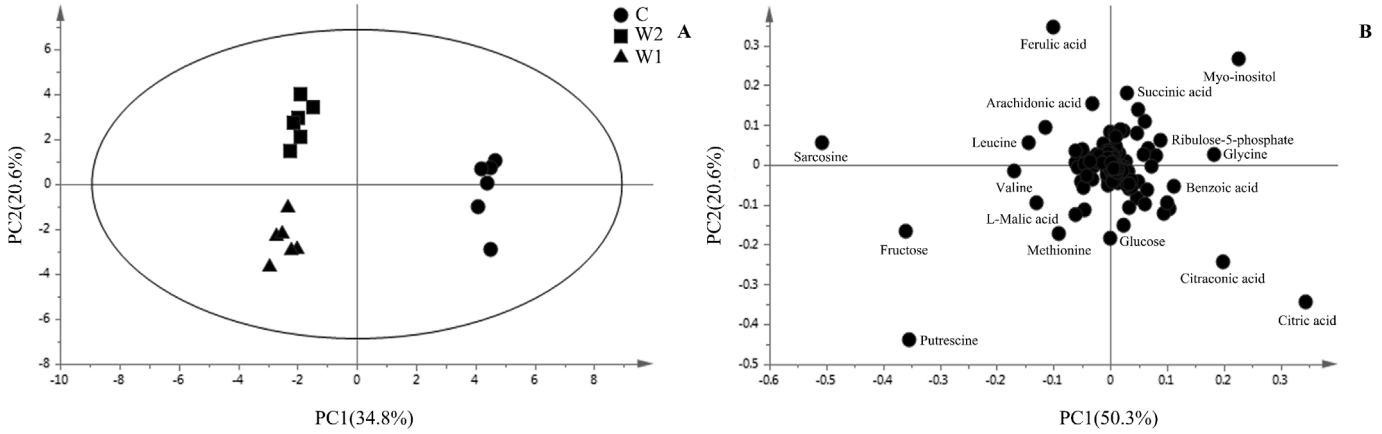

Fig. 2. Principal component analysis (PCA) of metabolic profiles from *Soja* leaves and loading plots of metabolites from the early flowering stage (six biological replicates). (A) PCA of leaves; (B) loading plot of leaves.

3.4.2. Metabolite levels in the two wild soybean genotypes

In the PCA, a comparison between W1 and W2 revealed that their metabolites were different. The metabolomics data showed 38 significantly different metabolites between W1 and W2 (Table 3).

The tyrosine, methionine, glycine, valine, norleucine, phenylalanine, isoleucine, aspartic acid and asparagine contents were lower in W2 than W1 ($P < 0.05$). However, the contents of other metabolites showed the opposite trend in the two wild soybean genotypes. Carbohydrates, polyols, organic acids, esters, fatty acids and secondary metabolites were greater in W2 than in W1. The carbohydrate metabolites included fucose, galactose, fructose, maltose and galactinol. Esters are formed by the esterification of inorganic or organic acids with alcohols; therefore, the organic acid, alcohol and ester contents were greater in W2. Additionally, the main substances were phytol, stigmasterol, myo-inositol, erythritol, resveratrol, succinic acid, pyruvic acid, citric acid, 3-hydroxy-3-methylglutaric acid, ribulose-5-phosphate, citraconic acid, threonic acid, palmitate and gluconic lactone ($P < 0.05$). The contents of fatty acids, such as linolenic acid, glycerol, stearic acid, palmitic acid, arachidonic acid and pentadecanoic acid, in W2 were greater than in W1. Ascorbate, 5-methoxytryptamine and ferulic acid are small molecular organic compounds produced by secondary metabolism that play important roles in plant growth and development, and accumulated more in W2.

3.4.3. Metabolite levels in W1 and C

A comparison between W1 and C revealed 34 specific differential metabolites (Table 4). The contents of amino acid metabolites, including alanine, L-ornithine, methionine, taurine, leucine, sarcosine, glycine and tryptophan, in C were significantly lower than in W1 ($P < 0.05$). The accumulations of erythrose, D-galactopyranoside, fucose and fructose in C were significantly greater than those in W1, which was similar to those of polyol, organic acid and fatty acid metabolites, such as threitol, phytol, inositol, erythritol, glycerol, arachidonic acid, palmitic acid and stearic acid. Citraconic acid, nicotinic acid, glucose-6-phosphate, benzoic acid, glycerol-1-phosphate and malonic acid contents were relatively greater in C than in W1, while the L-malic acid content was lower. The change trends of secondary metabolites and amino acids were the same, and the ascorbate, shikimic acid, putrescine and ethanolamine contents in W1 were greater than in C.

4. Discussion

Fully understanding the physiological mechanisms and metabolic levels of plants is the basis of crop improvement (Gu et al., 2012). Here, based on the analysis of the early flowering stage, significant differences in morphology, photosynthetic activity, mineral nutrition and metabolic level were found between W1 and W2, and W1 and C.

The wide geographical distribution and varied growth conditions of wild soybean varieties provide a rich genetic diversity (Li et al., 2014). W2, which has adapted to an external saline-alkali stress environment, showed no obvious morphological difference from W1, but had a significantly different photosynthetic activity and mineral nutrient contents. The photosynthetic rate of W2 was significantly greater than that of W1, indicating that W2 could produce more photosynthetic products and produce more energy while resisting the adverse environment. W2 could maintain a greater photosynthetic capacity than W1and could resist salt stress (Jiao et al., 2018). The mineral nutrient status of a plant greatly reflects its ability to adapt to adverse environmental conditions. There were different adaptation mechanisms based on the mineral nutrient contents of W2 and W1. The Zn and Mn contents in W2 were significantly greater than in W1. Zn can increase plant resistance to adverse environments by enhancing the stability of plant cell membrane systems under stress (Pandey et al., 2002). Mn can improve the activity of superoxide dismutase in plants, eliminate harmful substances and improve their resistance to stress (Kaminaka et al., 1999). To adapt to environmental stress, W2 reduced the toxic effects of stress by adjusting its absorption of mineral elements.

Plants experience various environmental stresses and, consequently, have created protective mechanisms to avoid or tolerate stress and reduce the associated damage (Chinnusamy et al., 2004). Plant metabolism can be reconfigured to maintain essential life activities, thereby achieving a new stable state, minimizing the harmful effects of the environment, and maximizing survival and growth (Couso and Fernández, 2012). The growth and cell metabolism of W1 are greater than those of W2 under salt and alkali stresses (Yang et al., 2017). The tyrosine, methionine, glycine, valine, norleucine, phenylalanine, isoleucine, aspartic acid and asparagine contents in W2 were lower than W1, indicating that the amino acid metabolism in W2 was weaker than in W1. In barley, amino acid metabolism is an energy-consuming process under low nitrogen stress (Quan et al., 2016). Additionally, the weaker amino acid metabolism in W2 than in W1 indicated that W2 could maintain a greater photosynthetic ability while reducing energy consumption. Carbohydrate, polyol and organic acid metabolism in W2 were significantly greater than in W1. Carbohydrates, polyols and small molecular organic acids can increase the osmotic potential and reduce injury under salt-stress conditions in plants (Li et al., 2017). Soluble carbohydrates are sources of carbon and energy. In addition, plants can use the state of glucose metabolism as a signal to regulate growth and development in response to abiotic stress (Akšić et al., 2015). Fructose is an indicator of plant stress resistance (Pilon-Smits et al., 1962). The accumulation levels of glucose and fructose in W2 were significantly

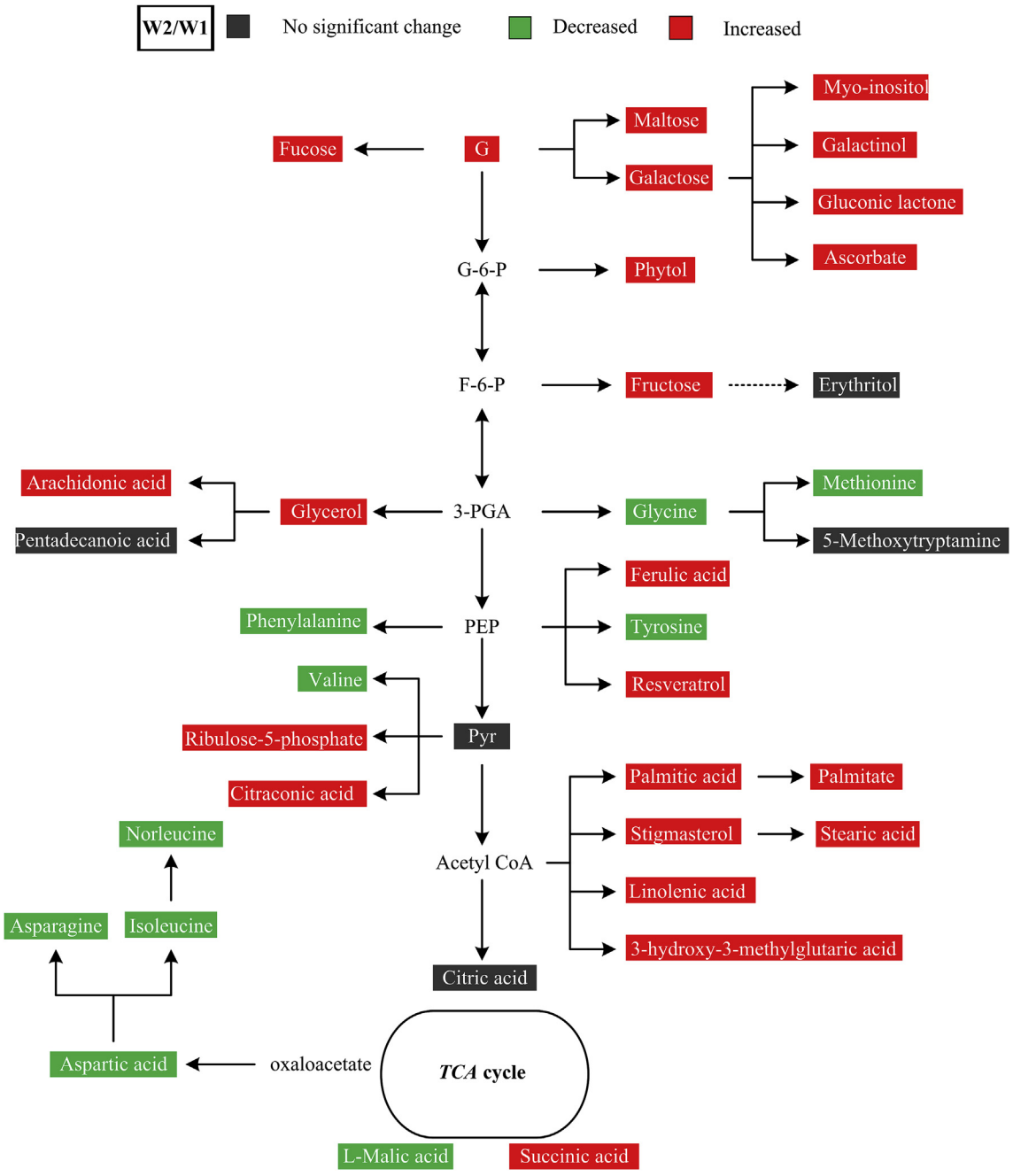

Fig. 3. Differences in the metabolic pathways of leaves between the salt-sensitive wild soybean and salt-tolerant wild soybean in the early flowering stage as assessed by a partial least square-discriminant analysis (PLS-DA). Red boxes denote significantly enhanced metabolites ($P < 0.05$); green boxes denote significantly reduced metabolites ($P < 0.05$); grey boxes denote unchanged metabolites ($P > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

greater than in W1, indicating a greater resistance to stress. Secondary metabolites have the ability to improve the antioxidant capabilities of plants. Secondary metabolites are accumulated in wild soybean to help resist salt stress and low nitrogen stress (Li et al., 2018). The accumulations of secondary metabolic intermediates, including ascorbate, 5-methoxytryptamine and ferulic acid, in W2 were significantly greater than in W1. Basic physiology is closely correlated with metabolic activities in plants (Wu et al., 2013). We analyzed the correlations among photosynthetic activity, mineral elements and significant differential metabolites according to Chong et al. (2018). The P_N, *Chl* a, *Chl* b, *Car* and B contents had remarkable positive correlations with the metabolism of amino acids and organic acids but significant negative correlations with carbohydrates and polyols, as well as fatty acid and secondary metabolism (Fig. S1, $P < 0.05$). In conclusion, we hypothesized that W2 develops its adaptability to external adverse environmental conditions through interactions among photosynthesis, ionic balance and metabolic profiles. This might be the result of the long-term adaptation of W2, which indicated that different ecotypes have certain effects on the changes in the chemical composition of metabolic groups in different wild soybean genotypes.

C is a main source of plant protein and vegetable oil worldwide. Its closest relative and predecessor is annual wild soybean (Li et al., 2014). After a long period of domestication and breeding, C was obtained to meet the demands of high yield and good quality (Lee et al., 2011). During domestication from wild soybean to C, the phenotype of the plant changed fundamentally. For example, the seed changed from

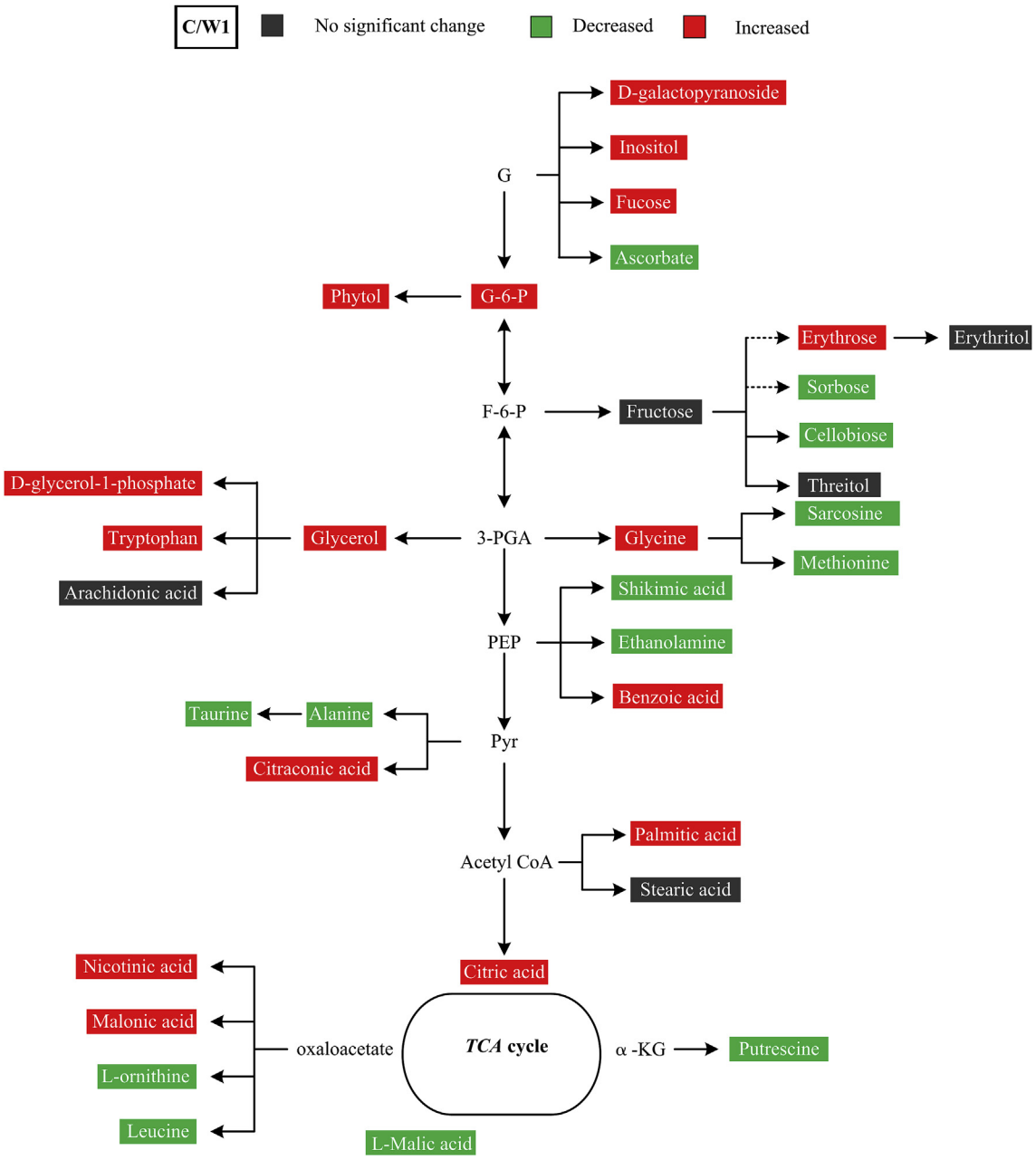

Fig. 4. Differences in the metabolic pathways of leaves between the salt-sensitive wild soybean and cultivated soybean in the early flowering stage as assessed by a partial least square-discriminant analysis (PLS-DA). Red boxes denote significantly enhanced metabolites ($P < 0.05$); green boxes denote significantly reduced metabolites ($P < 0.05$); grey boxes denote unchanged metabolites ($P > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

black to yellow, the seed became larger, and the plant became upright. A positive correlation between 100-grain weight and oil content exists (Yan et al., 2014). C's increased seed size allowed for a greater oil content, which was in line with consumer demand.

Different plant organs may be functionally integrated with each other and are often dependent (Midgley and Bond, 1989). Leaves are the principal organs of photosynthesis and assimilation in plants. The leaf area of C was greater than that of W1, and the photosynthetic activity of C was stronger than that of W1, indicating it had a greater photosynthetic ability. Thus, long-term acclimation and breeding involves the continuous evolution of photosynthetic organs and the continuous improvement of the photosynthetic ability (Li et al., 2006). In terms of mineral nutrition, the accumulations of $H_2PO_4^-$, K, Zn and B in C were greater than in W1, but those of NO_3^-, SO_4^{2-}, Ca, Mg, Fe, Cu, Mo and Mn were greater in W1 than in C. The B content is closely correlated with vertical growth in C (Shi et al., 2016).

The greatest advantage of metabolomics is that similarities and differences in the chemical compositions among different samples can be determined by statistical analysis. The PCA showed significant differences between C and W1 groups, which indicated that the metabolites of C were significantly different from those of wild soybean. C and W1 groups contained different metabolite levels because of long-term domestication, which was consistent with the physiological and morphological classifications. The protein content of wild soybean is also greater than that of C (Lan, 2010).

The alanine, L-ornithine, methionine, taurine, leucine, sarcosine, glycine and tryptophan contents in C were significantly lower than in W1. The metabolism of amino acids in C was lower than in W1, which

Table 3
Relative concentrations and fold changes of differential metabolites in the salt-sensitive wild soybean and salt-tolerant wild soybean in the early flowering stage.

Metabolite name	W1	W2	$Log_2^{(W2/W1)}$
Tyrosine	0.07 ± 0.01	0.05 ± 0.00	−0.40 *
Methionine	1.19 ± 0.04	1.00 ± 0.07	−0.25 *
Glycine	34.60 ± 0.08	24.38 ± 0.09	−0.51 *
Valine	30.24 ± 0.00	20.43 ± 0.02	−0.57 *
Norleucine	1.34 ± 0.06	0.77 ± 0.06	−0.80 **
Phenylalanine	0.30 ± 0.04	0.14 ± 0.05	−1.11 *
Isoleucine	9.09 ± 0.04	7.64 ± 0.01	−0.25 *
Aspartic acid	9.36 ± 0.06	5.79 ± 0.01	−0.69 *
Asparagine	4.66 ± 0.01	2.99 ± 0.07	−0.64 *
Glucose	0.21 ± 0.02	0.26 ± 0.02	0.28 *
Fucose	0.09 ± 0.01	0.17 ± 0.03	0.95 *
Galactose	1.39 ± 0.07	2.78 ± 0.05	1.00 *
Fructose	0.79 ± 0.12	1.51 ± 0.16	0.93 **
Maltose	2.87 ± 0.03	5.27 ± 0.62	0.88 *
Galactinol	0.38 ± 0.01	0.56 ± 0.07	0.55 *
Phytol	1.85 ± 0.44	4.22 ± 0.48	1.19 **
Stigmasterol	0.47 ± 0.05	0.68 ± 0.04	0.54 *
Myo-inositol	56.74 ± 0.00	107.00 ± 0.01	0.92 *
Erythritol	0.44 ± 0.05	0.53 ± 0.03	0.28
Resveratrol	0.08 ± 0.01	0.13 ± 0.02	0.80 *
Palmitate	0.12 ± 0.01	0.16 ± 0.01	0.41 *
Gluconic lactone	2.58 ± 0.17	4.92 ± 0.06	0.93 **
L-Malic acid	43.09 ± 0.07	31.07 ± 0.11	−0.47 *
Succinic acid	0.08 ± 0.01	0.11 ± 0.01	0.37 *
Pyruvic acid	22.10 ± 0.47	22.78 ± 0.71	0.04
Citric acid	18.80 ± 0.04	23.22 ± 0.05	0.30
3-hydroxy-3-methylglutaric acid	0.34 ± 0.07	0.79 ± 0.05	1.20 **
Ribulose-5-phosphate	0.10 ± 0.01	0.17 ± 0.02	0.78 **
Citraconic acid	0.78 ± 0.06	1.03 ± 0.06	0.40 *
Linolenic acid	8.11 ± 0.31	15.76 ± 0.04	0.96 *
Glycerol	0.16 ± 0.04	0.34 ± 0.08	1.12 *
Stearic acid	6.58 ± 0.01	10.01 ± 0.04	0.60 *
Palmitic acid	15.48 ± 0.01	25.49 ± 0.71	0.72 *
Arachidonic acid	0.20 ± 0.03	0.38 ± 0.06	0.89 *
Pentadecanoic acid	0.16 ± 0.02	0.19 ± 0.02	0.30
Ascorbate	0.27 ± 0.05	0.51 ± 0.06	0.89 *
5-Methoxytryptamine	1.42 ± 0.05	1.66 ± 0.08	0.22
Ferulic acid	0.99 ± 0.20	1.31 ± 0.05	0.39 *

Relative concentration values and standard deviations were increased 10,000 times. * and ** indicate significant ($P < 0.05$) and highly significant ($P < 0.01$) differences, respectively. W1: salt-sensitive wild soybean; W2: salt-tolerant wild soybean.

Table 4
Relative concentrations and fold changes of differential metabolites in the salt-sensitive wild soybean and cultivated soybean in the early flowering stage.

Metabolite name	W1	C	$Log_2^{(C/W1)}$
Alanine	0.23 ± 0.06	0.80 ± 0.22	−1.79 *
L-ornithine	0.23 ± 0.02	0.31 ± 0.02	−0.46 *
Methionine	0.72 ± 0.03	1.19 ± 0.04	−0.72 **
Taurine	0.20 ± 0.02	0.26 ± 0.02	−0.43 *
Leucine	1.35 ± 0.28	18.87 ± 0.77	−3.80 **
Sarcosine	65.78 ± 0.71	192.20 ± 0.63	−1.55 **
Glycine	3.28 ± 0.25	2.59 ± 0.08	0.34 *
Tryptophan	0.17 ± 0.04	0.06 ± 0.01	1.41 *
Erythrose	0.56 ± 0.08	0.27 ± 0.05	1.04 *
D-galactopyranoside	1.69 ± 0.24	0.09 ± 0.01	4.26 **
Fucose	0.21 ± 0.02	0.09 ± 0.01	1.21 **
Sorbose	0.29 ± 0.04	0.78 ± 0.10	−1.44 **
Cellobiose	0.07 ± 0.01	0.11 ± 0.01	−0.59 *
Fructose	1.05 ± 0.23	0.79 ± 0.12	0.41
Threitol	5.45 ± 0.67	3.97 ± 0.25	0.46
Phytol	4.24 ± 0.69	1.85 ± 0.44	1.20 *
Myo-Inositol	44.37 ± 0.91	23.63 ± 0.50	0.91 **
Erythritol	0.48 ± 0.03	0.44 ± 0.05	0.13
Citric acid	139.38 ± 0.36	18.80 ± 0.34	2.89 **
L-malic acid	23.74 ± 0.24	43.09 ± 0.07	−0.86 *
Malonic acid	0.21 ± 0.02	0.13 ± 0.02	0.67 *
Citraconic acid	1.32 ± 0.05	0.78 ± 0.06	0.76 **
Nicotinic acid	8.10 ± 0.40	3.81 ± 0.28	1.09 *
Glucose-6-phosphate	0.22 ± 0.04	0.10 ± 0.03	1.10 *
Benzoic acid	11.80 ± 0.09	0.51 ± 0.04	4.52 **
D-glycerol- 1 -phosphate	3.32 ± 0.01	1.01 ± 0.03	1.72 *
Glycerol	4.74 ± 0.03	2.92 ± 0.06	0.70 *
Arachidonic acid	0.24 ± 0.06	0.20 ± 0.03	0.23
Palmitic acid	16.07 ± 0.46	15.48 ± 0.06	0.05 *
Stearic acid	6.76 ± 0.04	6.58 ± 0.07	0.04
Ascorbate	0.25 ± 0.02	0.27 ± 0.05	−0.14 *
Shikimic acid	0.82 ± 0.06	1.04 ± 0.06	−0.35 *
Putrescine	0.91 ± 0.04	4.43 ± 0.87	−2.28 **
Ethanolamine	0.98 ± 0.04	1.15 ± 0.04	−0.24 *

Relative concentration values and standard deviations were increased 10,000 times. * and ** indicate significant ($P < 0.05$) and highly significant ($P < 0.01$) differences, respectively. W1: salt-sensitive wild soybean; C: cultivated soybean.

further confirmed earlier results (Yang and Ji, 1999; Zhang et al., 2008). Carbohydrates and lipids are basic metabolites and important biochemical characteristics that reflect the nutritional values of cultivated plants (Loskutov et al., 2017). Compared with C, wild soybean has a lower fat content (Lan, 2010). The fatty acid metabolism was stronger in C than W1, and the accumulations of fatty acid metabolites glycerol, arachidonic acid, palmitic acid and stearic acid in C were greater than in W1. Our results were consistent with previous studies, indicating that C was more suitable to human needs. Wild soybean is the ancestor of C, which has many excellent characteristics, and is an important source of major genes for resistance to pests, diseases and environmental stress (Dong et al., 2001). During the long-term artificial domestication, harmful mutations might increase, resulting in the loss of adaptability and stress resistance in soybean (Lu et al., 2006). The secondary metabolism of W1 in the early flowering stage was stronger than in C, which further indicated that the antioxidant-based ability to withstand adverse environmental conditions of C decreased after artificial cultivation. We analyzed the correlations among photosynthetic activity, mineral elements and significant differential metabolites according to Chong et al. (2018) (Fig. S1). The correlations among C's photosynthesis, mineral nutrition and cell metabolism were not as regular as those of W1, and cannot regulate life activities under external adverse environmental conditions, making it susceptible to adverse environmental conditions.

In general, soybean cultivation has met human demands on morphological and metabolic levels, but the environmental resistance of C has become weaker than that of wild soybean during acclimation. Wild soybean, especially W2, has a strong resistance to adverse environmental conditions. Therefore, it is necessary to improve the quality of soybean resistance by exploiting and utilizing wild soybean resources.

5. Conclusion

In this study, differences in photosynthetic activity, mineral nutrition and metabolomics among three soybean genotypes in the early flowering stage were compared. The survival and growth of soybean relies on the interplay between stress-adaptive morpho-physiological changes that occur at the whole-plant level and metabolic changes that occur at the cellular level. W2 had more physiological metabolic advantages than W1, which could maintain a greater photosynthetic activity, reduce energy consumption and absorb more of the favorable mineral elements Mg, Zn, Mo, Mn and B, and enhance carbohydrate, polyol, organic acid and fatty acid metabolism as well as secondary metabolism. As a result of domestication and breeding, C had a greater photosynthetic activity and can enhance fatty acid metabolism, which helps meet human demands. However, some excellent traits, such as amino acid metabolism and secondary metabolism, have been lost in this process, and the ability to withstand adverse environmental conditions was weakened. Thus, our results will be useful for the protection and utilization of wild soybean resources and the domestication or

improvement of C.

Contributions

MXL, JX, RG and LXS designed the study. MXL, RG and YL performed the research. MXL analyzed the data, and MXL, JX, SYW, HW, AU and LXS wrote the manuscript. All the authors reviewed the manuscript.

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Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.03.012.

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