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Variation of Wheat Root Exudates under Aluminum Stress

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The wheat (*Triticum aestivum* L.) cultivar *Yangzhou 158* was used as a reference. The wheat root exudates were collected using a hydroponic mode. The changes of the electrolytes, H^+, sugar, organic acids, amino acids, and secondary metabolites in wheat root exudates induced by aluminum (Al) were studied. The research results show that Al stress affects wheat root exudation. The secreted electrolytes and sugar increase with the increasing of the external Al^{3+} concentration. The total amount of secreted amino acids has a specific correlation with the external Al^{3+} concentration. At first, the amino acids secrete normally, but when Al^{3+} concentration is over 10 mg·L^{-1}, the amino acid constitution varies obviously. Under Al stress, some original secondary metabolites disappear gradually, and other new secondary metabolites release simultaneously. Increasing the external Al^{3+} concentration gradually stimulates the exudation of organic acids. The organic acid levels in the wheat root zone increase in response to Al treatments. Active Al ions are accumulated in wheat roots. This Al-dependent variation in wheat root exudates suggests a specific Al-induced response of the wheat.

KEYWORDS: Wheat (*Triticum aestivum* L.) rhizosphere; aluminum stress; plant root exudates

INTRODUCTION

Aluminum (Al) toxicity is largely due to the acidic precipitation in industrial regions. It is estimated that 40% of the arable soils of the world are acidic and therefore present Al phytotoxicity hazards (*1*). By interfering with a wide range of physical and cellular processes, the elevated concentrations of solvable Al in soil will result in the inhibition of root growth and reduce crop yields, representing one of the most important limitations to agricultural production worldwide (*2*−*6*). The Al phytotoxicity occurred generally at soil pH values below 5.5. Particularly in acidic red soil, the exchangeable Al ranges from 26.9 to 387 mg·kg^{-1} at $4.03 < pH < 5.50$ (*5*) and the Al concentration in solution at which symptoms of toxicity appear in plant species varies from >50 μmol·L^{-1} to <3000 μmol·L^{-1} (*6*). On the other hand, crop plants have evolved resistant mechanisms that enable them to tolerate active Al in acid soils (*3*). Developing Al-resistant and acid-soil tolerant cultivars could offer a less expensive, ecologically friendly and permanent solution. Thus, the potential for improving adaptation to acid and Al-toxic soils in wheat is promising. Since Al-induced root exudate formation has been widely used as a trait for the screening of cultivars for Al resistance, this potentially significant area indeed deserves detailed study (*4*). It can be anticipated to be a useful tool to help in the breeding of wheat cultivars with superior adaptation to acidic and Al-toxic soils in the future.

Study of Al bioavailability and detoxicity in a complicated rhizosphere is a frontier topic. There are some reports regarding the effects of Al on ecological action in plant rhizosphere recently (*7*−*14*): (1) Ma et al. reported that some plant roots could excrete a great quantity of organic acids such as oxalic, citric and malic acids, etc. Secreted organic acids play an important role in the detoxification of Al. Recently, the anion channel of citric and malic acids in plasma membranes has been characterized and a gene encoding a malic acid channel has been cloned (*8*). (2) The harmfulness to plants of Al^{3+} in acid soils primarily reflects the Al-induced inhibition of root function and the variation of plant root exudates. In an acid rhizosphere, increasing Al^{3+} concentration can inhibit plant growth significantly (*9*). After active Al^{3+} is inserted into a plant root zone, the adverse effect starts within 1 h with inhibition of the root growth (*10*). But if active Al^{3+} is inserted far from a root zone, inhibition of the root growth is not observed (*11*). (3) Rhizosphere is an important root−soil interface, and plant roots are most sensitive to it. Various organic compounds, nutrients, and toxic pollutants also enter into the rhizosphere, in which some microbial activities and normal root physiological action are greatly influenced by root exudates (*12*). Under normal growth conditions, plant roots can excrete electrolytes, H^+, sugar, organic acids, amino acids, enzymes, endogenous hormones, and some secondary metabolites. Under Al stress, plasma membranes of plant roots are injured to a certain extent (*13*). Increasing penetrability of the plasma membrane affects the exudation of various root substances. Therefore, study of the compositional changes of root exudates has definite significance

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for revealing Al resistance and detoxifying Al in response to Al stress (*14*).

Variation of wheat root exudates to Al stress is an interesting research area. Under environmental stress, the composition of wheat root exudates is regulated by complex plant genetic mechanisms. Regulation of these compositional changes is extremely important in order to make a considerable contribution to plant survival and lead to ameliorative growth contributing to plant adaptation. However, research on Al-induced variation of wheat root exudates is still in its infancy. The effects of Al on wheat root exudates are not very clear. In this present study, we are studying the basic chemical compounds that occur in the rhizosphere and their direct consequences for Al-resistant function of plant roots. Since wheat is one of the most widely cultivated crops in the world, it is important to study the influence of Al on it (*12*). Such Al-dependent variation of wheat root exudates can directly affect plant root activity. By observing the dramatic effect of root exudation on plant ecology of the rhizosphere, we can indirectly know which compound is specific to wheat Al tolerance, know how its ecological adaptability is elevated, and further understand the wheat's Al-tolerant mechanism. The wheat cultivar *Yangzhou 158*, one of the most widely cultivated crops in our local arable lands and well adapted to acid soils with high availability of phytotoxic Al species, is the study reference. Since the mechanism of Al resistance in this species is not established, we have tried to assess possible implications of root exudates in Al detoxification of this wheat in this study. Because of the difficulty of isolating root exudates from the dynamic and complicated rhizosphere microenvironment, the effective collection of wheat root exudates is very important. Therefore, a hydroponic mode was specially designed for the collection of root exudates. The external Al^{3+} adding procedure was used to investigate Al-dependent compositional changes of wheat root exudates. Organic acids, H^+, electrolytes, amino acids, sugar, and secondary metabolites were detected in wheat root exudates. This Al-dependent variation of the above-mentioned compounds in wheat root exudates may provide useful information for the detoxification mechanism of Al-resistant species in the rhizosphere.

MATERIALS AND METHODS

Samples. The seeds of wheat cultivar *Yangzhou 158* were purchased from Yangzhou Agricultural Science Institute of China. The uniform seeds were first surface sterilized in a 75% ethanol solution for 10 min, rinsed in tap water for 20 min, and then washed with deionized water three times. Seeds were soaked in deionized water for 12 h and then germinated for 12 h. Seedlings of similar size were cultivated using the hydroponic mode.

The macronutrient solution was prepared by mixing 50 mg of NH_4NO_3, 10 mg of KH_2PO_4, 25 mg of KCl, 30 mg of $CaCl_2$, 125 mg of $MgSO_4 \cdot 7H_2O$, and 5 mg of $FeCl_3$ in 1 L of deionized water. The micronutrient solution was prepared simultaneously. It consisted of HBO_3, 2.86 mg·L^{-1}; $CuSO_4 \cdot 5H_2O$, 0.08 mg·L^{-1}; $ZnSO_4 \cdot 7H_2O$, 0.22 mg·L^{-1}; $MnCl_2 \cdot 4H_2O$, 1.81 mg·L^{-1}; $H_2MoO_4 \cdot 4H_2O$, 0.09 mg·L^{-1}; and Fe·EDTA, 20 mg·L^{-1}. The composition of the nutrient solution used in our work is mainly based on the modification for the methods proposed by Eticha et al. (*4*) and Cheng et al. (*15*). The two solutions were mixed in a certain ratio. The mixed solution was divided equally into 12 parts. The different amounts of $Al(NO_3)_3$ were added, and then their pH values were adjusted to 4.5. A series of nutrient solutions containing Al^{3+} concentrations of 0, 0.2, 0.4, 0.6, 0.8, 1, 5, 15, 25, 40, 60, and 80 mg·L^{-1} were prepared. The nutrient solution without Al^{3+} was used as a control sample in a blank assay. The deionized water was purified using a Milli-Q water purification system (Millipore, Eschborn, Germany).

Wheat seedlings of similar size were transplanted into a 25 L plastic pot containing aerated nutrient solution (200 seedlings per pot) and incubated in a LRH-250-G growth chamber at 25 °C. First, the seedlings were cultivated with deionized water until their root elongation reached 2 cm. Then they were cultivated with the above-described nutrient solutions containing different Al^{3+} concentrations. About 30 mL of fresh nutrient solution was added every 12 h. Wheat seedlings were grown in a controlled culture box with a 12 h light/12 h dark cycle under 40 W·m^{-2} light. The light/dark temperatures were set at 25/20 °C, and relative humidity was kept at 65%. The culturing time was 15 days. Ultraviolet radiation was used to disinfect the growth chamber prior to use.

To collect root exudates, wheat roots were treated with deionized water. Wheat roots were exposed to pH 4.5 0.5 mmoL·L^{-1} $CaCl_2$ solutions with corresponding Al levels for 12 h and then washed with 100 mL of deionized water (200 seedlings per treatment). The treatment solution was placed on a shaker, centrifugally separated (60 rpm) for 2 h, and then filtrated. The filtrate was divided equally into two parts. One part was used to measure the rhizosphere pH and exosmic electrolytes, and the other part was concentrated to 50 mL at 4 °C under vacuum (*16*) and used to analyze the sugar, amino acids, organic acids, and secondary metabolites.

Analysis. The general analytical procedures involved in this study are as follows:

(1) Rhizosphere pH. The relevant pH values in the sample solution containing different Al^{3+}concentrations were determined by a PHS-25 digital display acidometer (Shanghai Precision Instruments Co., China).

(2) *Conductivity.* The conductivities of the root exudate solution were measured. Then, the roots of wheat seedlings were exposed to the root exudate solution and heated in a boiling-water bath for 20 min, and then cooled to room temperature. Their conductivities were measured with a DDS-11A_T conductivity meter (Shanghai XingJing Instruments Co., China) (*17*).

(3) Amino Acids. A 5 mL sample solution was hydrolyzed with 8 mL of 6 mol·L^{-1} HCl under vacuum at 110 °C for 24 h. After cooling, the hydrolysate was washed with deionized water, filtered (Whatman No. 2, USA), and dried at 60 °C (also under vacuum) in a rotary evaporator. The dried sample was then dissolved in 0.01 mol·L^{-1} HCl. The amino acids in the hydrolysate were separated and quantified by injecting 50 μL into a Hitachi 835-50 amino acid automatic analyzer (Hitachi, Japan) equipped with a 2.6 mm × 150 mm ion exchange column coated with resin 2619#. The column temperature was 53 °C. Sodium citrate buffers (pH 3.3, 4.3, and 6.3) were used as eluents with a flow rate of 0.225 mL·min^{-1}. The light absorbance of the amino acids was detected with a 166 detector (Beckman Instruments, USA) at 570 nm, and the amino acids were quantified by comparing them with amino acid profiles from external amino acid standards.

(4) Total Sugar. A 5 mL concentrated sample was hydrolyzed with 10 mL of 4% H_2SO_4 under vacuum at 110 °C for 1 h. After cooling, the hydrolysate was filtered (Whatman No. 2, USA) and dried at 60 °C (also under vacuum) in a rotary evaporator. The dried sample was then dissolved in 0.005 mol·L^{-1} H_2SO_4. The total sugar in the hydrolysate was separated and quantified by injecting 10 μL into a HPLC (Waters 600, USA) with an RI × 4 detector, equipped with a Sugar-pak 1. P/N 85188 column (Waters, USA). The column temperature was 85 °C. Milli-Q water was used as the mobile phase with a flow rate of 0.6 mL·min^{-1}.

(5) Monosaccharide. Monosaccharide in 25 μL of concentrated solution was separated and quantified by injecting 10 μL into a HPLC (Waters 600, USA) with an RI × 4 detector, equipped with a Sugar-pak 1. P/N 85188 column (Waters, USA). The column temperature was 85 °C. Milli-Q water was used as the mobile phase with a flow rate of 0.6 mL·min^{-1}.

(6) Organic Acid. The sample solution was passed through a cation exchange column (16 × 14 mm) filled with 5 g of Amberlite IR-120B resin (H^+ form, Shanghai Chemical reagent Co., China), followed by an anion-exchange column (16 × 14 mm) filled with 2 g of Dowex 1×8 resin (100−200 mesh, format form, Shanghai Chemical reagent Co., China). The organic acids retained on anion-exchange resin were eluted by 1 mol·L^{-1} HCl, and the eluate was concentrated. A 25 μL concentrated solution was injected onto a Biorad HPX-87H column (7.8 mm i.d. × 300 mm, 9 μm). The quantitative determination of organic acids had been carried out using Waters 600 HPLC (*18*).

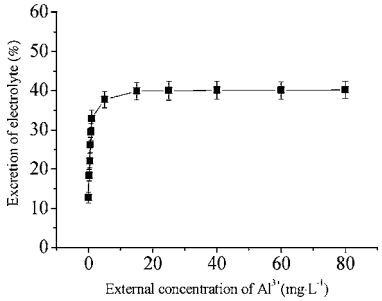

Figure 1. Electrolytes osmosized from wheat roots in response to Al stress. Seedlings were grown in nutrient solution until the root elongation of seedlings reached 2 cm and subsequently exposed to nutrient solution containing different Al levels for an additional 15 days before rhizosphere solutions were collected. Data are means ± SD ($n = 9$).

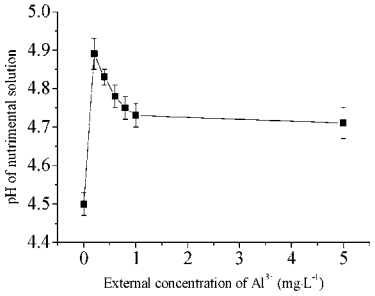

Figure 2. The dynamics of pH in rhizosphere solution in response to Al stress. Seedlings were grown in nutrient solution until the root elongation of seedlings reached 2 cm and subsequently exposed to nutrient solution containing different Al levels for an additional 15 days before rhizosphere solutions were collected. Each value is given for 200 wheat roots. Data are means ± SD ($n = 9$).

The mobile phase used was 0.005 mol·L^{-1} H_2SO_4 at a flow rate of 0.5 mL·min^{-1}. Detection was at a wavelength of 210 nm. The column temperature was 50 °C.

(7) Secondary Metabolite. Secondary metabolites in all samples were identified using a Finnigan Trace DSQ GC−MS (USA) in selected ion mode (SIM). The capillary column used was a DB-5MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The carrier gas was helium. A split−splitless injector in the splitless mode was used. The inject volume was 1 μL. The temperature program was programmed from 40 °C (initial time, 4 min) to 180 °C at a rate of 20 °C·min^{-1} holding for 1 min, from 180 °C to 245 °C at a rate of 2 °C·min^{-1} holding for 2 min, and from 245 °C to 280 °C at a rate of 20 °C·min^{-1} holding for 10 min. The temperature of the injector was held at 260 °C.

(8) Aluminum. To determine the level of Al in wheat roots, seedlings were separated into shoot and root and dried at 70 °C for 48 h. The roots were weighed, ground, acid-digested, filtered, and finally concentrated to a certain volume. The Al was determined by VerdFluor fluorescence spectrophotometry [Bole (B10-RAB), USA]. Nine replications were undertaken for each treatment.

Statistical Analysis. Each result shown in figures was the mean of at least nine replicated treatments. The significance of differences between treatments was statistically evaluated by standard deviation.

RESULTS

Electrolytes Osmosized from Wheat Roots. The percentages of electrolytes osmosized from roots, as shown in **Figure 1**, were calculated using the following equation: $\eta = (S_1/S_2) \times 100\%$, where S_1 is the conductivity measured at room temperature and S_2 is the conductivity measured after being heated in a boiling-water bath. When the external Al^{3+} concentration increased from 0 to 15 mg·L^{-1}, Al-induced conductivity increased transiently. When the external Al^{3+} concentration was more than 15 mg·L^{-1}, the increasing rate of conductivity decreased gradually. Under Al stress, the maximum percentage of osmotic electrolytes was approximately 3-fold of the blank assay (−Al: without Al addition).

Variation of pH in Root Exudates. The growth solution pH was initially adjusted to 4.5, and then not controlled during the experiment. Final solution pH levels are presented in **Figure 2**. Increasing rhizosphere Al^{3+} concentration from 0 to 2 mg·L^{-1} enhanced the comparable rate of H^+ secreted from the wheat roots to the utmost extent. The transient increase in pH was from 4.5 to 4.9. When the external Al^{3+} concentration was in excess of 2 mg·L^{-1}, the secretion of H^+ from the wheat roots gradually increased to the control level (−Al).

Secretion of Amino Acids in Root Exudates. The effects of nutrient solutions containing different Al^{3+}concentrations on the secretion of amino acids were different. The research results listed in **Figure 3** indicate that there were 15 amino acids to be detected in root exudates in the blank assay. They were asparagine (ASP), glutamic acid (GLU), glycin (GLY), arginine (ARG), threonine (THR), alanine (ALA), proline (PRO), tyrosine (TYR), valine (VAL), methionine (MET), isoleucine (ILE), leucine (LEU), phenylalanine (PHE), serine (SER), and lysine (LYS); but cystine (CYS) and histidine (HIS) were not observed simultaneously. When the rhizosphere Al^{3+} concentration was from 0 to 1.0 mg·L^{-1}, 15 amino acids excreted from the wheat roots normally. When the external Al^{3+}concentration was in excess of 1.0 mg·L^{-1}, the quality of excreted amino acids changed greatly. When the external Al^{3+}concentration was in excess of 25 mg·L^{-1}, ASP secretion was not detected and the exuded amounts of GLU, CYS, VAL, PHE, ARG were significantly higher compared with the data of the blank assay. When the external Al^{3+}concentration was 60 mg·L^{-1}, ASP, THR, SER, GLU, ALA, CYS and VAL were not detected, but the exuded amounts of GLY, TYR, PHE and ARG were especially high. The detectable releasing of HIS was found in external Al concentration of 60 mg·L^{-1}, and not detected in other Al treatments. CYS was not secreted at first, but subsequently some detectable releasing of ARG was observed with increasing external Al^{3+}concentration. Al stress on wheat caused elevation of PRO secretion.

Sugar Excreted from Wheat Roots. The concentration gradients of glucose, fructose, aldose, and the total sugar exuded from wheat roots were observed in different Al^{3+} treatments **(Figure 4)**. The exuded amounts of fructose and aldose were very low. Their variation did not change with increasing external Al concentration, but the exuded amounts of glucose and total sugar changed significantly. Compared with the blank assay, when the Al^{3+} concentration in nutrient solution was 1 mg·L^{-1}, the concentration difference of glucose and total sugar exuded from wheat roots was not obvious. When the Al^{3+} concentration in nutrient solution was 5, 15, 25, 40, 60, and 80 mg·L^{-1}, the percentages of glucose exuded from wheat roots were increased 9.5%, 20.2%, 55.4%, 71.70%, 119.5%, and 110.0%, and the percentages of total sugar exuded from wheat roots were increased 11.1%, 16.2%, 51.1%, 67.4%, 121.7%, and 149.2% compared with the data of the blank assay. Moreover, when the Al^{3+} concentration increased to 60 mg·L^{-1}, Al stress stimulated the secretion of sugars in wheat roots (*19*). The maximum secretion of glucose appeared. The level of total sugar also increased.

Organic Acids Excreted from Wheat Roots. The effects of different external Al^{3+} concentrations on the organic acid secretion are presented in **Figure 5**. Under Al stress, the wheat roots exude detectable malic and citric acids after being treated for 0.5 h for malic acid and 2−4 h for citric acid, respectively. The citric acid exuded from wheat roots increased with the

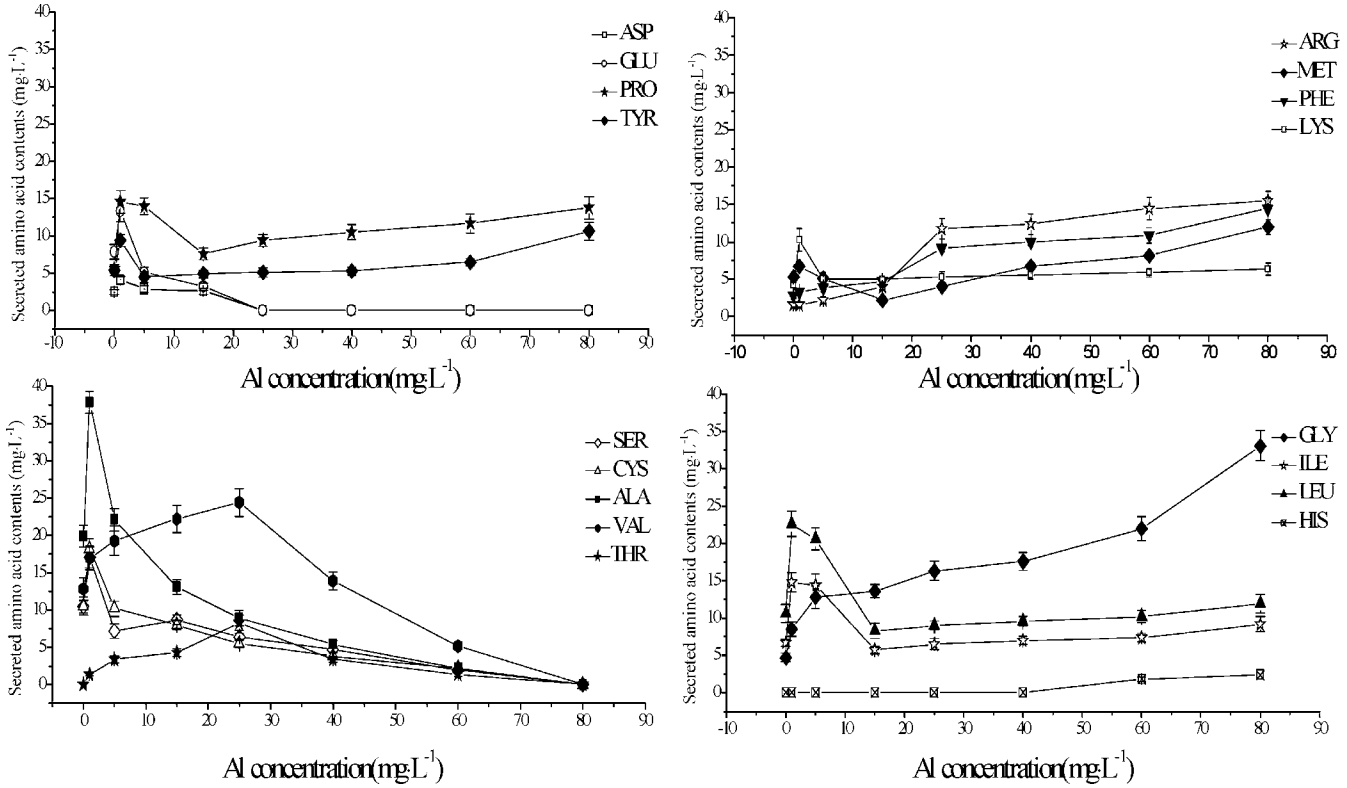

Figure 3. Secretion of amino acid contents in wheat roots under Al stress. Seedlings were grown in nutrient solution until the root elongation of seedlings reached 2 cm and subsequently exposed to nutrient solution containing different Al levels for an additional 15 days before root exudates were collected. Data are means ± SD ($n = 9$).

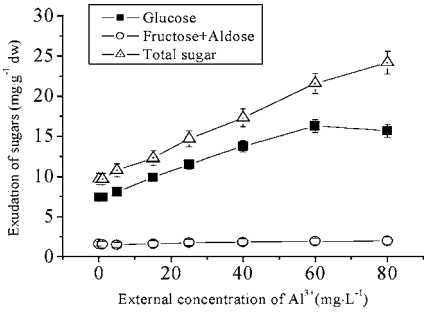

Figure 4. Variation of various sugars excreted from wheat roots under Al stress. Seedlings were grown in nutrient solution until the root elongation of seedlings reached 2 cm and subsequently exposed to nutrient solution containing different Al levels for an additional 15 days before root exudates were collected. Data are means ± SD ($n = 9$).

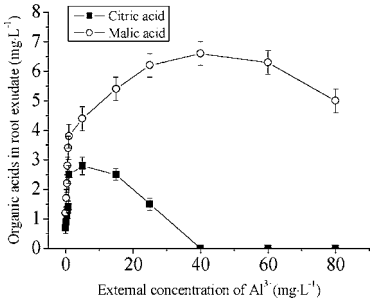

Figure 5. Organic acid exudation induced by different Al levels for wheat *Yangzhou 158*. Seedlings were grown in nutrient solution until the root elongation of seedlings reached 2 cm and subsequently exposed to nutrient solution containing different Al levels for an additional 15 days before root exudates were collected. Data are means ± SD ($n = 9$).

external Al^{3+} concentrations ranging from 0 to 5 mg·L^{-1}, and decreased with the external Al^{3+} concentrations ranging from 5 to 40 mg·L^{-1}. Once the external Al^{3+} concentration raised over 40 mg·L^{-1}, citric acid exuded from wheat roots was not detectable. Under the same conditions, the malic acid exuded from wheat roots increased with the external Al^{3+} concentrations ranging from 0 to 40 mg·L^{-1}. After the external Al^{3+} concentration raised over 40 mg·L^{-1}, malic acid exuded from wheat roots decreased slightly with increasing Al^{3+} concentration.

Absorption of Al by Wheat Roots and Root Growth. Al accumulation in root systems of wheat under normal growth was relatively low, with an average amount of 0.1 mg·g^{-1}. However, Al accumulation in root systems increased significantly with rhizosphere Al^{3+} concentration. When Al^{3+} concentration was more than 25 mg·L^{-1}, Al accumulation in root systems of wheat increased to 0.6 mg·g^{-1}. The root growth of wheat decreased with increasing external Al^{3+}concentration, as shown in **Figure 6**.

Effects of Rhizosphere Al^{3+} on the Secondary Metabolites Exuded by Wheat Roots. The wheat was grown in different nutrient solutions containing 0, 1, 5, 15, 25, 40, 60, and 80 mg·L^{-1} Al^{3+} respectively. The detectable kinds of secondary metabolites secreted were 13, 8, 7, 5, 4, 3, 2, and 2 for each corresponding Al level. The detectable secondary metabolites exuded by wheat roots were also found in the blank assay. But in response to Al stress, some original secondary metabolites were not detected. In addition, some other new secondary metabolites were also observed, as shown in **Table 1**. In the rhizosphere, under Al stress, the detectable secondary metabolites were 3-methyl-furandione- (*2*, *5*), pentadiene- (*1*, *4*), *N*-methyl ethylamine, *N*-methyl phenylethylamine, 3-nitrylpyrazole, 5-nitrylpyrazole, pentanal. But in the absence of Al, none of them were detected.

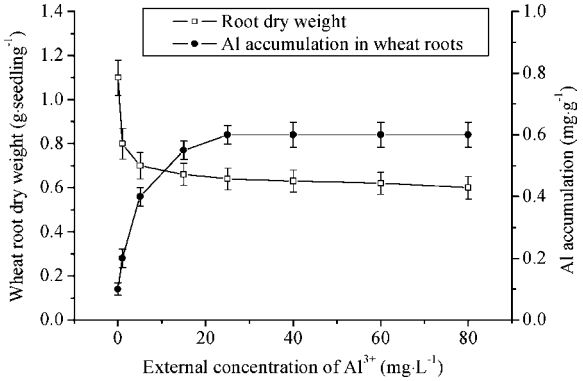

Figure 6. Correlation between wheat root weight and Al accumulation under Al stress. Seedlings were grown in nutrient solution until the root elongation of seedlings reached 2 cm and subsequently exposed to nutrient solution containing different Al levels for an additional 15 days before wheat roots were collected. Seedling were separated into shoot and root and dried at 70 °C for 48 h. Data are means ± SD (n = 9).

DISCUSSION

An increased understanding of the mechanisms involved in Al tolerance can help in the breeding of wheat that is adapted to acidic soils (*20*). The Al-tolerant mechanism is apparently the secretion of root exudates into the rhizosphere. Our work emphasizes wheat *Yangzhou 158's* response to Al stress. Al-stimulated root exudates are a useful system for studying how the Al signal expresses physiological responses underlying Al tolerance, and we believe that their compositional changes play a significant role in the transduction of Al signals and expressions of some physiological responses in the root apex of Al-tolerant wheat. In general, plants will produce more secondary compounds and exudates under environmental stress (*21*, *22*). Wheat root exudates mainly consist of electrolytes, H^+, sugar, organic acids, amino acids, and other secondary metabolites. With the external Al level being increased, the plant root defense system gradually shows greater tolerance to Al toxicity. In the plant−soil interface, active Al ions react with organic root exudates, for example, Al^{3+} chelates react with organic acids to alleviate Al toxicity. Simultaneously, new secondary metabolites are continuously released (*4*, *23*). Following is the discussion on the characteristics of Al-induced root exudation, which will benefit the understanding of the Al-tolerant mechanism of this wheat.

Osmosis of Electrolytes from Wheat Roots. Soil chemical factors including Al, Mn, and various cations such as Ca, Mg, Mo, and Si may greatly limit root growth in acid soils, exhibit a variety of nutrient-deficiency symptoms, and diminish crop production (*24*). Therefore, study of electrolytes osmosized from wheat roots is necessary for plant systems. These effects are further complicated by the interactions of rhizosphere Al with the electrolytes in wheat root exudates. This study revealed that osmosis of electrolytes greatly affected the quantity and quality of wheat root exudates. The Al-induced release of electrolytes from roots was observed when the external Al^{3+} concentration was less than 15 mg·L^{-1}. Perhaps this is caused by a physiological instinct of plants to adapt to an Al-stressed rhizosphere. Plants, mostly through their root system, take up or exchange various ions, which may alleviate various environmental stresses. For example, an affinity of root plasma membrane for Al^{3+} was stronger than that for Ca^{2+}. The binding sites of Ca^{2+} in plasma membranes of root cells were gradually replaced by Al^{3+}, which injured the integrality and selectivity of plasma membranes within root cells (*25*).

Variation of pH in Root Exudates. Many researchers have observed rhizosphere pH changes (*21*, *26*, *27*). The pH values of the soil solutions vary within a wide range (pH 3.8−7.1) (*6*). Root-induced changes in rhizosphere pH occur primarily as a consequence of differential rates in the uptake of cations and anions by plants (*28*). As can be seen in **Figure 2**, pH in the wheat rhizosphere increased up to 0.4 units compared with the blank assay. This alkalinization plays a major role in the bioavailability of the potentially toxic Al. Apparently, as solution pH increases, the activity of free Al^{3+} decreases, thereby leading to a decreased risk of Al phytotoxicity (*27*). Then, the rhizosphere pH had a positive correlation with Al tolerance. Nye proposed that, after exposure to Al^{3+} solution, the roots must then release HCO_3^- into the rhizosphere to maintain electrical neutrality, which made rooting in the adjacent region more alkaline (*29*). Increasing pH is probably related to rhizosphere Ca^{2+} concentration (*30*).

Secretion of Amino Acids in Root Exudates. Acid soils contaminated with active Al^{3+} also contained a number of organic ligands, particularly in the rhizosphere, and thus it is likely that only a fraction of active Al in soil exists in a chelated form. The presence of soluble Al-chelating substances can influence the fate and transport of Al. The formation of the complexes between Al^{3+} and low molecular weight organic ligands might affect the soil functionality, especially in the rhizosphere of acid soil. Our research results reveal that the Al-triggered exudation of amino acids is a specific response to Al stress (*31*). We have observed the variation of secreted amino acids with different Al treatments. Whether they influence plant growth or alleviate Al rhizotoxicity, however, is not clear. Based on our observations, we conclude that the research of secreted amino acids is of utmost importance for plant adaptive physiology, which may play a role in Al tolerance.

Excretion of Sugar from Wheat Roots. Figure 3 showed the changes in sugar excreted from the wheat roots under Al stress. Test results demonstrated a correlation between external Al levels and increased release of sugar. Al^{3+} may trigger the secretion of glucose and total sugar. This stimulation increased with increasing external Al^{3+} concentrations. However, this mechanism is not yet known, and little evidence for the formation of sugar species during Al treatment has yet been documented. Its role in stress environment should be studied further.

Excretion of Organic Acids from Wheat Roots. Organic acids in plant root exudates have been directly implicated in a number of soil processes such as plant metabolism, metal detoxification, and nutrient solubilization by plant roots during plant growth (*32*). Their quantitative role in these processes, however, has been difficult to establish due to the influence of many interdependent factors. These factors include solid phase sorption/desorption reactions, metal complexation reactions, leaching, and microbial degradation (*33*). Our study investigated how the secretion of organic acids changed in response to Al stress, which had an important significance for plant toxicology. In acid soils, Al-induced citric and malic acids were found in wheat roots. Our experimental results showed that the citric and malic acids exuded from wheat roots increased within the range of limited Al^{3+} levels. These organic acids could play an important role in degrading the soil Al poisoning. The toxicity of Al^{3+} could be generally eliminated once Al^{3+} was chelated with organic acids (*34*). Slightly toxic Al−ligand complexes are used to theoretically evaluate the effectiveness of ligands in Al detoxification (*35*). In the rhizosphere, malic and citric acids form stable complexes (M:L ratio 1:1) with Al, thereby

Table 1. Effects of Al on Secondary Metabolism Products Excreted from Wheat Roots[a]

Al concn ($mg \cdot L^{-1}$)	secondary metabolism products excreted by wheat roots
0	cyclohexanol, cyclohexanone, 1,2,3,5-tetramethylbenzene, 2-methylnaphthalene, dodecanal, hexadecane-1-alcohol, 1,13-tridecanediol diacetate, tetradecanal, octadecanal, 4-ethyl-1,2-dimethylbenzene, 1-ethyl-3,5-dimethylbenzene, 2-ethyl-1,4-dimethylbenzene, 1-ethyl-2,4-dimethylbenzene
1.0	cyclohexanol, cyclohexanone, 4-ethyl-1,2-dimethylbenzene, 1-ethyl-2,3-dimethylbenzene, 2-ethyl-1,4-dimethylbenzene, 1-methyl-4-(1-methylethyl)benzene, 1,2,3,5-tetramethylbenzene, heptahydrobenzo-cycloheptylene
5	cyclohexanol, cyclohexanone, 1-ethyl-2,4-dimethylbenzene, 1-methyl-4-(1-methylethyl)benzene, 1,2,3,4-tetramethylbenzene, 1,2,3,5-tetramethyl ethyl stearate, hexatriacontane
15	cyclohexanol, cyclohexanone, 1,2,3,5-tetramethyl ethyl stearate, *N*-methyl ethylamine, hexatriacontane
25	cyclohexanol, cyclohexanone, *N*-methyl phenylethylamine, 1,2,3,5-tetramethyl ethyl stearate
40	cyclohexanol, cyclohexanone, *N*-methyl phenylethylamine
60	cyclohexanol, *N*-methyl phenylethylamine
80	cyclohexanol, *N*-methyl phenylethylamine

[a] Seedlings were grown in nutrient solution until the root elongation of seedlings reached 2 cm and subsequently exposed to nutrient solution containing different Al levels for an additional 15 days before root exudates were collected.

preventing binding of Al^{3+} with intra- and intercellular compounds in roots. Therefore, such complex formation can alleviate Al toxicity.

Effects of Rhizosphere Al^{3+} on the Secondary Metabolites Exuded by Wheat Roots. The secretion of secondary metabolites was greatly influenced by rhizosphere stress, as shown in **Table 1**. The varieties of secondary metabolites decreased and their composition varied with increasing external Al^{3+} concentration, suggesting that further study on the Al-stimulated secretion of plant secondary metabolites is necessary. Interactions between Al and Al-induced secondary metabolites are important for the Al-detoxification effect, although their role is not clear. Our results indicate that Al exposure can alter the secretion of some secondary metabolites, and the new secondary metabolites should have a specific response to Al detoxification efficiency in the rhizosphere. These secondary metabolites have developed strategies to avoid or tolerate Al toxicity, and have progressively stimulated wheat to grow in acid soils. As shown in our experimental results, we think that original secondary metabolites do not resist Al toxicity, which gradually disappears with increasing external Al^{3+} level. But the new secondary metabolites appear to alleviate wheat Al toxicity; they should react with Al^{3+} to form less toxic species. Bourgaud et al. indicated that plant secondary metabolites play a major role in the adaptation of plants to their environment. These molecules contribute significantly to plant fitness by interacting with the ecosystems (*36*). However, the internal mechanism of secondary metabolites specific to Al toxicity remains largely unknown. This should be studied in more detail in the future.

Root Growth and Absorption of Al by Wheat Roots. Al limits crop production in acid soils. When the soil pH drops below 5, the rhizotoxic Al species, Al^{3+}, is solubilized into the soil solutions and inhibits root growth and function (*3*). **Figure 6** shows that, under Al stress, root growth and metabolization of plants were significantly influenced, and the dry weight of wheat roots decreased significantly. The initial and most recognizable symptom of Al accumulated in roots is inhibition of root growth, which results in a reduced and damaged root system (*12*). As a result, limited Al^{3+} migrated to stems and leaves above the ground, and sufficient Al^{3+} was left in the roots (*24*). Research carried out in our laboratory demonstrated that Al^{3+} triggers adaptive responses to environmental stress. Al accumulation in root systems increased significantly with rhizosphere Al^{3+} concentration. The root growth of wheat increased with increasing external Al^{3+}concentration (*20*). The root systems of wheat have the potential for an increase in Al absorption and may be adversely influenced by excess Al in acid soil. More Al might be mobilized and translocated into the wheat rhizosphere. Phytotoxicity of Al restricts crop productivity in acid soils (*5*, *37*). Plants should have the ability to cope with Al phytotoxicity, a major problem in many of the world's arable soils.

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