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Full Length Article

Gas Exchange and Ionomic Changes in Wild and Cultivated Soybean Seedlings under Salt Stress

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Abstract

A comprehensive acquaintance of photosynthesis and ionic homeostasis are essential for improvement of salt tolerance in crops. However, gas exchange, ion homeostasis and their effects on metabolites in soybean under salt stress have not been fully investigated. In this study, wild and cultivated soybeans were used to explore gas exchange and ionomic profile changes in order to reveal the salt tolerance mechanism in wild soybean. Under neutral salt stress (NS), wild soybean increased the net photosynthetic rate (p_N) and decreased Na^+ and Cl^- absorption and accumulated in the roots and reduced content in leaves than in cultivated soybean. Under alkaline salt stress, it inhibited the absorption of Cl^- to adapt salt stress. During neutral salt stress in wild soybean, p_N and carotenoids in leaves were positively correlated with fatty acid metabolism, but negatively correlated with amino acid, organic acid and antioxidant receiver operating characteristics (ROC) metabolisms in roots and Na^+ and Cl^- were negatively correlated with fatty acid metabolism in leaves and with TCA cycle in roots. Under alkaline salt stress, gas exchange was negatively correlated with amino acid metabolism in leaves, and with TCA cycle in roots and Na^+ was positively correlated with amino acid metabolism. Results indicated the restricted accumulation of toxic ions, regulated absorption of nutrient elements, maintained gas exchange and then caused changes in small molecule metabolites pathway are possible reasons for salt tolerance in wild soybean. © 2019 Friends Science Publishers

Keywords: Cultivated soybean; Gas exchange; Ionomics; Metabolite; Salt tolerance; Wild soybean
Abbreviations: AS - alkaline salt stress; *Car* - carotenoids; *chl* a - chlorophyll a; *chl* a/b - chlorophyll a/b; *chl* b - chlorophyll b; *chl* t - total chlorophyll content; C_i - CO_2 concentration; E - Transpiration rate; g_s - Stomatal conductance; NS - neutral salt stress; M - Cultivated soybean; PCA - principal component analysis; PC1 - the first principal component; PC2 - the second principal component; p_N - Net photosynthetic rate; W - Wild soybean; *WUE* - Water-use efficiency; ROC - receiver operating characteristics; TCA - the tricarboxylic acid cycle; RGR - the relative growth rates

Introduction

Cultivated soybean (*Glycine max*) originated in China and is one of the most popular crops in the world due to oil and dietary protein (Zhang *et al.,* 2011). Cultivated soybean is a typical glycophyte plant, and its growth and yield are greatly inhibited by salt stress. Wild soybean (*Glycine soja*) is widely distributed in China, Japan, Korea and other parts of East Asia (Xue *et al.,* 2014). As the close progenitor of cultivated soybean, wild soybean has evolved various ecotypes to adapt to adverse environments and has superior quality characteristics, such as genetic diversity for salt tolerance (Chen *et al.,* 2013a). It is possible and desirable to breed elite germplasm of cultivated soybean incorporating salt tolerance from wild soybean. With the development of industry and the economy in China, the degree of salinization of agricultural land is increasingly serious, with about a quarter of the total irrigated land in the world now damaged by salts (Taffouo *et al.,* 2010). Photosynthesis is the basis of plant growth and development, but is very sensitive to the external environment (Chen *et al.,* 2013b). Under short-term NaCl stress, the receptor side of photosystem II of soybean leaves suffers heavy injury (Ren *et al.,* 2009).

The uptake of ions and ion homeostasis are indispensable to normal growth of plants (Gupta and Huang, 2014; Zhu and Han, 2018). However, salt stress not only causes ion toxicity in plants, but also disrupts the absorption of ions including K^+, Mg^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} and B^{3+} (Munns, 2005; Tavakkoli *et al.,* 2011). Brini and Masmoudi (2012) reported that excessive accumulation of Na^+ and Cl^- harms plants, especially Na^+ for leguminous plants. It was reported that wild soybean could reduce the accumulation of Na^+ and Cl^- to improve salt tolerance (Shao *et al.,* 2016). Many studies have focused on the effects of salt stress on one or several kinds of ions, while few studies have clarified the response of ionome to salt stress in *soja* (Yu *et al.,* 2001; Arshi *et al.,* 2012; An *et al.,* 2017). Plant ionomics is a comprehensive study of inorganic elements in plants. Based

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on the integrality and complexity of ionic balance in plants, it is very important to analyze the mechanism of salt tolerance in plants by means of ionomics.

Salt stress severely limits the growth and yield of crops by damaging mechanisms, including photosynthesis and mineral nutrition and the metabolic products of plants change as a result (Farooq *et al.*, 2017; Yang *et al.*, 2017). Our previous studies revealed the physiological and molecular differences between wild soybean (W; Huinan06116) and cultivated soybean (M; Jinong24) using metabolomics under two types of salt stress. The results demonstrated 40 differential metabolites among the two genotypes under neutral salt stress and 43 metabolites under alkaline salt stress for leaves and correspondingly 47 and 62 metabolites in roots (Zhang *et al.*, 2016; Li *et al.*, 2017). However, the photosynthetic characteristics, ionomics and their effects on metabolites in wild soybean and cultivated soybean under salt stress remained obscure. In this study, we investigated the changes of gas exchange and ionomics and demonstrated correlations among changes in gas exchange, ions and metabolites in wild soybean and cultivated soybean under two types of salt stress. Our aim was to determine the physiological adaptive mechanisms of wild soybean under salt stress, which is more salt tolerant than cultivated soybean. The study will help protect and utilize of wild soybean resources and provide a theoretical basis for improving salt resistance of cultivated soybean.

Materials and Methods

Plant Materials

Seeds of wild soybean (W; Huinan06116) and cultivated soybean (M; Jinong24) from the same latitude in the northeast of China were taken from Jilin Academy of Agriculture Science, China.

Stress Treatments

Seeds of W and M were sown in 14-cm diameter plastic pots containing 2.5 kg of washed sand and were grown by irrigating with water. Plants were grown in an outdoor experimental field, with temperatures of 18.5 ± 1.5/26 ± 2°C for day/night at Northeast Normal University, Changchun, Jilin.

Salt treatment was initiated on plants when third compound leaves were emerged. In the salt-treated group, W and M were exposed to neutral salt stress based on 1×Hoagland's nutrient solution (NaCl and Na_2SO_4, at a 1:1 molar ratio; NS) and alkaline salt stress (Na_2CO_3 and $NaHCO_3$, at a 1:1 molar ratio; AS). For the first two days, 15 m*M* Na^+ was added; for the next two days, 30 m*M* Na^+ was added and finally, in the next two weeks, 45 m*M* Na^+ was added. In the control group, soybeans were cultivated under normal conditions (1× Hoagland solution). W and M were respectively divided into three groups: control, NS and AS groups. Each group consisted of eight pots: four pots for measuring basal biomass and photosynthesis and determination of ion contents after drying; and four pots for metabolomic analyses.

Photosynthetic Indices Measurements

The first day after the salt stress, P_N, g_s, C_i and E of leaves were determined using a portable open flow gas exchange system LI-6400 (LICOR, USA) at 10:00 AM; the respective results were expressed as $umol \cdot m^{-2} \cdot s^{-1}$, $mol \cdot m^{-2} \cdot s^{-1}$, $umol \cdot mol^{-1}$ and $mmol \cdot m^{-2} \cdot s^{-1}$. *WUE* was calculated as the ratio of p_N/E. Air temperature and humidity were about 24°C and 50%, respectively. Gas exchange parameters were measured in fully expanded leaves: 36 data points including nine repeats for each plant per biological replicate pot were recorded. 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. Spectrophotometric (SpectrUV-754, Shanghai Accurate Scientific Instrument Co.) determination at 440, 645 and 663 nm of each sample was performed three times, using the formulae of Holm (1954).

Growth Indices Measurements

Shoot height, root length, dry weight (DW) and the relative growth rates (RGRs) of plant materials were determined according to Li *et al.* (2017).

Ion Content Measurements

Dry roots and leaves were grinded, and 50 mg of plant materials 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 supernatant was collected. Subsequently, the course was repeated twice and three times the supernatants were collected in tubes; then their volume was made up to 10 mL. Unified supernatants were used to determine Cl^-, SO_4^{2-}, NO_3^-, $C_2O_4^{2-}$ and $H_2PO_4^-$ concentrations by 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 m*M*, DIONEX, Sunnyvale, C.A., U.S.A.). An inductively coupled plasma atomic emission spectrometer (Prodigy, Leeman, U.S.A.) was used to determine the concentrations of Na^+, K^+, Mn^{2+}, Ca^{2+}, Fe^{2+}, Zn^{2+}, B^{3+} and Mg^{2+}. Each sample was repeated three times.

Metabolite Profiling Analysis

For metabolite profiling analysis, metabolites were extracted from soybean leaves and roots (100 ± 5 mg) and their contents were determined using an Agilent 7890 GC system

coupled to a Pegasus HT time-of-flight MS, as described by Li *et al.* (2017).

Data Processing Analysis

We analyzed the changes of the gas exchange parameters and ions under salt stress by calculating the FD (fold changes). The gas exchange parameters and ions were prepared by a principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), orthogonal partial least squares discriminant analysis (OPLS-DA) and loading plot were performed by SIMCA-P 13.0 software package (Umetrics, Umea, Sweden) using normalized data. Use Metaboanalyst 4.0 (http://www.metaboanalyst.ca/) did a correlation analysis, and explained the effects of gas exchange parameters and ionomics on metabolites (Xia and Wishart, 2016).

Results

Growth Response of Soybean under Salt Stress

Compared with the control, both species of salt-stressed seedlings had obviously inhibited growth and biomass. The numbers of leaves and roots per plant decreased, the leaf area became smaller, old leaves turned deep yellow and root color deepened after salt stress. Additionally, shoot height, root length, DW of roots and RGR of roots decreased significantly under both salt stresses ($P < 0.05$). The amplitude of the inhibition was higher in cultivated than in wild soybean.

Gas Exchange and Photosynthetic Pigment Contents of Soybean under Salt Stress

There were obvious genotypic differences in p_N, E, g_s, C_i, *WUE* and photosynthetic pigment contents in two genotypes after both salt treatments. PCA clearly separated between samples within treatments and among genotypes (Fig. 1). The control and treatment samples in leaves were clearly separated by PC1, representing 82.6% of the total variation (Fig. 1A). The major determinants that contributed to PC1 were p_N, E, g_s and *chl* b (Fig. 1B). PC2 clearly distinguished the samples in leaves, explaining 11.5% of the variation (Fig. 1A). The major contributions of factors to PC2 were p_N, *WUE*, *chl* a and *chl* t (Fig. 1B).

Under NS, p_N reduced significantly by 6.45% in cultivated soybean; however, this increased in wild soybean (Table 1). E and g_s in wild soybean reduced significantly by 18.21 and 18.04%, respectively, in comparison with the respective controls and correspondingly decreased by 21.60 and 37.06% in cultivated soybean. Notably, contents of *chl* a, *chl* b and *chl* t significantly increased by 12.97, 39.74 and 17.01% in W, respectively, and in *chl* a/b and *Car* contents reduced by 19.15 and 8.25%, respectively. The *chl* a, *chl* a/b, *chl* t and *Car* increased significantly by 26.36, 10.48, 23.80 and 21.07% in M, respectively.

Under AS, p_N, E and g_s in W decreased significantly by 70.42, 72.84 and 81.08%, respectively, compared with the control, and correspondingly decreased by 98.02, 92.39 and 96.76% in M. The *WUE* reduced significantly by 79.67% in M. The *chl* a, *chl* a/b, *chl* t and *Car* decreased significantly by 12.97, 20.99, 9.45 and 24.74% compared with the control group in W, respectively (Table 1) and correspondingly reduced significantly by 13.98, 13.97, 9.66 and 6.89% in M. NS promoted accumulation of photosynthetic pigments in W, but was opposite under AS. Under both salt stresses, the C_i content did not significantly differ in W, but decreased in M.

Ionomics Changes of Soybean under Salt Stress

The control and treatment samples in leaves were clearly separated by PC1, representing 57.6% of the total variation (Fig. 2A). The major determinants which contributed to PC1 were Na^+, Ca^{2+} and NO_3^- (Fig. 2B). PC2 clearly distinguished the samples in leaves, explaining 19% of the variation (Fig. 2A). The major contribution of factors to PC2 was dominated by Fe^{2+}, B^{3+}, $H_2PO_4^-$ and $C_2O_4^{2-}$ (Fig. 2B). Control and treatment samples in roots were clearly separated by PC1, representing 44.6% of the total variation (Fig. 2C), with the major determinants being Na^+, Fe^{2+}, NO_3^- and K^+ (Fig. 2D). PC2 clearly distinguished the samples in roots, explaining 21.7% of the variation (Fig. 2C), with major contributions by Ca^{2+}, SO_4^{2-} and B^{3+} (Fig. 2D).

Under NS, the Na^+ and Cl^- contents in leaves and roots in both genotypes increased significantly (Table 2). Compared with the control group, the accumulation of Na^+ and Cl^- in the leaves and roots of M increased more than W and the accumulation in the roots accumulated more than in the leaves under NS. The contents of SO_4^{2-}, Zn^{2+} and Mn^{2+} in leaves increased significantly. The Fe^{2+} contents in leaves and roots of W and M and B^{3+} contents in leaves of M increased significantly ($p < 0.05$). The NO_3^- and K^+ contents significantly decreased, especially in roots of W. Except for M leaves, the Mg^{2+} and Ca^{2+} contents decreased significantly.

Under AS, the Na^+ contents significantly increased in leaves and roots in both genotypes, with significantly greater increases than in NS, especially in M (Table 2). The K^+ and NO_3^- contents significantly decreased in W and M. In leaves and roots, K^+/Na^+ ratios were 523.9 and 29.79% in W, respectively, and correspondingly 412.57 and 16.8% in M, showing a greater K^+/Na^+ ratio in W. The contents of B^{3+} in leaves and Fe^{2+} in roots of W increased significantly by 14.46 and 181.81% compared with controls, respectively.

Effect of Gas Exchange on Metabolites in Leaves and Roots

Metabolites included 11 species in leaves and 14 species in roots in W, 7 species in leaves and 18 species in roots in M under NS, 14 species in leaves and 32 species in roots in W,

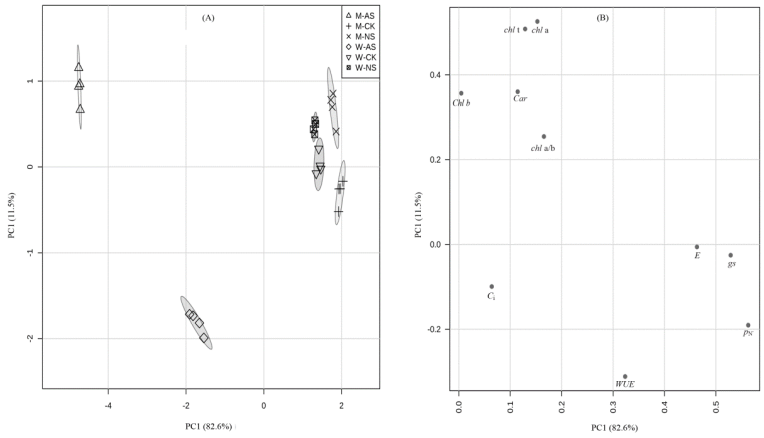

Fig. 1: Leaves gas exchange variation analysis using the PCA under control and two types of salt stress and the loadings of gas exchange to the PC1 and PC2. (**A**) Leaves gas exchange variation among samples, (**B**) the loadings of gas exchange to the PC1 and PC2 in leaves. CK: controls; NS: neutral salt stress; AS: alkaline salt stress; PC1: the first principal component; PC2: the second principal component

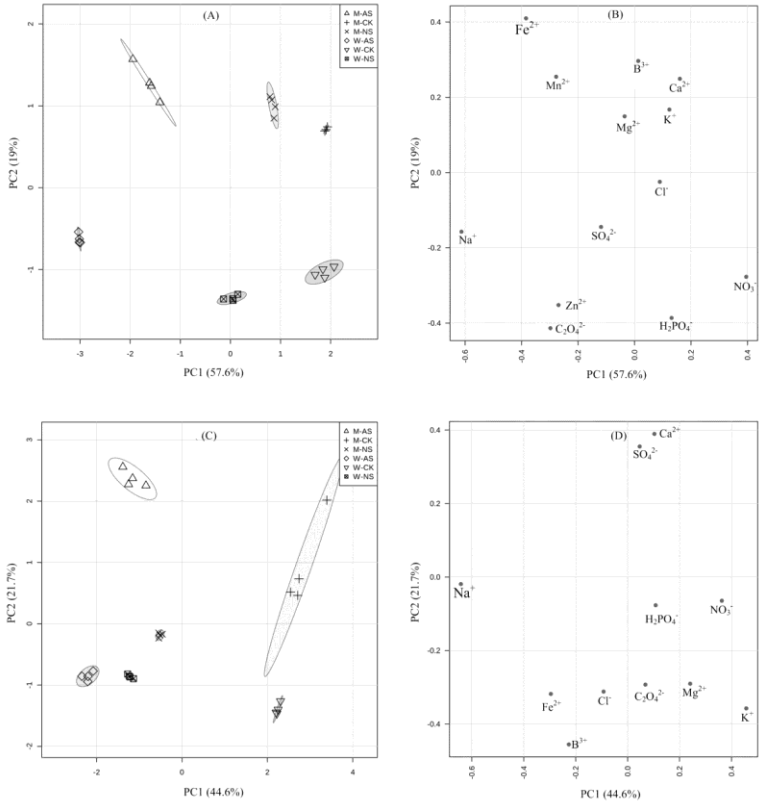

Fig. 2: Tissue ions variation analysis using the PCA under control and two types of salt stress conditions and the loadings of ions to the PC1 and PC2. (**A**) leaf ions variation among samples; (**B**) the loadings of ions to the PC1 and PC2 in leaves; and (**C**) Root ions variation among samples; (**D**) the loadings of ions to the PC1 and PC2 in roots. CK: controls; NS: neutral salt stress; AS: alkaline salt stress; PC1: the first principal component; PC2: the second principal component

16 species in leaves and 30 species in roots in M under AS. To understand the effect of gas exchange parameters on metabolites, correlations between gas exchange and metabolites were determined (Fig. 3 and 4; $P < 0.05$).

Under NS, in leaves p_N, E, g_s and *Car* had significantly positive correlations with 4 kinds of fatty acids, and negatively with tyrosine, isoleucine; *chl* t had significantly negatively with asparagine, tyrosine, and positively with linolenic acid, stearic acid in W. The p_N, *chl* t and *Car* were significantly negatively correlated with 7 kinds of metabolites; E and g_s were significantly positively correlated with 4 kinds of organic acids in M. In roots, gas

Table 1: Gas exchange parameters in leaves of two soybean genotypes under two types of salt stress

Parameters	W					M				
	CK	NS	AS	$FDlog_2^{(NS/CK)}$	$FDlog_2^{(AS/CK)}$	CK	NS	AS	$FDlog_2^{(NS/CK)}$	$FDlog_2^{(AS/CK)}$
p_N (umol·m^{-2}·s^{-1})	8.89±0.07a	9.19±0.02a	2.63±0.08b	0.05	-1.76*	13.10±0.11a	12.25±0.07b	0.26±0.00c	-0.10*	-5.66*
E (mmol·m^{-2}·s^{-1})	6.62±0.09a	5.13±0.01b	1.70±0.11c	-0.29*	-1.88*	7.64±0.01a	5.99±0.05b	0.58±0.00c	-0.35*	-3.72*
g_s (mol·m^{-2}·s^{-1})	0.36±0.01a	0.30±0.01b	0.07±0.01c	-0.29*	-2.40*	0.51±0.01a	0.32±0.01b	0.02±0.00c	-0.67*	-4.95*
WUE (umol·mol^{-1})	1.42±0.03a	1.79±0.00a	1.57±0.05a	0.33	0.14	2.19±0.02a	2.26±0.04a	0.44±0.01b	0.05	-2.30*
C_i (umol·mol^{-1})	280.50±1.34a	273.25±0.52a	268.50±3.06a	-0.04	-0.06	272.00±1.78a	260.50±1.59ab	251.00±1.08b	-0.06*	-0.12*
Chl a (mg·g^{-1}DW)	8.48±0.15b	9.58±0.46a	7.38±0.06c	0.18*	-0.20*	8.08±0.42b	10.21±0.22a	6.95 ±0.18c	0.34*	-0.22*
Chl b (mg·g^{-1}DW)	1.56±0.03a	2.18±0.54a	1.72±0.06a	0.59	0.14	2.17 ±0.26a	2.48±0.25a	2.30±0.06a	0.19	0.08
Chl a/b(mg·g^{-1}DW)	5.43±0.12a	4.39±0.67b	4.29±0.09c	-0.31*	-0.34*	3.72±0.31ab	4.11±0.33a	3.02±0.04b	0.14*	-0.30*
Chl t (mg·g^{-1}DW)	10.05±0.16b	11.76±0.99a	9.10±0.10c	0.23*	-0.14*	10.25±0.63b	12.69±0.46a	9.26±0.24b	0.31*	-0.15
Car (mg·g^{-1}DW)	2.91±0.05a	2.67±0.03b	2.19±0.05c	-0.13*	-0.41*	2.61±0.06b	3.16±0.02a	2.43±0.07bc	0.28*	-0.11*

The data are the means from four biological replicates. The fold changes was calculated using the formula $log_2^{(salt/control)}$. Values were presented as the mean ± standard deviation of four biological replicates. CK: control treatment; NS: neutral salt stress; AS: alkaline salt stress; W: wild soybean; M: cultivated soybean
*Significant difference at $P < 0.05$

Table 2: Ions contents (mmol·g^{-1} DM) in leaves and roots of two soybean genotypes under two types of salt stress

		W					M				
		CK	NS	AS	FD $log_2^{(NS/CK)}$	FD $log_2^{(AS/CK)}$	CK	NS	AS	FD $log_2^{(NS/CK)}$	FD $log_2^{(AS/CK)}$
Leaves	Na^+	9.43 ± 0.00c	15.51 ± 0.00a	25.17 ± 0.01b	0.72*	1.42*	6.95 ± 0.00c	13.25 ± 0.00b	35.15 ± 0.01a	0.93*	2.34*
	Cl^-	9.05 ± 0.00b	19.37 ± 0.01a	9.00 ± 0.00b	1.10*	-0.01	8.81 ± 0.00b	25.41 ± 0.00a	8.56 ± 0.01b	1.53*	-0.04
	NO_3^-	34.68 ± 0.00a	25.76 ± 0.01b	11.99 ± 0.00c	-0.43*	-1.53*	31.62 ± 0.00a	20.06±0.00ab	10.14 ± 0.02b	-0.66*	-1.64*
	K^+	150.36 ± 0.01a	134.64± 0.01b	134.71±0.01b	-0.16*	-0.16*	160.49±0.00a	142.91±0.00b	140.02±0.00c	-0.17*	-0.19*
	Mg^{2+}	28.57 ± 0.00a	22.80 ± 0.00b	29.74 ± 0.00a	-0.33*	0.06	27.48 ± 0.00a	28.86 ± 0.00a	28.26 ± 0.00a	0.07	0.04
	Ca^{2+}	31.00 ± 0.00a	26.87 ± 0.00b	26.31 ± 0.00b	-0.21*	-0.24*	35.10 ± 0.00a	35.83 ± 0.00a	29.58 ± 0.00b	0.03	-0.25*
	SO_4^{2-}	18.37 ± 0.00c	20.30 ± 0.00b	23.64 ± 0.00a	0.14*	0.36*	14.99 ± 0.01b	23.08 ± 0.01a	16.96±0.01ab	0.62*	0.18*
	Mn^{2+}	0.02 ± 0.00c	0.03 ± 0.00b	0.04 ± 0.00a	0.90*	1.24*	0.03 ± 0.00c	0.03 ± 0.00b	0.04 ± 0.00a	0.22*	0.50*
	Zn^{2+}	0.02 ± 0.00b	0.04 ± 0.00ab	0.05 ± 0.00a	0.74*	1.12*	0.02 ± 0.00a	0.02 ± 0.00a	0.03 ± 0.00a	0.00	0.13
	B^{3+}	0.39 ± 0.00b	0.38 ± 0.00b	0.44 ± 0.00a	-0.04	0.19*	0.48 ± 0.00ab	0.52 ± 0.00a	0.43 ± 0.00b	0.10*	-0.16*
	Fe^{2+}	0.05 ± 0.00c	0.08 ± 0c.00b	0.18 ± 0.00a	0.56*	1.92*	0.07 ± 0.00b	0.09 ± 0.00b	0.26 ± 0.00a	0.37	1.87*
	$H_2PO_4^-$	18.64 ± 0.00a	18.46 ± 0.00a	12.90 ± 0.00b	-0.01	-0.53*	13.80 ± 0.00a	12.87±0.00ab	12.30 ± 0.00b	-0.10*	-0.17*
	$C_2O_4^{2-}$	2.83 ± 0.00b	3.08 ± 0.00b	4.91 ± 0.00a	0.12	0.79*	1.75 ± 0.00b	1.69 ± 0.00b	2.76 ± 0.00a	-0.05	0.65*
Roots	Na^+	13.39 ± 0.00c	80.31 ± 0.01b	110.20 ±0.04a	2.58*	3.04*	6.20 ± 0.00c	117.97±0.01a	83.57 ± 0.03b	4.25*	3.75*
	Cl^-	14.86 ± 0.00b	22.23 ± 0.00a	12.29 ± 0.00b	0.58*	-0.27	9.86 ± 0.00b	29.31 ± 0.00a	9.51 ± 0.00b	1.57*	-0.05
	NO_3^-	69.56 ± 0.00a	27.97 ± 0.01b	20.41 ± 0.00c	-1.31*	-1.77*	47.74 ± 0.00a	34.77 ± 0.00b	30.36 ± 0.01b	-0.46*	-0.65*
	K^+	113.99 ± 0.00a	28.13 ± 0.00b	32.83 ± 0.02b	-2.02*	-1.80*	96.04 ± 0.01a	41.20 ± 0.00b	14.10 ± 0.00c	-1.22*	-2.77*
	Mg^{2+}	44.11 ± 0.01a	21.90 ± 0.00b	22.24 ± 0.01b	-1.01*	-0.99*	27.29 ± 0.01a	25.03 ± 0.00b	18.03 ± 0.02c	-0.12*	-0.60*
	Ca^{2+}	16.16 ± 0.00a	14.00 ± 0.00b	14.59 ± 0.01b	-0.21*	-0.15*	18.78 ± 0.00a	12.83 ± 0.00b	26.90 ± 0.01c	-0.55*	0.52*
	SO_4^{2-}	17.88 ± 0.01a	12.40 ± 0.00b	11.15 ± 0.01b	-0.53*	-0.68*	17.27 ± 0.00c	28.92 ± 0.00b	31.24 ± 0.00a	0.74*	0.85*
	B^{3+}	0.12 ± 0.00a	0.09 ± 0.00b	0.14 ± 0.00a	-0.39*	0.23	0.04 ± 0.00a	0.05 ± 0.00a	0.06 ± 0.00a	0.35	0.79
	Fe^{2+}	0.15 ± 0.00b	0.29 ± 0.00b	0.43 ± 0.00a	0.95	1.49*	0.09 ± 0.00c	0.17 ± 0.00a	0.13 ± 0.00b	0.87*	0.44*
	$H_2PO_4^-$	17.54 ± 0.00a	16.13 ± 0.00a	9.25 ± 0.01b	-0.12	-0.92*	13.08 ± 0.00b	18.77 ± 0.00a	13.27 ± 0.01b	0.52*	0.02
	$C_2O_4^{2-}$	3.41 ± 0.00a	2.56 ± 0.00c	3.03 ± 0.00b	-0.41*	-0.17*	2.67 ± 0.00b	3.31 ± 0.00a	2.06 ± 0.00c	0.31*	-0.37*

The data are the means from four biological replicates. The fold changes was calculated using the formula $log_2^{(salt/control)}$. Values were presented as the mean ± standard deviation of four biological replicates. CK: control treatment; NS: neutral salt stress; AS: alkaline salt stress; W: wild soybean; M: cultivated soybean
*Significant difference at $P < 0.05$

exchange, *chl* t and *Car* were significantly negatively correlated with 14 metabolites metabolites in W. Eight kinds of amino acids and 5 kinds of organic acids in roots had significant negative correlations with p_N, *chl* t and *Car*; p_N, *chl* t and *Car* were significantly positively correlated with naringin, gallic acid in M.

Under AS in leaves, gas exchange, *chl* t and *Car* were significantly negatively correlated with 14 kinds of metabolites in W. 16 kinds of metabolites in M were significantly negatively correlated with p_N, E and g_s and positively with *chl* t and *Car* in M. In roots, gas exchange, *chl* t and *Car* were negatively correlated with 8 kinds of amino acids, 5 kinds of organic acids and two kinds of antioxidant receiver operating characteristics (ROC) substances and positively with 11 kinds of sugar alcohol and 5 kinds of organic acids in W. 10 kinds of amino acids and digalacturonic acid, myristic acid, gallic acid were significantly negatively correlated with p_N, E and g_s, and positively with *chl* t and *Car*. 6 kinds of glycols and 4 kinds of organic acids were significantly positively correlated with gas exchange and negatively with *chl* t and *Car* in M.

Effect of Ionomics on Metabolites in Leaves and Roots

To understand the effect of ionomics on metabolites, we found significant correlations between ions and metabolites in W and M (Fig. 3 and 4; $P < 0.05$). Under NS in leaves, glucose, three kinds of organic acids and four kinds of fatty acids were significantly positively correlated with K^+, Ca^{2+}, Mg^{2+}, B^{3+}, NO_3^- and $H_2PO_4^-$, and negatively correlated with

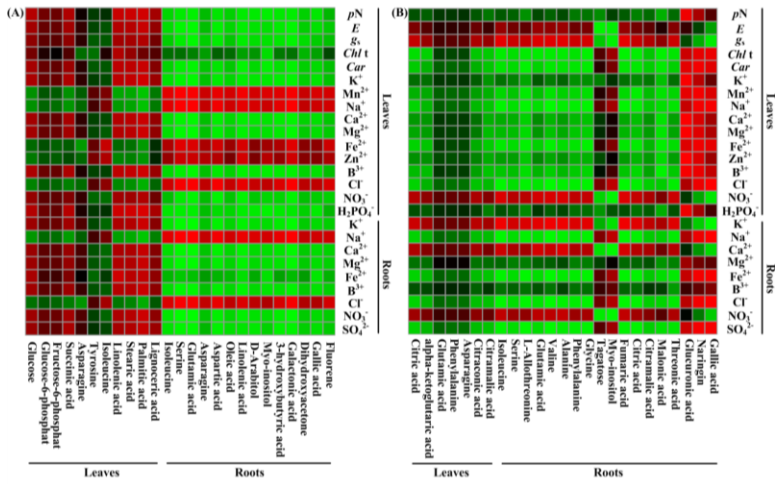

Fig. 3: The correlation between gas exchange and metabolites, between ions and metabolites in tissue of W and M under neutral salt stress. (**A**) W- neutral salt stress; (**B**) M- neutral salt stress. Green color indicates a significantly negative correlation at the $P < 0.05$ level, while red color indicates a significantly positive correlation at the $P < 0.05$ level, respectively. W: Wild soybean; M: Cultivated soybean

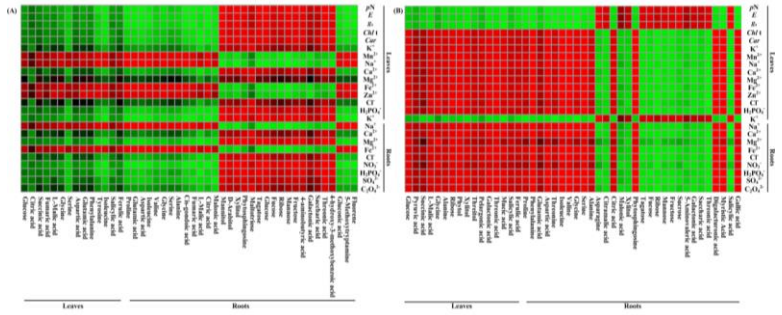

Fig. 4: The correlation between gas exchange and metabolites, between ions and metabolites in tissue of W and M under alkaline salt stress. (**A**) W- alkaline salt stress; (**B**) M- alkaline salt stress. Green color indicates a significantly negative correlation at the $P < 0.05$ level, while red color indicates a significantly positive correlation at the $P < 0.05$ level, respectively. W: Wild soybean; M: Cultivated soybean

Na^+, Cl^-, Mn^{2+}, Fe^{2+} and Zn^{2+} in W. In roots, 14 kinds of metabolites were significantly positively correlated with Na^+, Cl^-, and negatively correlated with K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, B^{3+}, NO_3^- and SO_4^{2-} in W. Eight kinds of amino acids and 5 kinds of organic acids were significantly negatively correlated with Na^+, Cl^-, Mg^{2+}, Fe^{2+}, B^{3+} and SO_4^{2-} and positively correlated with K^+, Ca^{2+} and NO_3^- in cultivated soybean.

Under AS in leaves, 14 kinds of metabolites were negatively correlated with Cl^-, K^+, Ca^{2+}, Mg^{2+} and $H_2PO_4^-$, and positively with Na^+, Mn^{2+}, Fe^{2+} and Zn^{2+} in W. Na^+, Cl^-, K^+, Mn^{2+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+} and $H_2PO_4^-$ were positively with 16 kinds of metabolites in cultivated soybean. In roots, 8 kinds of amino acids and 5 kinds of organic acids and 2 kinds of antioxidant ROC substances were positively correlated with Na^+ and Fe^{2+}, and negatively with Cl^-, K^+, Ca^{2+}, Mg^{2+}, NO_3^-, $H_2PO_4^-$, SO_4^{2-} and $C_2O_4^{2-}$, 11 kinds of sugar alcohol and 5 kinds of organic acids were negatively correlated with Na^+ and Fe^{2+}, and positively with Cl^-, K^+, Ca^{2+}, Mg^{2+}, NO_3^-, $H_2PO_4^-$, SO_4^{2-} and $C_2O_4^{2-}$ in wild soybean.

Discussion

Seedling growth and biomass are the most visible signs for measuring plant responses to various stresses (Qiu *et al.,* 2011; Rahnama *et al.,* 2011). Plant biomass is generally inhibited under salt stress (Shi and Sheng, 2005). Osmotic stress can lead to decrease in plant cell wall extensibility (Pitann *et al.,* 2009). Our experiments showed that growth and biomass of wild and cultivated soybeans were severely inhibited under both salt stresses, and the inhibition of cultivated soybean was greater, confirming the previously demonstrated stronger salt tolerance of wild compared with cultivated soybean.

The detrimental influences caused by two types of salt stress on plant growth could occur due to the damage to photosynthesis and ion imbalances (Latef and Tran, 2016). Photosynthesis is a sensitive physiological process that is inhibited by salt stress, mainly manifested in decreased p_N (Chen *et al.,* 2013b). Under both salt stresses, p_N decreased significantly especially for AS, and the amplitude of p_N

decrease was greater in cultivated than wild, indicating that cultivated soybean was more damaged than wild under salt stress. The g_s decreased significantly and C_i did not significantly change under both salt stresses in wild species. In addition, under AS, *chl* a significantly decreased possibly related to the decline of p_N, so the reason for the decrease of p_N in wild species was non-stomatal limitation (Zheng *et al.*, 2013). The decrease of p_N in wild soybean under salt stress might be due to accumulation of toxic ions in cells and chloroplast structure destruction, resulting in damage to photosynthetic organs and decreased photosynthetic activity of mesophyll cells. Nevertheless, the change trends of C_i and g_s were consistent – they all decreased in cultivated soybean. The reason for the decrease of p_N in cultivated line was stomatal limitation. The *WUE* in cultivated line decreased significantly under AS, but was not significantly different for W, showing the greater sensitivity of cultivated soybean to salt than wild type. In addition to inhibiting photosynthetic rate, salt stress also hinders chlorophyll synthesis or accelerates decomposition (Zhang *et al.*, 2010). NS promoted synthesis of *chl* a, illustrating that the inhibitory effect of AS on wild soybean chlorophyll synthesis and accumulation was stronger than NS. The depression of photosynthetic pigments in both cultivars under AS was consistent with previous report (Jia *et al.*, 2017).

Ions can be used as osmotic adjustment substances, which can reduce cell water potential, improve plant water absorption ability and reduce salt stress damage; however, toxic ions can lead to nutrient deficiency in plants and physiological growth and metabolism are seriously affected (Maathuis and Amtmann, 1999; Farooq *et al*., 2015). In this study, both Na^+ and Cl^- contents increased under NS, but Na^+ content increased and Cl^- decreased under AS. Compared with respective control, the accumulation of Na^+ increased more in cultivated than in wild type in leaves and roots under both salt stresses showed that cultivated soybean was more damaged under salt stress. Under AS, the relative Na^+ content of roots/leaves was greater in wild than cultivated soybean, indicating that wild plants could store Na^+ in roots and so reduce the damage to photosynthetic organs. Roots have a special transport mechanism for the transfer of Na^+ and some plants have salt resistant structures such as salt glands to avoid damage to the photosynthetic apparatus (Xue *et al.*, 2011). We found that wild soybean could accumulate less toxic ions (Na^+ and Cl^- in NS; Na^+ in AS) than cultivated soybean and adjust the distribution in leaves to improve salt tolerance.

In present study, the Zn^{2+} and Mn^{2+} contents were higher in leaves of wild than cultivated soybean under both salt stresses. Zn^{2+} contributes to plant tolerance to environmental stress factors (Cakmak, 2000). Mn^{2+} activates several enzymes of the shikimic acid pathway and subsequent pathways, leading to biosynthesis of aromatic amino acids (*e.g*., tryosine) and various secondary products (*e.g*., lignin and flavonoids) (Burnell, 1988; Hughes and Williams, 1988). K^+ is involved in very important metabolic activities in plants such as protein synthesis and ribosome functions, but Na^+ can inhibit K^+ absorption (Zhu, 2003). Under AS, Na^+ accumulated and K^+ decreased, with greater change in cultivated than wild type, showing that wild soybean could maintain a high K^+/Na^+ to resist salt stress. The stress was greater for AS than NS, indicating that the toxicity of Na^+ and Cl^- was lower than the combination of Na^+ and high pH. Wu *et al.* (2014) reported that wild soybean could reduce Na^+ and Cl^- contents by their faster efflux, lower the K^+ efflux and maintain a high K^+/Na^+ ratio under salt stress (Taffouo *et al.*, 2010). Under AS, Mg^{2+} increased in leaves and the Mg^{2+} content was greater in wild than cultivated soybean, showing that wild could produce more photosynthetic pigments to reduce the damage to photosynthesis (Rao *et al.*, 1987). Fe^{2+} showed a trend of increase, with greater trend for wild than cultivated, because wild soybean could transfer Fe^{2+} through the absorption, accumulation and translocation mechanisms of Fe^{2+} in roots, and protected the photosynthetic apparatus and maintained normal growth (Abdelhamid *et al.*, 2013).

Different metabolisms are closely related to salt tolerance of plants under salt stress, and change was obviously affected by plant photosynthetic function and ion contents (Han *et al.*, 2014). Under NS in leaves, p_N was positively correlated with fatty acid metabolism; under AS, gas exchange and pigment were negatively correlated with TCA cycle, amino acid and organic acid metabolism in leaves of wild soybean. Under NS and AS, in roots, gas exchange and pigment were negatively correlated with antioxidant ROC in wild species. P_N compensation increased under NS and suppression smaller than cultivated soybean under AS in wild soybean revealing that photosynthetic products could provide a more abundant carbon chain for fatty acid metabolism, amino acid metabolism, organic acid metabolism, TCA cycle and increase antioxidant ROC to alleviate salt damage and then resist the salt stress. TCA cycle enhancement could provide plants with more energy to resist salt stress (Hu *et al.*, 2014; Li *et al.*, 2017). ROC could prevent the accumulation of oxygen free radicals and peroxides in plants, eliminate excessive harmful active oxygen, reduce the damage to plants, and enhance the salt tolerance of plants (Phang *et al.*, 2008). Under NS, in roots, p_N was significantly positively correlated with antioxidant ROC in cultivated soybean. Under AS, in leaves, TCA cycle was positively with pigment in cultivated plants. The decrease in photosynthesis leads to inhibition of the TCA cycle, indicating that the plant's energy supply was insufficient, and the synthesis of antioxidant ROC was inhibited, indicating that cultivated soybean was seriously injured under salt stress.

Under NS in wild type, in roots, amino acid, fatty acid, sugar alcohol, organic acid metabolisms and antioxidant ROC were positively correlated with Na^+. Under AS, TCA cycle, amino acid, organic acid metabolisms and antioxidant ROC were positively with Na^+ in wild soybean. It was further shown that under salt stress, the accumulation and

toxic effects of Na^+ stimulated the metabolic reactions of specific substances in wild soybean including enhanced the TCA cycle and antioxidant ROC. These metabolic reactions could significantly increase wild soybeans against salt stress (Li *et al*., 2019). However, cultivated soybeans lack this ability and suffer severe salt damage.

Conclusion

Soybean growth depends on the interaction between adaptive morphological changes occurring at the plant level and metabolic changes occurring at the cellular level (Li *et al.*, 2019). Wild soybean had higher salt tolerance through regulating gas exchange, accumulating lesser toxic ions than cultivated soybean, increased K^+/Na^+ ratio, absorbing beneficial nutrient elements, adjusting ion distributions in different organs. And gas exchange and ion balance adjusted metabolic pathway of metabolites in wild soybean to adapt salt stress. This study will help protect and utilize of wild soybean resources and provide a theoretical basis for the development of salt-tolerant soybean.

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