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Salt stress-induced changes in soil metabolites promote cadmium transport into wheat tissues

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ARTICLE INFO

Article history:
Received 24 March 2022
Revised 6 June 2022
Accepted 6 June 2022
Available online 18 June 2022

Keywords:
Saline stress
Cadmium availability
Wheat growth
Metabolite profile
Rhizosphere

ABSTRACT

Soil salinity is known to improve cadmium (Cd) mobility, especially in arid soils. However, the mechanisms involved in how salt stress-associated metabolic profiles participate in mediating Cd transport in the soil-plant system remain poorly understood. This study was designed to investigate the effects of salinity-induced changes in soil metabolites on Cd bioavailability. Sodium salts in different combinations according to molar ratio ($NaCl:Na_2SO_4$=1:1; $NaCl:Na_2SO_4:NaHCO_3$=1:2:1; $NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$=1:9:9:1; $NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$=1:1:1:1) were applied to the Cd-contaminated soils, which increased soil Cd availability by 22.36% and the Cd content in wheat grains by 36.61%, compared to the control. Salt stress resulted in soil metabolic reprogramming, which might explain the decreased growth of wheat plants and increased Cd transport from the soil into wheat tissues. For example, down-regulation of starch and sucrose metabolism reduced the production of sugars, which adversely affected growth; up-regulation of fatty acid metabolism allowed wheat plants to maintain a normal intracellular environment under saline conditions; up-regulation of the tricarboxylic acid (TCA) cycle was triggered, causing an increase in organic acid synthesis and the accumulation of organic acids, which facilitated the migration of soil Cd into wheat tissues. In summary, salt stress can facilitate Cd transport into wheat tissues by the direct effect of salt-based ions and the combined effect of altered soil physicochemical properties and soil metabolic profiles in Cd-contaminated soils.

Introduction

As the largest wheat producer and consumer in the world, China produced 131.68 million tons of wheat in 2020. In the North China Plain (NCP), which is the main wheat producing region in China, sewage water is often used to irrigate farmland due to the scarcity of water resources in recent years. Wastewater irrigation can solve the problem of water scarcity to some extent, and at the same time transfer nutrient elements such as nitrogen, phosphorus, potassium, low molecular organic compounds, and other medium/micro-nutrients to the agricultural soil (Pedrero et al., 2010; Gatta et al., 2015). However, sewage water often contains harmful elements such as heavy metals, and soil heavy metal contamination due to the long-term use of sewage irrigation has become a prominent issue in this area.

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https://doi.org/10.1016/j.jes.2022.06.017
1001-0742/

Among the heavy metal contaminants, cadmium (Cd) is one of the most toxic and mobile contaminants in surface soils that results from by wastewater irrigation, and Cd is present in soil solutions mainly as Cd^{2+} and Cd chelates (Lux et al., 2011). Previous studies have shown that Cd toxicity alters plant growth, reduces photosynthesis and mineral nutrition, and can even lead to plant death (Qi et al., 2020). Due to its high mobility in the environment, Cd is easily transferred through plants into the food chain. Cd can accumulate in the human body, leading to serious medical conditions such as renal tubular dysfunction, osteoporosis, and cancer (Akesson et al., 2014; Sui et al., 2018). In addition, wastewater also contains a large number of soluble salts. Long-term wastewater irrigation may lead to increased salinity of shallow groundwater and the build-up of salts on the soil surface through evaporation, resulting in soil salinization. Soil salt stress has been shown to reduce plant growth, biomass, and mineral nutrient uptake (Nawaz et al., 2015). For example, soil salinity can lead to nutrient disorders in wheat by limiting water uptake by the roots, resulting in reduced growth due to ionic toxicity (Moez et al., 2016). It has also been found that high salt levels also inhibit photosynthetic pigment production (Pan et al., 2020). Soil salinity can have an important effect on Cd bioavailability, because salt-based ions can form complexes with Cd and increase Cd mobility (Zhang et al., 2016; Abbas et al., 2018). Moreover, salt-based cations can compete with Cd for soil sorption sites, allowing Cd desorption from soil particles, which also increases Cd mobility. In addition, salinity affects the ionic strength and pH of the soil solution to alter Cd bioavailability (Raiesi et al., 2018).

Soil metabolomes determined by analysis of soil metabolites can be highly informative of the actual soil biological or environmental changes that occur during stimulation or perturbation (Obata and Fernie, 2012; Van Dam and Bouwmeester, 2016). In recent years, metabolomics analysis has developed into a powerful tool for studying soil metabolite profiles including plant root secretions and soil microbial metabolic activities (Li et al., 2019; Miura et al., 2020). Soil metabolites include exudates released by plants and microorganisms, which can further alter Cd mobility (Li et al., 2013) and change the soil redox potential (Shi et al., 2011) and metal complexation (Xu et al., 2017), thus affecting the rate of Cd transfer to plants. Under salt stress conditions, the soil microbial community structure might change, inducing changes in their metabolites; plants growing on these soils need to adapt to saline conditions by adjusting the levels of osmoregulatory substances in their tissues and changing the metabolic profiles of their root secretions (Wang et al., 2011). Analysis of soil metabolites to characterize the soil metabolome can provide insights into the response of microorganisms and plant roots to salt stress. However, current research into the effect of soil saline stress on Cd mobility has generally excluded analysis of soil metabolic profiles.

We thus hypothesized that addition of sodium salts alters soil metabolites that participate in mediating Cd transport into wheat tissues in arid, Cd-contaminated soils. The main objective of this study was to investigate the effects of salinity-induced changes in soil metabolites on Cd bioavailability. The experimental design involved adding several different types of sodium salts to the soils. After planting the wheat, changes in Cd availability and soil metabolite profiles were analyzed. The results of our study will contribute to the understanding of the mechanisms involved in the changes in Cd availability by soil metabolites in response to salinity stress.

1. Materials and methods

1.1. *Preparation of pot soil*

The soils used in the experiments were collected from two different locations in the North China Plain (NCP), the main wheat producing area in China, and included the upper 20 cm of agricultural soils in Baoding city (38°76′N, 115°47′E) and Xinxiang city (35°18′N, 113°54′E). The soils from both of these sites are contaminated with Cd due to long-term wastewater irrigation, as sewage effluents originating from the industrial wastewater and domestic sewage have been used for irrigation purposes, long-term fertilization and pesticide applications also contribute to the intensification of soil Cd pollution. The basic physical and chemical properties of the soils are shown in Table 1. Soil samples were randomly collected from five points in as "S" pattern and mixed. The collected soil samples were air dried at room temperature, plant residues, stones, and other debris were removed, and the soils were then ground to pass through a 2 mm sieve. Six kilograms of air-dried soil was mixed thoroughly with nitrogen, phosphorus, and potassium fertilizers (2.6 g urea, 1.6 g KH_2PO_4, and 2.5 g KNO_3) and placed in plastic pots (27 cm in diameter × 35 cm in height). Seeds of the wheat cultivar 'Liaochun 18' were surface sterilized in hydrogen peroxide for 20 min, rinsed five times with deionized water, and allowed to germinate in Petri dishes containing deionized water.

1.2. *Pot experimental design and sampling*

According to the characteristics of the main salt components in the saline fields of the NCP, the sodium salts were configured according to different molar ratios in order to simulate the actual saline environment: (1) wheat planting with no salt added as the control (CK) treatment, and wheat planting with sodium salts added to the soil in the following molar ratios, (2) NaCl:Na_2SO_4=1:1 (T1), (3) NaCl:Na_2SO_4:$NaHCO_3$=1:2:1 (T2), (4) NaCl:Na_2SO_4:$NaHCO_3$:Na_2CO_3=1:9:9:1 (T3), and (5) NaCl:Na_2SO_4:$NaHCO_3$:Na_2CO_3=1:1:1:1 (T4). In this study, different sodium salts were applied, because the salt-based cations contained in the wastewater used for wastewater irrigation are mainly Na^+. Characteristics of sewage wastewater historically used for irrigation in this study were shown in Wang et al. (2019). It should be noted that in this study, the treatment without wheat was not set up, further more investigation about the comparison of soil metabolomic profiles and physicochemical properties with and without planting is suggested. The concentration of sodium salts added in the different treatments in both soils was 9 g/kg. In this study, a completely randomized zonal design was performed for all pots, with each treatment repeated three times. For the above treatments, the different sodium salts were first mixed in a fixed ratio and dissolved in an appropriate amount of

Table 1 – Chemical and physical properties of the test soils.

Soil code	Location	Soil type	pH	Na (g/kg)	EC (mS/cm)	CEC ($cmol^+/kg$)	SOC (%)	Cd concentration (mg/kg)	Cd concentration after planting (mg/kg)
S1	Baoding	Cinnamon soil	7.15±0.42	6.68±0.31	0.18±0.01	14.65±0.62	0.91±0.06	1.32±0.11	1.31±0.13
S2	Xinxiang	Cinnamon soil	7.30±0.15	8.31±0.05	0.16±0.01	16.65±1.29	2.05±0.16	1.84±0.16	1.83±0.25

S1: Baoding, S2: Xinxiang, EC: electrical conductivity, CEC: cation exchange capacity, SOC: soil organic carbon.

deionized water, and then mixed evenly with the soil. Irrigation was applied to the soil pots to maintain 70% of the maximum field water holding capacity (MWHC) for 30 days to reach equilibrium. The germinated seeds were sown in the soil of each pot, after which the soil was maintained at 70% MWHC to sustain normal wheat growth. The pots containing the wheat seedlings were placed in a greenhouse at the Chinese Academy of Agricultural Sciences in Beijing, China, from April 3 to August 1, 2021, for the entire growth period of approximately 120 days (from seedling to maturity) at a temperature of 25°C/20°C (day/night) under the natural photoperiod.

Metabolomic response to plant stress can be evaluated in vegetative or reproductive stages, but soil metabolites, soil physicochemical properties, and Cd uptake and transport by wheat should correspond to each other, so soil samples were taken after 120 days (maturity stage). Direct detection of root exudates is indeed more responsive for plant response to stresses, but considering that soil microorganisms also play an important role in plant stress resistance. In this study, rhizosphere soil metabolites including secretions from root tissues and metabolites produced by microbial activity were analyzed. We first collected the rhizosphere soil using the following method; wheat roots were shaken to remove loose soil leaving a layer of soil approximately 1 mm thick on the root surface, after which the roots were immersed in sterile deionized water and shaken with sterile forceps to release the remaining soil (Edwards et al., 2015). The rhizosphere soil solution was then centrifuged at 10,000 × *g* for 1 min at 4°C, the supernatant was removed, and the residue was collected. Approximately 1 g of rhizosphere soil was stored at -80°C for DNA extraction and microbial analysis. The wheat roots, stems, and grains were then harvested separately. The plant part samples were washed with tap water, thoroughly rinsed with distilled water, and then placed in an oven at 70°C and dried to a constant weight. The dried wheat samples were threshed manually and the dry biomass of each part, the roots, stems, leaves, and seeds were measured separately, ground, and stored for further analysis. Finally, soil samples were collected in the pots and dried naturally at room temperature for subsequent determination of soil physicochemical properties and Cd content.

1.3. *Chemical analyses*

Soil pH and electrical conductivity (EC) were determined in a soil water suspension (1:2.5 soil/water ratio, *m*/*V*) with a pH meter (PHS-3C, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China) and a digital conductivity meter (DDS-11A, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China), respectively. The soil cation exchange capacity (CEC) was analyzed using the ammonium acetate exchange method. The soil organic carbon (SOC) content was analyzed by potassium dichromate oxidation colorimetry. The soil Cd concentration was determined using an atomic absorption spectrometer (AAS, Perkin-Elmer, AAnalyst 300, USA). Concentrations of Ca^{2+} and Mg^{2+} were measured by AAS, and Na^+ and K^+ concentrations were determined by flame photometry (M410, Sherwood, UK).

In this study, the available Cd in the soil was determined using the diethylenetriaminepentaacetic acid (DTPA) extraction method. Briefly, a fresh soil sample (10.00 g) was mixed with 25 mL of 0.05 mol/L DTPA extraction solution at pH 7.3, shaken at 25°C and 180 r/min for 2 hr, and then filtered. The Cd concentration in the filtrate was determined by AAS. The oven-dried roots, shoots, and grain samples were ground to a fine power and then digested with 8mL HNO_3 and 3 mL H_2O_2 at 180°C for 50 min using a microwave digestion apparatus (Milestone MLS 1200 Mega, Italy). Quality assurance and control of the analyses were ensured by using sample replicates, reference material (National Research Center for Certified Reference Materials, China) and blanks. The Cd concentrations in the digestion solutions were then determined using AAS, the detection limit of AAS for Cd was 0.1 mg/L.

1.4. *Metabonomic analysis*

For metabolite extraction, soil samples (200 mg) were weighed and transferred to 2 mL Eppendorf tubes. Each sample was then mixed with 0.6 mL of 2-chloro-l-phenylalanine (4 mg/L) dissolved in methanol (-20°C) as an internal standard along with 100 mg of glass beads. The tubes were kept at -20°C for 2 min, ground with a high-throughput tissue grinder for 90 sec at 60 Hz and ultrasonicated for 30 min at room temperature, after which they were placed on ice for 30 min. Samples were then centrifuged at 4°C for 10 min at 12,000 × *g*, and the supernatants were filtered through a 0.22 μm membrane prior to liquid chromatography-mass spectrometry (Thermo Fisher Scientific Inc., USA) (LC-MS) analysis.

Chromatographic separation was accomplished using a Thermo Ultimate 3000 system (UltiMate3000, Thermo Fisher Scientific Inc., USA) equipped with an ACQUITY UPLC® HSS T3 (150 × 2.1 mm, 1.8 μm, Waters) column at 40°C. The temperature of the autosampler was 8°C. Gradient elution of analytes was carried out with 0.1% formic acid in water (C) and

0.1% formic acid in acetonitrile (D) or 5 mmol/L ammonium formate in water (A) and acetonitrile (B) at a flow rate of 0.25 mL/min. Aliquots of each sample (2 μL) were injected into the column after equilibration. An increasing linear gradient of solvent B (*V/V*) was used as follows: 0-1 min, 2% B/D; 1-9 min, 2%-50% B/D; 9-12 min, 50%-98% B/D; 12-13.5 min, 98% B/D; 13.5-14 min, 98%-2% B/D; 1420 min, 2% D-positive model (14-17 min, 2% B-negative model).

The ESI-MSn experiments were performed using a Thermo Q Exactive Focus mass (Q Exactive Focus, Thermo Fisher Scientific Inc., USA) spectrometer with spray voltages of 3.8 and -2.5 kV in positive and negative modes, respectively. Sheath gas and auxiliary gas were set at 30 and 10 arbitrary units, respectively. The capillary temperature was 325°C. The analyzer scanned over a mass range of *m/z* 81-1000 for full scan at a mass resolution of 70,000. Data dependent acquisition (DDA) MS/MS experiments were performed with HCD scan. The normalized collision energy was 30 eV. Dynamic exclusion was implemented to remove some unnecessary information in the MS/MS spectra.

1.5. *Statistical analysis*

The sodium adsorption ratio (SAR) and exchangeable sodium percentage (ESP) were calculated using the following Eqs. (1) and (2):

$$\mathrm{SAR} = \frac{[\mathrm{Na^+}]}{\sqrt{\frac{[\mathrm{Ca^{2+}}]+[\mathrm{Mg^{2+}}]}{2}}} \quad (1)$$

$$\mathrm{ESP} = \frac{[\mathrm{Na^+}]}{[\mathrm{K^+}]+[\mathrm{Na^+}]+[\mathrm{Ca^{2+}}]+[\mathrm{Mg^{2+}}]} \times 100\% \quad (2)$$

The metabolites detected in this study were annotated into the Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp/) database to reveal the potential functions and interactions of the metabolites. The KEGG IDs of the differentially-expressed metabolites (DEMs) was used for enrichment pathway analysis to reveal the response mechanisms of metabolic pathways between the different treatment groups. The correlations between the soil metabolites and soil solution properties were determined by the Mantel test, and redundancy analysis (RDA) was performed using the R vegan package.

All experimental data were analyzed using SPSS 16.0 for Windows (IBM, USA). Significant differences between the various treatments were determined by one-way analysis of variance (ANOVA) at $p<0.05$. The means and standard deviations were calculated using the data from three replicates for each treatment.

2. Results

2.1. *Soil properties and soil-available Cd*

Table 2 shows the changes in soil properties for the four salinity treatments. In both soils, the pH increased significantly, compared to the control, in all treatments except for T1. The largest increases were in the T4 treatments, which increased by 1.02 and 1.17 units in Baoding (S1) and Xinxiang (S2), respectively. Electrical conductivity (EC) is an important chemical indicator of soil salinity, and the addition of sodium salts significantly increased the soil EC values, with the largest increases in the T1 treatments of 2.50- (S1) and 2.21-fold (S2) compared to the control. In both soils, the cation exchange capacity (CEC) was significantly decreased in the with sodium salt treatments compared to the control, with the lowest CEC values in the T4 treatments in both cases, with decreases of 8.07% (S1) and 7.42% (S2) (Table 2). The soil organic carbon (SOC) content decreased in all treatments compared to the control, with maximum decreases of 6.67% (S1) and 5.74% (S2) in the T4 treatments. The addition of sodium salts increased the sodium adsorption ratio (SAR) by 38.95%-64.55% (S1) and 14.81%-52.32% (S2), respectively, compared to the control. Similarly, the exchangeable sodium percentage (ESP) values for the treatments after adding the sodium salts increased by 25.14%-40.81% (S1) and 17.90%-33.51% (S2), respectively (Table 2). As SAR and ESP are mainly influenced by Na^+ concentration in soil. When the concentration of sodium salts in both T3 and T4 were 9 g/kg with different molar ratios, comparatively, the T4 treatment contained more moles of Na^+. Therefore, SAR and ESP were highest under T4 treatment.

In this study, we found that the treatments in which sodium salts were added to the soil changed the DTPA-extractable Cd content in the soil compared to the control, and this change was similar in the two soils. For example, the DTPA-extractable Cd content was significantly increased by 11.73%-18.19% (S1) and 16.68%-22.36% (S2) in the T1 and T2 treatments compared to the CK (Fig. 1). However, the DTPA-Cd content decreased by 11.89% (S1) and 12.13% (S2) in the T4 treatments (Fig. 1), while the difference between the T3 treatment and the CK was not significant.

2.2. *Cd transfer and accumulation across wheat tissues*

2.2.1. *The effects of the different treatments on wheat physiological*

Table 3 shows that salinity stress significantly reduced the plant height of wheat. The reduction of plant height ranged from 6.72% to 13.31% (S1), 6.31% to 13.51% (S2). The fresh weight of each tissue (seed, stem, and root) of wheat was also significantly reduced by 17.56%-20.83%, 8.02%-14.28%, and 16.38%-28.66% (S1); 24.95%-30.78%, 6.12%-14.47%, and 10.60%-26.26% (S2), respectively. The dry biomass of all wheat tissues decreased significantly in the treatments with added sodium salts (Table 3). In S1, the biomass of grain, shoots and root was reduced by 27.13%-33.55%, 21.64%-33.95%, and 19.22%-29.09%, respectively, in the salt treatments compared to the control. Similarly, in S2, grain, shoot and root biomass was reduced by 27.82%-31.84%, 17.61%-32.74%, and 13.86%-27.16%, respectively, in the salt treatments compared to the control. In addition, the dry mass of wheat tissues in the T4 treatment was the lowest in both soils, indicating that this treatment had the strongest growth inhibitory effect on wheat growth. Thus, wheat growth was negatively affected by the addition of different sodium salts. The measurement of Fv/Fm, panicle number and other physiological indicators can reflect the response of

Table 2 – Changes in the chemical and physical properties of the two soils for the different salt treatments.

Soil code	Treatment	pH	EC (mS/cm)	CEC (cmol$^+$/kg)	SOC (%)	SAR (mmol/L)	ESP (%)
S1	CK	7.62±0.07a	0.78±0.03a	13.13±0.45b	1.80±0.02d	4.57±0.36a	12.13±0.77a
	T1	7.71±0.05a	2.73± 0.60c	12.67±0.42ab	1.76±0.01c	6.35±0.04b	15.18±0.04b
	T2	7.87±0.01b	2.27±0.16bc	12.63±0.55ab	1.72±0.01b	6.63±0.16b	16.04±0.19c
	T3	8.26±0.12c	1.71±0.19b	12.63±0.50ab	1.70±0.06ab	6.81±0.02b	16.37±0.18c
	T4	8.64±0.12d	1.61±0.19b	12.07±0.06a	1.68±0.02a	7.52±0.57c	17.08±0.22d
S2	CK	7.73±0.09a	0.69±0.03a	14.83±0.45b	2.96±0.06c	6.55±0.65a	16.65±0.39a
	T1	7.78±0.06a	2.22±0.17d	14.13±0.42a	2.89±0.01b	7.52±0.15b	19.63±0.30b
	T2	7.99±0.10b	1.83±0.08c	14.07±0.55a	2.87±0.01b	7.76±0.06bc	19.99±0.05bc
	T3	8.48±0.03c	1.65±0.09b	13.77±0.50a	2.84±0.01ab	8.22±0.24c	20.62±0.30c
	T4	8.90±0.06d	1.56±0.20b	13.73±0.06a	2.79±0.03a	8.94±0.29d	22.23±0.93d

Values are means ± SD (n=3). Different lowercase letters indicate a significant difference between salt treatments at $p<0.05$. These comparisons were performed separately for the parameters in each column. SAR: The sodium adsorption ratio, ESP: exchangeable sodium percentage.

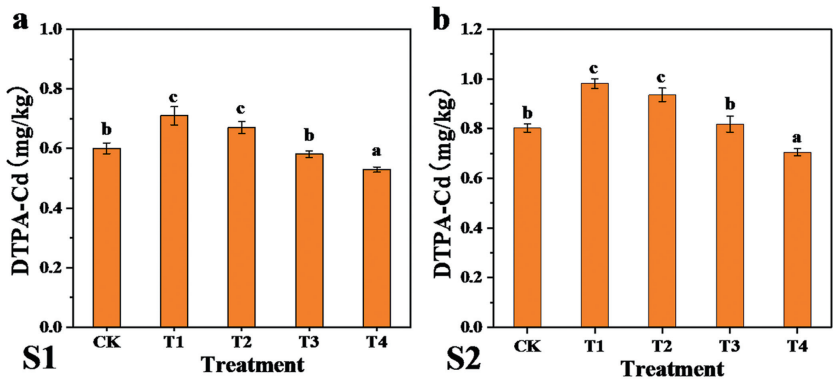

Fig. 1 – Changes in diethylenetriaminepentaacetic acid (DTPA) extractable Cd contents for Baoding (a) and Xinxiang (b) soils in the different salt treatments. Data is the average of three replicates. Error bars show the standard deviations. Lower-case letters indicate significant differences between the treatments at $p<0.05$. Significant differences between the various treatments were determined by one-way ANOVA at $p<0.05$. The means and standard deviations were calculated using the data from three replicates for each treatment. S1: Baoding, S2: Xinxiang.

wheat growth to salt stress more clearly, so we should focus on the measurement of these indicators in future studies.

2.2.2. *Cd accumulation in wheat tissues*

In this study, the Cd content of wheat roots increased significantly in each salt treatment compared to the control in both soils (S1 and S2) (Fig. 2). The Cd content in wheat roots in the control was 2.067 mg/kg (S1) and 2.484 mg/kg (S2), while the added salt treatment increased the root Cd contents to 2.401-2.615 mg/kg (S1) and 3.013-3.394 mg/kg (S2). The Cd content in wheat roots increased most significantly in the T1 treatment, with relative increases of 31.30% (S1) and 36.61% (S2). At the same time, the addition of salt to the soil also increased the Cd contents of the wheat shoots and grains. Compared to the control, the Cd contents in wheat stems and leaves increased by 12.09%-27.77% (S1) and 12.06%-28.15% (S2). The Cd contents in the wheat grains increased by 11.17%-26.15% (S1) and 11.57%-20.82% (S2), although the grain Cd contents in the T4 treatment were not significantly different compared to the control. These results indicate that soil salinization (increased soluble salts concentration) affects not only wheat growth and productivity but also wheat quality (Cd contamination).

2.3. *Rhizosphere metabolite profiles*

In order to compare the effects of salt stress on soil rhizosphere metabolites, multivariate data analysis using partial least squares discriminant analysis (PLS-DA) was used to discriminate metabolite changes in response to salt stress in the different treatments. The scoring plot (Fig. 3a) showed that all groups with added sodium salts were significantly different from the control group, and that the largest separation was between the T1 treatment and the CK, indicating that the T1 treatment had the greatest effect on soil metabolites. These results suggest that salt stress changes the metabolite profiles in wheat rhizosphere soils. Moreover, in KEGG pathway enrichment analysis, metabolic pathways with significant associations among treatments were identified at $p<0.05$ with impact values >0.1. In this study, six major metabolic pathways were significantly enriched, and these were closely associated with specific, differentially abundant metabolites among the treatments (Fig. 3b). The significant metabolic pathways identified included the tricarboxylic acid (TCA) cycle, fatty acid biosynthesis, glycine, serine and threonine metabolism, propanoate metabolism, starch and sucrose metabolism, and glyoxylate and dicarboxylate metabolism.

Table 3 – Fresh and dry weights of each tissue (grains, shoots, and roots) and plant height of wheat in the different sodium salt treatments.

Soil code	Treatment	Plant height (cm)	Fresh weight (g/pot)			Dry weight (g/pot)		
			Grain	Shoot	Root	Grain	Shoot	Root
S1	CK	77.4±2.4c	17.14±0.70b	83.55±1.55c	16.54±0.66c	10.91±0.51c	35.82±0.69c	9.73±0.43c
	T1	72.2±1.8b	14.13±0.92a	76.85±1.92b	13.83±0.76b	7.95±0.32b	28.07±0.87b	7.86±0.13b
	T2	70.5±2.2ab	14.09±0.61a	75.36±1.89b	13.79±0.43b	7.93±0.18b	27.77±0.91b	7.79±0.19b
	T3	68.4±1.2a	13.79±0.45a	73.26±2.18ab	11.82±0.72a	7.65±0.23b	26.53±1.07b	7.12±0.33a
	T4	67.1±3.1a	13.57±0.17a	71.62±1.32a	11.80±1.28a	7.25±0.08a	23.66±0.43a	6.90±0.48a
S2	CK	79.2±2.7c	16.83±0.36b	82.40±2.01c	14.43±0.44c	10.46±0.36c	32.25±0.72d	9.02±0.25c
	T1	74.2±2.0ab	12.63±0.53a	77.36±1.22b	12.90±0.62b	7.55±0.26b	26.57±0.27c	7.77±0.43b
	T2	73.5±2.6ab	12.45±0.58a	75.71±2.26b	12.30±0.72b	7.40±0.34b	24.58±1.24b	7.36±0.35b
	T3	69.4±2.2a	11.71±0.16a	74.12±1.12b	10.88±0.28a	7.26±0.06a	23.80±0.41b	6.80±0.12a
	T4	68.5±3.1a	11.65±0.60a	70.48±0.98a	10.64±0.84a	7.13±0.11a	21.69±0.77a	6.57±0.32a

Values are the means ± SD (n=3). Different lowercase letters indicate significant differences between salt treatments at $p<0.05$. The comparisons were performed separately for the parameters in each column.

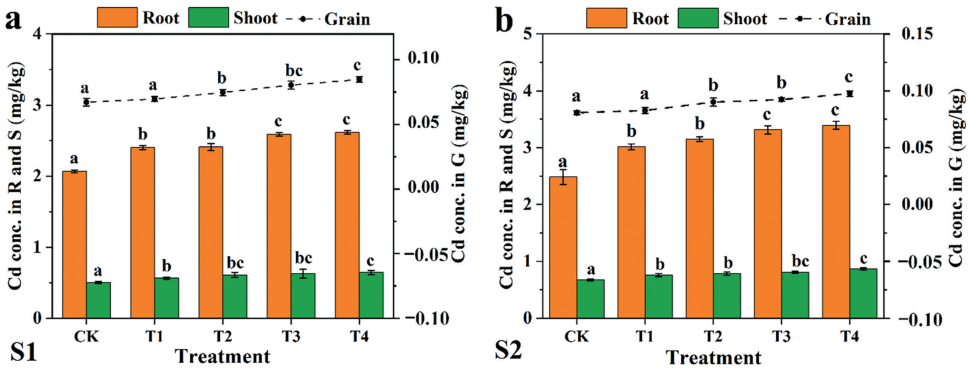

Fig. 2 – Effects of the different sodium salt treatments in Baoding (a) and Xinxiang (b) soils on the accumulation of Cd in the roots, shoots, and grains of wheat. Data are the average of three replicates. Error bars show the standard deviations. Significant differences between the various treatments were determined by one-way ANOVA at $p<0.05$. The means and standard deviations were calculated using the data from three replicates for each treatment. R: root; S: shoot; G: grain.

Random forest analysis showed the most significant soil metabolites in the rhizosphere soil in the different treatments (Fig. 3c), and it seemed that metabolites such as D-ribose, starch, and glucose decreased significantly with increasing extent of soil salinity-alkalinity. In contrast, metabolites such as homoserine, oxalic acid and glycine increased. Notably, D-ribose, starch had the largest average decrease in accuracy (Fig. 3c), suggesting that these metabolites contributed significantly to the separation between control and saline treatments.

3. Discussion

3.1. *Influence of salt stress on soil Cd availability and phytotoxicity*

Changes in the soil physicochemical properties were assessed in the four different salt treatments at the end of the wheat reproductive period (Table 1). The results showed that exposure to sodium salts increased soil pH, EC, SAR, and ESP values to varying degrees, which were mainly influenced by the salt-based ions added to the soils. In contrast, the CEC and SOC values were somewhat decreased by the addition of salts. This is because the increased salt concentration causes Na^+ to occupy ion exchange sites in the soil (Ramakrishna and Viraraghavan, 2005), and Na^+ also stimulates the mineralization of organic matter (Mavi and Marschner, 2017). The addition of sodium salts to both soils resulted in changes in the DTPA-extractable Cd content. In general, the DTPA-extractable Cd characterizes the biological effectiveness of Cd and represents the active state of Cd. In the present study, the effective soil Cd content in the T1 and T2 treatments increased significantly. This is because Cl^- and SO_4^{2-} form uncharged or negatively charged complexes with Cd (e.g., $CdCl_2^0$, $CdCl^+$, and $CdSO_4^0$), thus weakening the electrostatic attraction to Cd and increasing its freedom. At the same time, the added Na^+ competes with the Cd adsorption sites, thus promoting the desorption of Cd from the soil colloid (Wang et al., 2013). In contrast, the available Cd content decreased significantly in the T4 treatment. This is related to the CO_3^{2-} and HCO_3^- that was added to the soil; carbonate ions can increase soil pH and increase the negative charge on the soil surface, thus enhancing the adsorption of Cd^{2+} (Malandrino et al., 2011). In addition, the

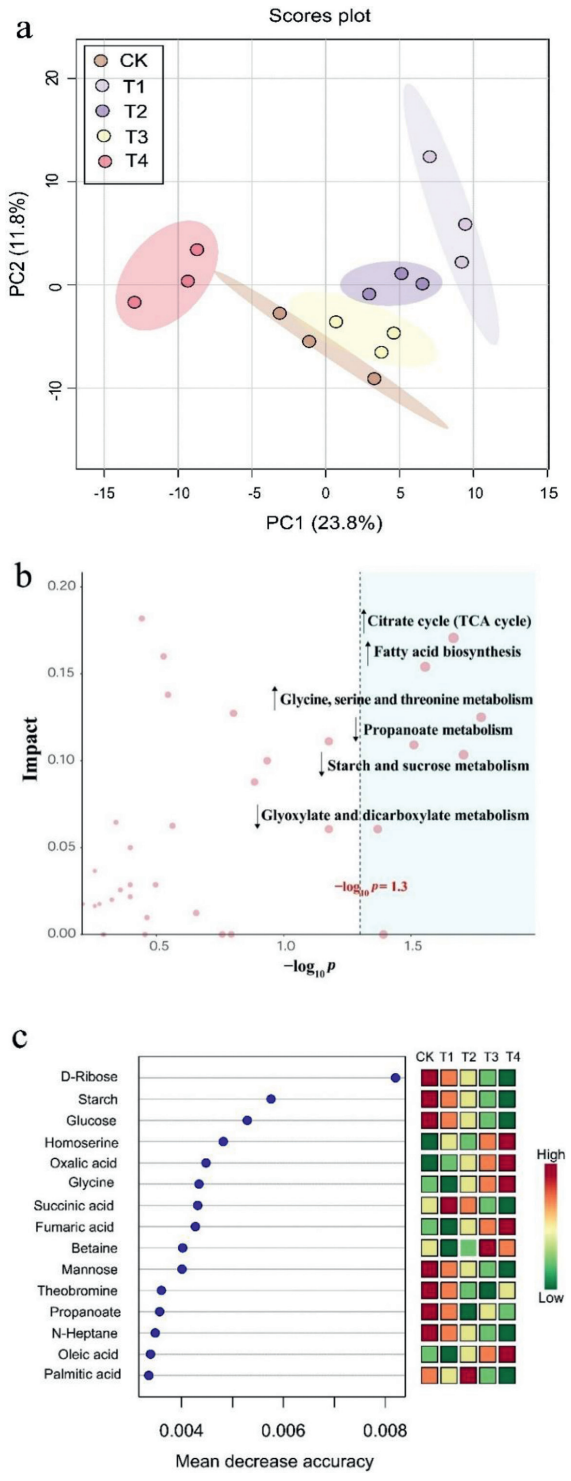

Fig. 3 – A partial least-squares discriminant analysis (PLS-DA) score plot (a), an enrichment over-representation analysis showing remarkable differences (indicated by circle size) in metabolic pathways (upregulation and downregulation are indicated by arrows) (b) and random forest analysis (permutation-based mean decrease accuracy) showing the most highly significant soil metabolites (c) that were enriched in the different treatments. Data are expressed as the mean values from soils S1 and S2. TCA: tricarboxylic acid.

hydrolysis of Cd^{2+} under alkaline conditions produces $CdOH^+$, which is more readily adsorbed by the soil colloid compared with Cd^{2+} (Hale et al., 2012). Finally, CO_3^{2-} and HCO_3^- have a precipitating effect on Cd, thus reducing the soil available Cd content (Mugwar and Harbottle, 2016).

Both salt stress and Cd contamination affect the growth and development of wheat plants. The experimental results showed that salt stress significantly reduced the biomass of roots, stems, and grains in wheat (Table 3). The biomass of all wheat parts tended to decrease with increasing salinity, and all reached the minimum biomass in the T4 treatment. The decline in wheat biomass resulting from Cd and salt stress may be due to severe structural disorders in the different plant parts, such as damage to the nucleus, chloroplasts, and mitochondria (Abbasi et al., 2015). In addition, Na^+ and Cl^- in the soil exert osmotic stress on wheat roots, triggering physiological water deficit and impeding nutrient uptake by the roots, resulting in a decrease in wheat biomass (Ismail et al., 2014). At the same time, high levels of CO_3^{2-} and HCO_3^- in the soil cause an increase in soil pH, which severely affects wheat growth and disrupts the ionic balance (Guo et al., 2017). Alkaline salts such as Na_2CO_3 and $NaHCO_3$ have been shown to cause more severe damage to plants than do neutral salts (e.g., NaCl and Na_2SO_4) (Javid et al., 2012).

Salt stress increased the Cd levels in different parts of the wheat plants (Fig. 2a and b), possibly due to salinity-induced osmoregulation, which triggers biogeochemical processes of Cd mobilization in the rhizosphere soil and Cd uptake and translocation in plants (Xu et al., 2017). A similar study found that increased salinity enhanced Cd uptake by soybean (Ashrafi et al., 2014). Furthermore, Cd concentrations in the roots were higher than in other plant parts (Fig. 2), which may be one of the protective mechanisms in plants (Rizwan et al., 2018), and it was found that intracellular chelators can sequester Cd in vesicles after chelation, preventing Cd^{2+} from being transported laterally by the coplasmic pathway, reducing the amount of Cd^{2+} reaching the xylem and phloem, and thus weakening Cd transport to the aboveground parts (Seregin and Kozhevnikova, 2008). At the same time, Cd toxicity is one of the main environmental conditions that leads to reduced photosynthesis and transpiration in plants, which in turn affects Cd transport. Similarly, a study by Abbas et al. (2018) found that salt stress increased Cd uptake by wheat and enhanced its accumulation in the grains. It has been suggested that the salt-induced increase in Cd accumulation may be due to the accumulation of Cl in wheat tissues, which may mobilize Cd in the shoots and increase its transfer to the grains (Ozkutlu et al., 2007).

3.2. *Metabolite profiles of wheat rhizosphere soil under salt stress*

In addition to salt stress, the changes in the concentration and composition of plant root secretions also alters Cd bioavailability. Rhizosphere soil metabolites include secretions from root tissues and metabolites produced by microbial activity, which have multiple functions to influence abiotic and biotic processes in the soil (Rodriguez et al., 2019). In plant rhizosphere, compounds released by the root system can influence the structure of the rhizosphere microbial community, and

thus soil microbial metabolism. In turn, root exudation is influenced by biotic and abiotic factors, including environmental factors and soil microbial activity (Zhalnina et al., 2018). Notably, plant and microbial metabolites are associated with the stability of heavy metals in soils. In the present study, we found that the treatments with added salts were significantly separated from the control, so we can conclude that wheat rhizosphere metabolism is strongly affected by salinity. Interestingly, the T1 treatment was the most distant from the CK compared with the other three treatments, suggesting that strong salinity is one of the main factors that affects wheat rhizosphere metabolism. The KEGG pathway analysis showed that six major metabolic pathways were significantly enriched, closely related to varying levels of specific metabolites in the samples (Fig. 3b), and the major metabolic pathways were specifically linked (Fig. 4). We then found that metabolites such as D-ribose, starch, and glucose contributed significantly to the separation between the CK and the treatments using random forest analysis (Fig. 3c).

When plants are subjected to osmotic stress and ionotoxicity under salt stress conditions, a range of side effects occur, such as nutrient deficiencies and oxidative stress (Zhu, 2003), which severely inhibit plant growth. While there are important response mechanisms such as detoxification, resistance could defend the plant from the effects of salt stress. For example, amino acids play a very important role in osmoregulation and maintenance of cell membrane stability as precursors for protein and secondary metabolite synthesis (Widodo et al., 2009). Specifically, in this study, homoserine and glycine accumulated in the wheat rhizosphere soil under saline conditions, and their levels increased with increasing salinity, suggesting that homoserine and glycine play a role in the response of wheat to salt stress. Homoserine is an intermediate in the biosynthesis of threonine, isoleucine, and methionine, and the accumulation of homoserine may lead to elevated levels of these amino acids (Ta et al., 1984). Threonine and glycine are important in the synthesis of the broad defense molecule glutathione, which is often secreted during stress conditions (Zhang et al., 2018). The increased production of serine as well as glycine in this study (Fig. 3c) suggests the initiation of antioxidant defense pathways in specific microbial communities (Zhang et al., 2020). Notably, glycine can mediate the adsorption of heavy metals to soil components and the formation of glycine-Cd complexes, which increases the availability of Cd adsorption sites (Zhang et al., 2019). In addition, in response to saline conditions, plants synthesize and accumulate compatible solutes in the cytoplasm that can help them to maintain normal physiological and biochemical processes. Similarly, glycine and betaine can scavenge reactive oxygen species (ROS) and inhibit lipid peroxidation, thereby preventing damage at the cellular level (Taji et al., 2002; Liang et al., 2018).

Sugar metabolism is a key factor in the plant response to salt stress. Many sugars, including monosaccharides, disaccharides, and polysaccharides, are metabolic substrates, and these provide energy for plant growth and development (Keunen et al., 2013). In addition, they play an important role in removing excess reactive oxygen species to maintain a normal cellular redox state (Krasensky and Jonak, 2012). Sugar accumulation and metabolic reprograming in response to salt stress is one of the most common responses by which plants adapt to salt-induced dehydration (Kumari et al., 2018). In the present study, the down-regulation of starch and sucrose metabolism, fructose and mannose metabolism, and caffeine metabolism due to salt stress reduced the soil content of certain sugars (Figs. 3c and 4), and affected photosynthesis in wheat, leading to reduced glycolysis and sugar production. Notably, proteins and sugars are usually the main components of microbial biofilms, which play an important role in the persistence and colonization of microorganisms under stressful environments (Booth et al., 2011). The down-regulation of these metabolites further suggests that the normal physiological activities of plant tissues are negatively affected by salt stress.

Fatty acid metabolism is involved in all aspects of plant physiology, including growth and development and interactions with the environment. Fatty acids usually play beneficial roles in the repair of damaged membranes and in maintaining an intracellular environment conducive to the function of key proteins during stress (Zhang et al., 2018; Zhao et al., 2018). Our study shows that the level of oleic acid was down-regulated in response to salt stress. However, the up-regulated TCA cycle directly increased the release of fumaric acid from the roots (Fig. 3c), while up-regulation of the TCA cycle may be induced by increases in the levels of the precursors of oxalic acid and oleic acid (Figs. 3c and 4). These organic acids can regulate the osmotic balance between the cytoplasm and the surrounding environment and alleviate salt stress by neutralizing excess cations such as Na^+ and Ca^{2+} (Yang et al., 2007; Widodo et al., 2009). Also, in a study in barley, Shen et al. (2016) found that the TCA cycle is up-regulated by salt stress. This phenomenon is attributed to the adaptation of plants to salt stress, and the TCA cycle is the main energy metabolism pathway, producing large amounts of energy compounds (ATP and NADH) as well as precursors for amino acid synthesis. Plants obtain an optimal energy supply for growth through increased TCA cycle activity. More importantly, organic acid accumulation caused by salt stress leads to rhizosphere soil acidification that then promotes soil Cd transport, which ultimately promotes Cd uptake into wheat tissues. In addition, low molecular weight organic acids secreted by the root system can chelate Cd in the soil to form a "Cd-low molecular weight organic acid" complex, which facilitates the uptake of Cd by the plant (Aravind and Prasad, 2005; Quartacci et al., 2005). Therefore, the changes in the amounts and composition of plant root secretions also promotes the uptake of Cd by the plant under salt stress conditions.

3.3. *The relationship between metabolite profiles in wheat rhizosphere soil and Cd bioavailability*

In order to determine whether the changes in wheat rhizosphere soil metabolites in response to salt stress could facilitate the migration of Cd into wheat tissues, we analyzed the correlation between environmental factors and soil metabolites (Fig. 5). The results showed that soil DTPA-extractable Cd and pH were the most significant environmental factors associated with changes in soil metabolite content. Some metabolites, especially low molecular weight organic acids, showed significant positive correlations with SAR and ESP, as well as

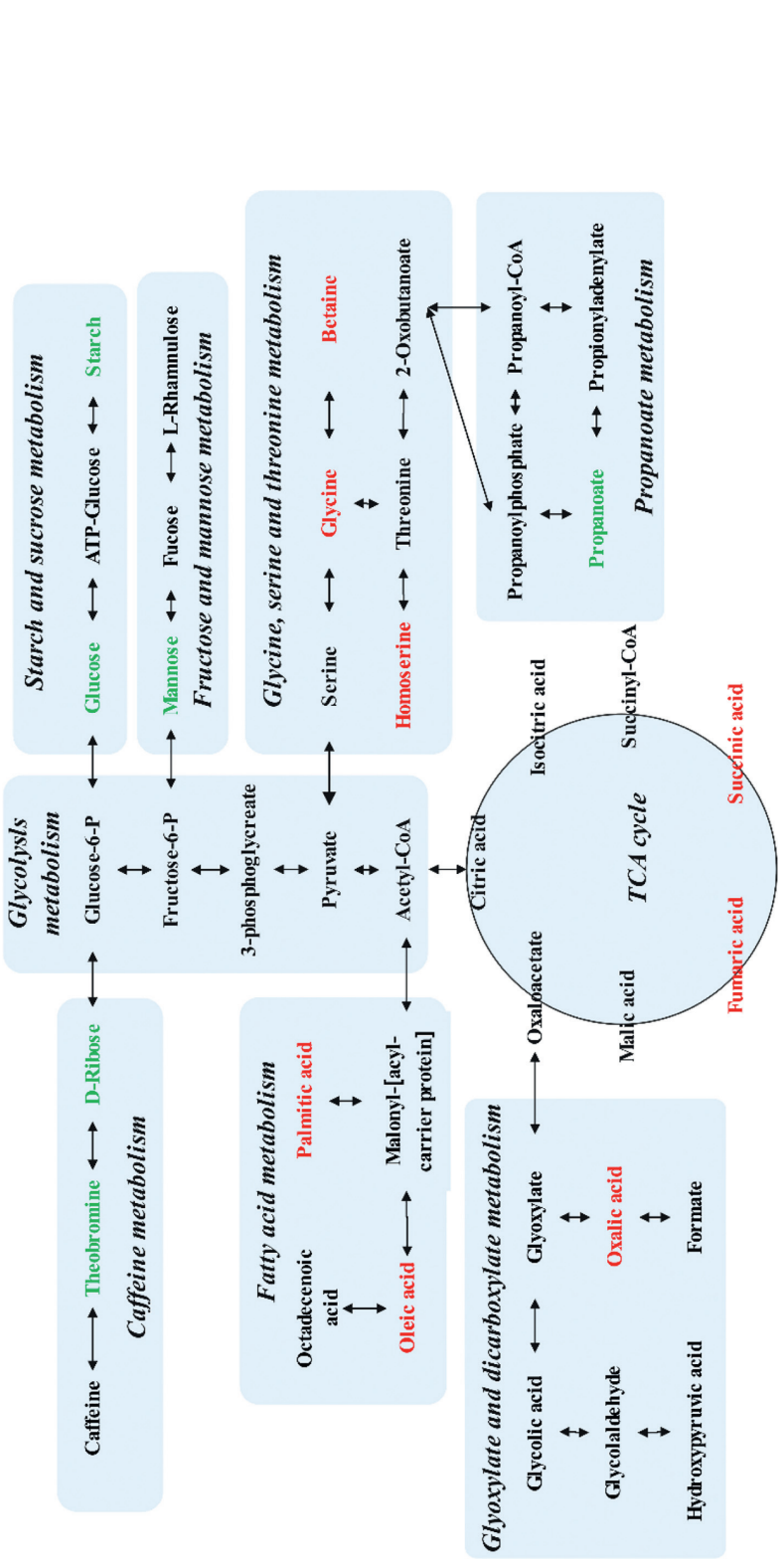

Fig. 4 – Schematic diagram showing the metabolic pathways in soil microbes exposed to saline conditions, with the up- and down-regulated metabolites shown in red and green, respectively.

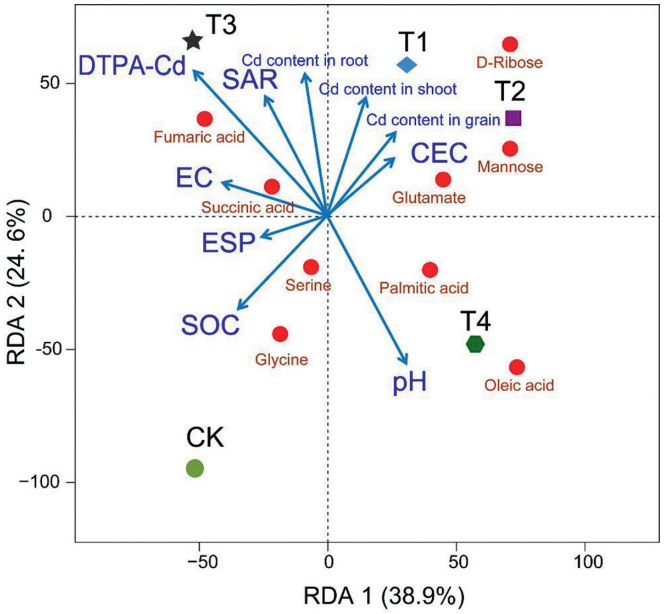

Fig. 5 – Redundancy analysis (RDA) between soil environmental variables and soil metabolite content. Blue arrows indicate relevant parameters that have a strong and significant effect on soil metabolites.

with soil DTPA-extractable Cd content. Thus, we can infer that soil salinity promotes Cd migration into wheat tissues through the increased secretion of soil organic acids. Similarly, it has been reported that salinity increased the release of malic and fumaric acids from roots in amaranth, leading to rhizosphere soil acidification and soil Cd activation, increasing Cd accumulation (Guo et al., 2018). Organic acids, especially citric and oxalic acids, not only acidify the soil but can form soluble complexes and chelate Cd ions, thus promoting Cd activation (Liu et al., 2015). This is due to the fact that malic, citric, and oxalic acids containing two or three carboxyl groups can form organic acid complexes with Cd that have a 5- or 6-membered ring structure, which increases Cd mobility in soil-plant systems (Qin et al., 2004).

4. Conclusions

In this study, we found that salinity altered soil physicochemical properties (e.g., pH, CEC, EC, and OC) and Cd availability in the soil. This may increase the movement of Cd into the wheat plant to some extent. Salt stress also increased the uptake and translocation of Cd in wheat; addition of sodium salts to the soil increased the Cd content of wheat seeds by 16.14%-31.30% (S1) and 21.31%-36.61% (S2) compared to the control, resulting in decreased wheat biomass accumulation. The growth response in wheat could be attributed to the fact that exposure to different salts resulted in the reprogramming of soil metabolites. For example, down-regulation of starch and sucrose metabolism can reduce the production of sugars, which adversely affects wheat growth. Up-regulation of fatty acid metabolism allowed the wheat plants to maintain a normal intracellular environment during salt stress; this triggered an up-regulation of the TCA cycle, causing an increase in organic acid synthesis and the accumulation of organic acids, which facilitated the migration of soil Cd into wheat tissues. The results of our study provide reasonable insights into understanding the mechanisms involved in the changes in Cd availability by soil metabolites in response to salt stress.

Declaration of Competing Interest

The authors declare that they have no competing interests that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 42177010 and 41877387) and Fundamental Research Funds for Central Non-profit Scientific Institution (No. 1610132021008).

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