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Boron reduces aluminum-induced growth inhibition, oxidative damage and alterations in the cell wall components in the roots of trifoliate orange

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ABSTRACT

Aluminum (Al) toxicity is a major restriction for crops production on acidic soils. The primary symptom of aluminum toxicity is visible in the roots of plants. Recently, several studies reported the alleviation of Al toxicity by the application of Boron (B), however, the information how B alleviates Al toxicity is not well understood. Thus, we investigated the ameliorative response of B on Al-induced growth inhibition, oxidative damages, and variations in the cell wall components in trifoliate orange roots. The results indicated that plants under Al stress experienced a substantial decrement in root length and overall plant growth. The supply of B improved the root elongation by eliminating oxidative stress, membrane peroxidation, membrane leakage, and cell death produced under Al toxicity. Moreover, accumulation of Al on the cell wall and alteration in the cell wall components might be one of the causes resulting in the quick inhibition of root elongation under B-starvation circumstances by providing susceptible negative charges on pectin matrix for binding of Al. The results provide a useful understanding of the insight into mechanisms of B-induced mitigation of Al toxicity especially in the trifoliate orange that might be helpful in the production of crops on acidic soils.

1. Introduction

In acidic soils, aluminum (Al) toxicity is one of the major limiting causes for crop development and productivity around the world, and approximately 50% of the world's total land area comprises of soils pH ≤ 5 (Von Uexküll and Mutert, 1995). In plants, aluminum toxicity results in the rapid interruption of root elongation, which can be noticed within a short period after exposure to Al (Llugany et al., 1995). Cell elongation and cell division contribute to the root developmental processes, and Al stress can influence both processes. However, the interruption of cell elongation rather than cell division is considered a major cause of root growth inhibition (Horst and Klotz, 1990). The primary targets of Al stress are the roots, particularly the root tips (Ryan et al., 1993), later, the commencement of the partial or complete inhibition of main root and lateral root growth. Root apices comparatively accumulate more Al than any other parts of the roots particularly under retarded root growth condition (Kochian, 1995). Aluminum stress accumulates reactive oxygen species (ROS) including H_2O_2 and O_2^-, enhanced production of ROS ultimately results in the oxidative damages and thus root inhibition (Jones et al., 2006). The accumulation of H_2O_2 and O_2^- not only induces lipid peroxidation of membranes, DNA damage but also cell death by interrupting intracellular process (Pan et al., 2001). In contrast, in hyper-accumulator tea plants, aluminum is bio-stimulant and promotes growth (Hajiboland et al., 2013). However, a higher concentration of aluminum can lead to negative growth defects, creating a serious threat to the production of high economic crops (Kochian et al., 1995). Higher accumulations of aluminum in the tea can circulate into the food chain and could be a possible threat to humans and animals (Ashenef, 2014).

Boron (B) is a micro-nutrient, and indispensable element for higher plants in maintaining the proper development and expansion of tissues (Loomis and Durst, 1992). Boron insufficiency culminates in the cessation of root growth. The major role of B is related to the primary cell wall synthesis, where it cross-links the pectic polysaccharide RG-II(rhamnogalacturonan II) (Brown and Hu, 1997; O'neill et al., 2001). Boron is typically present as boric acid [$B(OH)_3$] in the soil solution (Shorrocks, 1997), and is susceptible to be leached in a condition of high rainfall (Yan et al., 2006). The acidic condition eventually leads to B deficiency in plants; subsequent variations on cell wall structure with transformed physical characteristics (Ryden et al., 2003), and excessive accumulation of pectin and demethylesterified pectin (Yu et al., 2002). Some studies have reported that B is an essential part of cytoskeleton

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and plasma membranes, and maintains their structure and functions (Cakmak and Römheld, 1997; Brown et al., 2002).

Several reports have proposed that apoplastic binding of Al (especially pectin matrix) is the chief cause of Al toxicity (Chang et al., 1999). Al^{3+} ions (polyvalent cation) quickly and tightly bind to unmethylated pectin (Blamey et al., 1990). The demethylesterified pectin bears negative charged binding places and the binding of Al to the cell wall of roots consequently undergoes root injury, oxidative stress and poor uptake of nutrients. In barley roots, 85 − 90% of the total accumulated Al was reported to be linked with cell wall (Clarkson, 1967) resulting in the modification of the cell wall composition through inducing variations in the cell-wall polysaccharides. Al stress induces the rigidity and thickness of cell wall through the accumulation of the hemicellulose and polysaccharides. Long-term exposure to aluminum provokes lipid peroxidation and imbalanced antioxidant enzyme activities through the excessive buildup of oxidative stress (Cakmak and Horst, 1991). It has been reported that pectin, Al accumulation, and oxidative stress exhibit close relationship with the extent of Al toxicity (Schmohl and Horst, 2000). Cell wall pectin is comprised of glucuronic acid units and is a complex polysaccharide. The methyl-esterification of pectin and carboxylic groups present on the pectin regulate the negatively charged sites and eventually determines CEC of the cell wall and the amount of Al^{3+} it can hold tightly (Eticha et al., 2005). It is still not clear how Al toxicity induces root inhibition, and it is needed to be explored (Horst, 1995).

Boron induced alleviation of Al toxicity has long been confirmed in several reports (Ruiz et al., 2006; Li et al., 2017; Yan et al., 2018) by reduction uptake and mobilization of Al ions and eliminating oxidative stress and root injuries (Stass et al., 2007). Other studies also proposed that improved physiological mechanisms also contribute an important part in Al tolerance (Jiang et al., 2009; Yu et al., 2009). Al-stressed and B-starved plants display same symptoms of stress on root growth, so, it was suggested that Al might induce B deficiency, and an increased amount of B could improve Al toxicity (Lukaszewski and Blevins, 1996; LeNoble et al., 1996a, 1996b; Blevins and Lukaszewski, 1998). Until now, this statement has been verified in many studies. If this hypothesis could be confirmed in citrus under acidic soils, B would be a practical candidate for the improvement of soil acidity and subsequently Al toxicity, since B addition is comparatively less expensive than existing approaches of soil-acidity amendments (calcium carbonate, liming). There are several agronomic practices that are supposed to alleviate Al toxicity including liming by raising soil pH. But this method requires time and is costly, also not considered effective for the subsoil acidity. Increasing pH might interfere with the uptake of essential nutrients (Alva et al., 1986).

In China, citrus is an important fruit crop and usually cultivated in the acidic soils particularly in the areas of Jiangxi, Ganzhou (planting area ranks first in the world), where the low pH (82.1% acid soil, pH ≤ 5), B deficiency and Al toxicity disorders substantially affected the growth and yield of citrus (Han et al., 2008). Trifoliate orange is an important rootstock in China. None of the reports provides enough information on the influence of B in the amelioration of Al toxicity. The aim of this study was to investigate the ameliorative effect of B on Al-induced growth inhibition, oxidative damages, and variations in the cell wall components in the roots of trifoliate orange seedlings.

2. Materials and methods

2.1. Plant material and growth conditions

The present study was carried out at "Huazhong Agricultural University", Wuhan. The seeds of the trifoliate orange (*Poncirus trifoliata* L. Raf.) were cultured under control conditions. The seeds of trifoliate were obtained from the commercial fruit nursery of Ganzhou, Jiangxi. The seeds were immersed in water after surface sterilizing for 1 min in diluted (3–7% active chloride) sodium hypochlorite. The seeds were germinated in an incubator at 27 °C between two wet layers of clothes. After one week of emergence of 2–3 new leaves, the uniform size trifoliate orange seedlings with 4–5 cm root length and 10–12 cm stem length were randomly chosen and transferred to 18-L black pots containing macro and micronutrients solution of the following composition; 2 mM KNO_3, 0.10 mM K_2HPO_4, 0.10 mM $MgSO_4$, 1.23 mM Ca $(NO_3)_2$, 4.45 μM $MnCl_2$, 0.16 μM $CuSO_4$, 0.18 μM Na_2MoO_4, 0.8 μM $ZnSO_4.7H_2O$, and 13 μM Fe-EDTA. The orange seedlings were cultured under control conditions at 23/21 °C day/ night temperature, 16/8 h of day/night cycle and 75% relative air humidity. The experiment comprised of two Al (0 and 300 μM as $AlCl_3{\cdot}6H_2O$) and two B (0 and 10 μM as H_3BO_3) treatments. Boron and Al concentrations were selected through a pre-experiment on citrus seedlings. The nutrient solution pH was maintained at 4.0 with 1 *M* NaOH and 0.1 *M* HCl every day. Free Al^{3+} activity was estimated by a GeoChem-PC v1.0 speciation software package. The nutrient solution was changed after every 3 days, and aeration was provided for 20 min after every 4 h intervals. After 60 days (when seedlings showed a reasonable growth and some visual symptoms of Al toxicity), the seedlings were harvested and all parts of the plant were washed with deionized water for the quantification of cell wall components, ROS (reactive oxygen species), B and Al concentrations. The root elongation and histochemical analysis were estimated at 15, 30, 45, and 60 days intervals (DAT) after the initiation of B and Al treatments. In order to measure the dry weight, the rest of samples were placed in an oven at 70 °C until a constant weight. The experiment was laid out in a completely randomized design (CRD) with two B and two Al treatments, and each treatment had 4 independent replications. For easily elaborating analysis results, the B and Al treatments were grouped as B-Al- (0 μM B and 0 μM Al), B-Al+ (0 μM B and 300 μM Al), B + Al- (10 μM B and 0 μM Al), and B + Al + (10 μM B combined with 300 μM Al). The B + Al- treatment was considered control treatment (CK).

2.2. Extraction of cell wall

The root cell wall (CW) was extracted as reported by Wu et al. (2017) method. The root segments (100 root segments (0–10 mm) from each replication) were homogenized into liquid N_2 with pestle and mortar. The homogenized roots were washed with ice-cold water. The resulting residue was then washed with 80% ethanol, (once) methanol: chloroform mixture, and finally washing was carried out with acetone. After each step, the centrifugation process was conducted at 10,000 × *g* for 10 min. The cell wall insoluble pellet was regarded as CWM and was dried at 75 °C before being weighed, and some part of it was stored at 4°C for future usage. The CWM was employed for further analysis of B, Al, and fractionation of cell wall components.

2.3. Quantification of cell wall pectic and degree of methyl-esterification of pectin

The extracted cell wall (CW) from trifoliate orange roots was employed for the assessment of pectin by the procedure of Blumenkrantz and Asboe-Hansen (1973) with slight modifications. The CWM was treated with acetate buffer (pH 6.50, .05 *M*) containing 20 mM CDTA (trans-l, 2 diaminocyclohexane-N, N, N', N' tetraacetic acid) in a horizontal shaker for 12 h at 25 °C. The resultant homogenate was subjected to centrifugation process at 10,000 × *g* for 12 min at 4 °C, and then washed with cold ion-exchanged water. The CW was used to estimate the uronic acid (UA). The galacturonic acid was utilized for constructing a standard curve. The degree of methyl esterification of pectin (DM) was measured by Anthon and Barrett (2004) method with slight alterations. The preparation of reagents and stock solutions were as follows; the cell wall pectin material was treated with NaOH solution at 25 °C for 30 min subsequently by treating with (0.75) H_2SO_4, (3 mg ml^{-1}) MBTH, (0.2 M) Tris-HCl, and alcohol-oxidase, for 20 min at 30 °C. Finally, ammonium ferric sulfate and sulfaminic acid were used