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Ameliorative effects of boron on aluminum induced variations of cell wall cellulose and pectin components in trifoliate orange (*Poncirus trifoliate* (L.) Raf.) rootstock☆

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ABSTRACT

Aluminum (Al) phytotoxicity is a major limitation in the production of crops in the soils with pH ≤ 5. Boron (B) is indispensable nutrient for the development of higher plants and B role has been reported in the alleviation Al toxicity. Trifoliate orange rootstock was grown in two B and two Al concentrations. The results of the present study showed that Al toxicity adversely inhibited root elongation and exhibited higher oxidative stress in terms of H_2O_2 and O_2^- under B-deficiency. Additionally, the X-ray diffraction (XRD) analysis confirmed the increase of the cellulose crystallinity in the cell wall (CW). Al-induced remarkable variations in the CW components were prominent in terms of alkali-soluble pectin, 2-keto-3-deoxyoctonic acid (KDO) and the degree of methyl-esterification (DME) of pectin. Interesting, B supply reduced the pectin (alkali-soluble) under Al toxicity. Moreover, the results of FTIR (Fourier transform infrared spectroscopy) and ^{13}C-NMR (^{13}C nuclear magnetic resonance) spectra revealed the decrease of carboxyl groups and cellulose by B application during Al exposure. Furthermore, B supply tended to decrease the Al uptake, CW thickness and callose formation. The study concluded that B could mitigate Al phytotoxicity by shielding potential Al binding sites and by reducing Al induced alterations in the CW cellulose and pectin components.

1. Introduction

Aluminum (Al) is the most abundant element and accounts for about 8% of the total crust (Delhaize and Ryan, 1995). Approximately, 40% of the world and 21% of China's cultivated land is severely affected by Al toxicity (Kochian, 1995). Aluminum ionizes to produce phytotoxic ions (Al^{3+}) that are easily taken up by plant roots in the acidic soil (pH ≤ 5.5) (Kinraide, 1991). Aluminum toxicity not only plays major role in the inhibition of root elongation and restricting plant growth but also creates an obstacle to the uptake of essential nutrients, ultimately reducing crop yields (Ryan et al., 1995; Ryan et al., 2010; Matsumoto and Motoda, 2013). The binding of Al^{3+} to the cell wall (CW) in root cortical and epidermal cells affects the function and structure of CW (Hoson et al., 2003). The CW of roots has been reported as a chief site for the Al accumulation and barrier to Al toxicity (Horst, 1995; Ma, 2007). Several studies have reported the increase of cellulose under Al exposure in pumpkin (Van et al., 1994) and wheat (Hossain et al., 2005). The accumulation of CW cellulose increases the CW thickness, thus reduces CW rigidity and extensibility (Van et al., 1994; Hossain et al., 2005), ultimately resulting in the inhibition of root cell elongation. Additionally, Al toxicity induces the modification of cytoskeletal dynamics, plasma membrane surface, and alteration in the membrane permeability. Excessive reactive oxygen species (ROS) including H_2O_2 and O_2^- accumulation can also be induced as a consequence of Al stress (Sivaguru et al., 2003), leading to cell damage and death. On the contrary, as an Al hyper-accumulator tea plants, Al is bio-energizer and promotes growth (Hajiboland et al., 2013).

The role of Boron (B) has been described in the growth of higher plants and the development of the primary CW (Matoh, 1997). The B cross-links the pectic polysaccharide rhamnogalacturonanII (RG-II) and supports the binding of RG-II-B-RG-IIdimers, thus ensuring stable CW structure (Matsunaga et al., 1997). Previous studies indicated that rhamnolipid galacturonic acid chitosan formed by 12 different sugar residues in different glycosidic bond links, each of which single-stranded RG-IIcontaining two 2-keto-3-deoxyoctonic

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acid (KDO), usually named the KDO as RG-IIcharacteristic glycosyl (Thomas et al., 1989). Thus, KDO in pectin indirectly represents the amount of RG-IIin the CW.

The B plays a key role in the CW structure (Horst et al., 2010; O'Neill et al., 2004), B-deficiency and Al toxicity have similar negative symptoms on root growth elongation. Recently, it has been reported that B supply decreases ROS fluorescence at the root tip (Corrales et al., 2008; Riaz et al., 2018a), indicating elimination of oxidative stress. Furthermore, pectin is a key part of the CW, holding free carboxyl groups that promotes binding of Al (Tabuchi and Matsumoto, 2001; Li et al., 2016). Recent studies have demonstrated that CW pectin content and the degree of methyl-esterification (DME) of pectin are involved in the binding of Al (Li et al., 2009; Li et al., 2016). The cross-linking of RG-IIby B reduces the adsorption of Al^{3+} on pectin and decreases the toxicity of Al to plants (Li and Yu, 2013). Boron alleviates the Al toxicity associated with the pectin and the DME, thus influencing the adsorption and desorption ability of the Al (Horst et al., 2010). The effect of B on Al toxicity has been validated in many aspects, however, due to the complexity action of B on Al, most of the suggested mechanisms are still greatly hypothetical.

China is one of the origins of citrus and has a long history of cultivation. Due to acidity, the Al phytotoxicity is considered a major limitation in citrus production. There is a little information about the resistance of citrus to Al and the mitigation mechanisms of Al toxicity by B supply. Trifoliate orange rootstock is an important rootstock of citrus in China (Wu et al., 2017).

The objective of the present study was to explore, Al-induced alterations in the pectin characteristics, exactly variations on KDO and DME (1), the variations in the structure of CW components by the approaches of ^{13}C-NMR, TEM (Transmission electron microscope) and FTIR (2), and in an attempt to gain a new insight into the mechanisms of B induced alleviation of Al toxicity in the root of trifoliate orange (3).

2. Materials and methods

2.1. Plant material and treatments

The present experiment was conducted in the greenhouse of Huazhong agriculture university, China. The young rootstock of citrus (*Poncirus trifoliate* L. Raf.) was purchased from commercial nursery of Jiangxi (Ganzhou, China). All the selected seedlings were equally vigorous with uniform root length (6.5–7.5 cm), stem height (11–12 cm) and leaves (6–7) before starting the experimental treatments. Prior to transfer to plastic pots, the black pots (4L) were immersed in 1 M HCl and washed with ultrapure water. The modified Hoagland and Arnon (1950) was used with following macro and micronutrients concentrations i.e., 2.00 mM KNO_3; 0.50 mM $MgSO_4$; 1.23 mM $Ca(NO_3)_2$; 0.14 mM Na_2HPO_4; 0.32 mM NaH_2PO_4; and micronutrients: 4.45 μM $MnCl_2$; 0.80 μM $ZnSO_4$; 0.16 μM $CuSO_4$; 0.18 μM Na_2MoO_4; and, 37.40 μM EDTA-Fe. The concentrations of B and Al were selected through preliminary experiments on citrus seedlings. The experimental treatments contained two B levels (i.e., 0.1 (−B) and 10 μM (+B (adequate), as H_3BO_3) and two Al levels [i.e., 0 (−Al) and 1 mM $AlCl_3 \cdot 6H_2O$ (+Al)]. The Al^{3+} activity in the nutrient solution was calculated by the Debye-Hückel Equation (Manov et al., 2002), and the actual Al^{3+} chemical activity in solution was 512.27 μM. The pH of the culture solution was maintained at 4.5 using 0.1 M HCl and 1 M NaOH every day. The nutrient solution containing 0.5 mM $CaCl_2$ was aerated for 20 min at 4 h intervals and renewed once a week, and was supplied at one-quarter-strength for a week, subsequently half strength and finally at the full strength. The experiment was carried out in the complete randomized design with four treatments, and each treatment had four independent replications. The seedlings were grown in the hydroponics for 10 weeks. At the end of the experiment, roots, stem, and leaves were oven-dried at 75 °C until a constant weight and then were ground to fine powdered.

2.2. Hematoxylin staining

The hematoxylin staining was performed in the root tips (0–10 mm) by the Polle et al. (1978) method. Briefly, the root tips were stained in the 0.2% (w/v) hematoxylin (0.02% (w/v) potassium iodide) solution for 15 min. The root tips after washing with distilled water were photographed with a digital camera.

2.3. Histochemical determination of ROS and FDA-PI fluorescence staining

The cell activity was carried out by the FDA-PI fluorescence staining (Widholm, 1972) method. Approximately 10 mm lateral roots of trifoliate orange rootstock were stained with 20 μL FDA (fluorescein diacetate), 30 μL PI (propidium iodide) solution in 3 mL PBS solution for 10 min at room temperature in a dark. Finally, before observing under a fluorescence microscope, the roots were washed with PBS 3 times. The H_2O_2 and O_2^- signals were assessed by the DCF-DA and DHE fluorescent probe, respectively (Rodríguez-Serrano et al., 2006). The root portions were photographed by a stereo fluorescence microscope (Olympus SZX16), and images were exported to Adobe Photoshop CS6 for measuring the fluorescence intensity.

2.4. Callose determination in the roots

For callose determination, the root portions (0–10 mm) were dipped in ethanol (95%) overnight at 4 °C. Then, the root portions were homogenized in NaOH (4 M) and incubated at 80 °C for 20 min. The callose content was determined by fluorescent spectrometer (RF-5301PC) at emission (485/40 nm), an excitation wavelength (400/30 nm) and sensitivity (2) by aniline blue as the color reagent (Harald et al., 1985). The calibration curve was constructed by pachyman (USA) standard.

2.5. Quantification of B and Al

The fine powdered samples of root, stem and leaves were dry-ashed in a muffle furnace at 500 °C for 4 h. The B concentration was measured by the curcumin (Dible et al., 1954) method at 540 nm after digesting in 0.1 M HCl. The Al content was determined by a colourimetric method (You, 1997).

2.6. Extraction of CW materials

The CW was extracted according to Hu and Brown (1994). Briefly, samples of root and leaves were homogenized in liquid nitrogen and centrifuged at 3000 × g for 10 min. The supernatant was discarded and the pellet was washed with 80% ethanol (three times), methanol: chloroform mixture (1:1, v/v) (once), finally washed with acetone. The final insoluble pellet was dried and defined as CW material and stored for subsequent analysis of CW components (pectin, KDO, FTIR and XRD analysis).

2.7. Analysis of root CW pectin and its components

Root CW material was employed to quantity pectin by Redgwell and Selvendran (1986) method. The chelator soluble and alkali-soluble pectin were extracted with 50 mM imidazole solution (pH 7.0) and 50 mM Na_2CO_3 "containing 20 mM CDTA, trans-l, 2-