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Elevation of arginine decarboxylase-dependent putrescine production enhances aluminum tolerance by decreasing aluminum retention in root cell walls of wheat

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HIGHLIGHTS

- Al stress increases ADC activity and Put accumulation in root tips of wheat plants.
- Put can alleviate Al-induced wheat seedling root inhibition.
- Al accumulation in wheat root tips and root tip cell walls were decreased by Put.
- Put decreases the cell wall polysaccharides contents.
- Put increases the degree of pectin methylation in cell walls.

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ABSTRACT

Aluminum (Al) stress induces putrescine (Put) accumulation in several plants and this response is proposed to alleviate Al toxicity. However, the mechanisms underlying this alleviation remain largely unknown. Here, we show that exposure to Al clearly increases Put accumulation in the roots of wheat plants (*Triticum aestivum* L. 'Xi Aimai-1') and that this was accompanied by significant increase in the activity of arginine decarboxylase (ADC), a Put producing enzyme. Application of an ADC inhibitor (D-arginine) terminated the Al-induced Put accumulation, indicating that increased ADC activity may be responsible for the increase in Put accumulation in response to Al. The D-arginine treatment also increased the Al-induced accumulation of cell wall polysaccharides and the degree of pectin demethylation in wheat roots. Thus, it elevated Al retention in cell walls and exacerbated Al accumulation in roots, both of which aggravate Al toxicity in wheat plants. The opposite effects were true for exogenous Put application. These results suggest that ADC-dependent Put accumulation plays important roles in providing protection against Al toxicity in wheat plants through decreasing cell wall polysaccharides and increasing the degree of pectin methylation, thus decreasing Al retention in the cell walls.

1. Introduction

Aluminum (Al) is the most abundant metal in the earth's crust, occurring mainly as harmless oxides and aluminosilicates [1]. Aluminum constitutes one of the major environmental metal pollutants known to contaminate soils and originates from sources such as industrial mining, wrapping materials, food additives, and medicines [2]. As soils become acidic by acid precipitation and overuse of ammonia- and amide-containing fertilizers, phytotoxic Al forms are released into the soil solution and become major constraints for crop growth and yield [3,4]. Excessive Al in the diet

Abbreviations: Al, aluminum; ADC, arginine decarboxylase; Put, putrescine; PCA, perchloric acid; HC1, hemicellulose 1; HC2, hemicellulose 2; ODC, ornithine decarboxylase; Spd, spermidine; Spm, spermine; GalA, galacturonic acid.

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may impair kidney function, and cause Alzheimer's and other neurological diseases [5]. It follows that Al is not only a phytotoxic substance limiting crop productivity but also a hazard to human health because of Al transfer to humans through the food chain. Therefore, the mechanisms of Al accumulation and tolerance in plants needs urgent clarification to develop strategies for preventing its accumulation, in order to alleviate Al toxicity in plants and minimize health risks associated with high Al-containing foods.

Al causes a myriad of physiological, biochemical, and molecular effects in plants. For example, Al inhibits root elongation [1], and induces nutrient deficiencies [6,7] and oxidative stress [8–10] in plants. Fortunately, some plant species have evolved special mechanisms to withstand Al toxicity [11–14], such as Al exclusion by inducing an efflux of organic acids that detoxifies Al through chelating toxic Al ions in the rhizosphere [1,13,15,16] and alteration of the composition of the cell wall, which contains highly negatively charged sites that may serve as a sink for Al [17–19]. Recently, several studies have reported that Al stress alters polyamines profiles in plants [20,21] and this response was also proposed to alleviate Al toxicity. However, evidence in support of this hypothesis is still limited.

Polyamines, including putrescine (Put), spermidine (Spd) and spermine (Spm) are important signaling molecules in plants and are involved in several processes such as defense against pathogens, abiotic stress tolerance, and morphogenesis and organ development [22–24]. The cellular polyamine contents are highly regulated and various abiotic stresses, such as salt and heavy metal stress, lead to accumulation of one or more of the polyamines [25,26]. Put plays an important role as a precursor for the biosynthesis of higher polyamines [27], the levels of which are proposed to be homeostatically regulated and more tightly than those of Put [28]. Generally, Put is known to increase under various stress conditions, whereas Spd and Spm frequently remain unchanged or show minor changes [29,30]. The link between Put and Al stress was documented based on the observation of increased Put accumulation in Al-stressed plants [21,31,32] and the improved plant Al tolerance by exogenous Put application [32,33]. However, the mechanisms of how Al induces Put accumulation and how Put associates with Al tolerance remain elusive. Put is present in various cellular components, including cell wall fractions, and may directly interact with cell wall components [20,34,35] or affect cell wall composition [36,37]. As mentioned above, the cell walls are not only the primary Al-binding site in root cells but also serve as the first barrier for cellular Al uptake. In plants, almost 70–90% of the cellular Al is accumulated in the cell wall [19,38,39]. On the other hand, Al bound to the cell wall polymers negatively affects wall structure and function, making it more rigid and reducing cell expansion and mechanical extensibility, thus inhibiting root elongation [19,40]. Considering the role of the cell wall in Al toxicity and Al absorption, it is possible that Put accumulation might interfere with cell wall properties to alleviate Al toxicity.

In this study, wheat plants were used to investigate the mechanism by which Al induces Put accumulation, the role of Put in regulating cell wall composition and the association of Put with Al resistance. We found that arginine decarboxylase (ADC)-dependent Put production decreased aluminum retention in root cell walls by lowering the contents of polysaccharides and the degree of cell wall demethylation, thus enhancing the Al tolerance of wheat plants.

2. Materials and methods

2.1. Plant material and treatments

Wheat (*Triticum aestivum* L. 'Xi Aimai-1') seeds were surface sterilized for 20 min in a 1% NaClO solution, and soaked in distilled water overnight. Next, the seeds were germinated at 25 °C for 12 h before transplanted into plastic screens floating on 0.5 mM $CaCl_2$ solution (pH 4.3 ± 0.1) for 3 d. Plants were grown in a controlled-environment chamber with a 12 h light (25 °C)/12 h dark (22 °C) regime, photon flux density of 300 μmol m^{-2} s^{-1}, and relative humidity of 70%. The solution was renewed daily. Then, the seedlings were used for various experiments. Different concentrations of $AlCl_3$, putrescine (Put), and D-arginine were added to the basal solution (0.5 mM $CaCl_2$, pH 4.3 ± 0.1) for the different treatments. At selected time points after treatments, the roots were rinsed with distilled water several times and the root tips (1 cm) were excised for further analysis. Elongation of the primary root was also measured with a ruler before and after treatments at the selected times points ($n > 20$). Relative root elongation (RRE) was defined as the percentage of the root elongated in the treatment solutions compared to the elongation observed in the control solution.

2.2. Al content determination in root tips and root tip cell walls

For Al content determination in root tips, excised root tips were directly suspended in 10 mL of 2 M HCl with occasional shaking for 24 h. The Al content bound to root cell walls was estimated by homogenizing the root tips with ice-cold distilled water, according to Yang et al. [41]. The homogenate was centrifuged at 13,000 × *g* for 10 min, and the precipitate was then washed 3 times each with 80% ethanol, methanol:chloroform mixture (1:1), and 100% acetone, respectively. After drying, the precipitate was resuspended in 2 M HCl with occasional shaking for 24 h. The Al concentrations in the HCl solution were determined by inductively coupled plasma mass spectrometer (ICP/MS) Agilent 7500A (Agilent, Palo Alto, CA, USA) according to Xu et al. [42].

2.3. Determination of polyamines by high performance liquid chromatography (HPLC)

The concentrations of free polyamines were estimated following the methods described by Flores and Galston [43] with slight modifications. Briefly, root apexes were homogenized in 5% cold perchloric acid (PCA), and incubated on ice for 1 h, and then centrifuged at 12,000 × *g* for 20 min at 4 °C. The pellet was extracted twice with 5% PCA. Then, the supernatants were collected and derivatized using the benzoylation method. A 150 μL aliquot of PCA extracts was mixed with 1 mL of 2 M NaOH, followed by addition of 10 μL benzoyl chloride, vortexing for 20 s, and incubation for 25 min at 37 °C. Then, 2 mL of saturated NaCl was added immediately to stop the reaction. Benzoyl-polyamine derivatives were extracted with 2 mL of diethyl ether. After centrifugation at 1500 × *g* for 5 min, 1 mL of the ether phase was collected, evaporated to dryness, and dissolved in 200 μL of methanol. After filteration through 0.25 μm nylon membranes, 10 μL of the methanol solution of benzoyl polyamines was analyzed using a HPLC system (Agilent 1200, USA) and UV detector under the following conditions: 4.6 mm × 150 mm Elipse XDB–C18 reverse-phase column (Agilent, USA); particle size, 5 μm; column temperature, 30 °C; mobile phase, 65% methanol; flow rate, 0.6 mL min^{-1}; and detection wavelength, 254 nm. Three polyamine standards of Put, Spd, and Spm in the form of hydrochlorides (Sigma) were benzoylated simultaneously.

2.4. Analysis of arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) activities

ADC and ODC were extracted and determined according to the methods described by Palma et al. [44]. Root tips were homogenized in 100 mM potassium phosphate buffer (pH 8.0) containing