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Boron deficiency is correlated with changes in cell wall structure that lead to growth defects in the leaves of navel orange plants

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ABSTRACT

Boron (B) is an essential microelement for vascular plants. Although it has frequently been reported that B deficiency leads to abnormal cell wall structure based on microscopic observation, what exactly occurs in the architecture of cell wall under this condition remains unknown. Navel orange plants that had been treated with different amounts of B were studied through chemical and instrumental (X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectroscopy (FTIR)) analyses. Curling of the leaves and leaf chlorosis were observed only in the upper leaves of B-deficient plants. Boron deficiency significantly increased the relative hemicellulose and cellulose concentrations, and decreased covalently bound pectin in both upper and lower leaves. The results from XPS spectra suggested that the chemical states of carbon and oxygen were changed by B deficiency, and these changes were more serious in the upper leaves. The band at 3417 cm^{-1} in the upper leaf walls shifted to 3398 cm^{-1} due to B deficiency, suggesting that the mode of hydrogen bonding was changed by B deficiency (only in the upper leaves). The intensity and shape of the vibrations at 1200–900 cm^{-1} (the fingerprint region of polysaccharides) varied substantially between B-deficient plant cell walls and the control walls, indicating that B deficiency induced changes in both the amount and assembly of component polymers of cell wall. These results imply that the amount of wall components is not decisive for B deficiency symptoms in orange plants, but that rather structural changes within these fractions are important.

1. Introduction

Boron (B) is an essential microelement for vascular plants, and its deficiency causes various metabolic disorders and growth defects, mainly in young and growing portions of plants (Loomis and Durst, 1992). In China, B deficiency is frequently observed in citrus orchards, and is responsible for considerable loss of productivity and quality (Yang et al., 2013; Han et al., 2008). As one of the most widespread deficiencies among plant micronutrients in agriculture (Shorrocks, 1997), B deficiency causes a wide range of symptoms including cessation of root elongation, reduced leaf expansion, death of growing meristems, diminished photosynthesis, inhibition of flower development, decreased fruit and seed set, and male sterility, depending on the plant species and developmental stage (Dell and Huang, 1997; Goldbach et al., 2001). Boron deficiency also causes a wide array of physiological and biochemical changes in cell wall structure, membrane integrity and function, enzyme activity, and a wide range of changes in plant metabolites such as phenolics, ascorbate and glutathione (Brown et al., 2002; Dordas and Brown, 2005).

The role of B as a structural element of plant cell walls and its implications for plant growth have been well investigated. It has been discovered that most of the B in the wall is present as a borate diol diester, which cross-links two chains of rhamnogalacturonan II (RG-II), suggesting that a physiologically important function of B is to cross-link cell wall pectin (Ishii and Matsunaga, 1996; Kobayashi et al., 1996). Such a cross-linked pectic network is likely to play a role in regulating the mechanical and biochemical properties of the wall (Fleischer et al., 1999). Boron cross-linking with RG-II in cell walls increases the mechanical strength of the cell wall and maintains turgor-driven plant cell growth (O'Neill et al., 2001). The results of numerous studies have shown that B deficiency results in changes in cell wall structure, e.g. swelling of the cell walls and the formation of small and irregularly shaped cells (Brown and Hu, 1997; Matoh, 1997; Ishii et al., 2001).

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Cell walls are important for the structural integrity of the cell and, indeed, for the whole plant. They determine the shape and size of the cells as well as other important properties such as texture, mechanical strength, resistance to pathogenic microorganisms, and the capacity to bind and sequester toxic ions and molecules (Held et al., 2011; Hayot et al., 2012). The plant cell wall is a highly complex and dynamic structure made up of cellulose, hemicelluloses, pectins, lignin, and incrusting substances such as cutin, suberin, wax, and numerous proteins and inorganic molecules (Showalter, 1993). Although it has frequently been reported that boron deficiency leads to abnormal cell wall structure based on microscopic observation, what exactly occurs in the architecture of cell wall under this condition remains unknown.

X-ray photoelectron spectroscopy (XPS) is a surface analytical method that provides quantitative elemental analysis of all elements (except H and He) within a few surface nanometres of materials (Matuana et al., 2001). In addition to XPS's ability to recognize elements, the binding energies in the XPS spectrum yield information on the chemical state of the element as well. Although the chemical bond contributions are included in the same peak, they can be deconvoluted using Gaussian or Lorentzian (or combined) peak fit. The ratios of the deconvoluted peak areas correspond to the ratios of the bonds in atoms (Kontturi, 2005). Therefore, XPS can be used to examine the chemical states of carbon and oxygen in B-deficient plant cell walls.

Fourier-transform infrared spectroscopy (FTIR) can detect molecular vibration and provide information about the chemical composition of virtually all substances. It has been extensively applied in plant cell wall analyses (Séné et al., 1994; Chen et al., 1998). Mid-infrared spectroscopy detects, within underivatized cell walls, the vibrations of all molecular bonds, for which the component atoms differ in electronegativity, including asymmetric bonds such as C—H and O—H and particular functional groups, such as esters, amides, and carboxylates (McCann et al., 2007). However, very few studies have used FTIR to examine changes in plant cell wall structure due to B deficiency. FTIR has proven to be useful in studying compositional changes in plant cell walls during development (McCann et al., 1997) and upon exposure to organic contaminants (Dokken et al., 2002). Therefore, it can potentially be used to determine changes in cell wall architecture induced by B deficiency.

In spite of a wealth of information about the polysaccharide structures in plant walls in general (Cosgrove, 2005), very little is known about how the various components respond to B deficiency and whether the changes in wall structure are associated with the occurrence of B deficiency symptoms. Interest in the significant role of B in cell walls over the past decade demands appropriate methods to study the composition of wall polysaccharides in plants. In this study, we used XPS and FTIR in combination with chemical analysis to study structural changes and gain novel insight into cell wall structure and composition in navel orange leaves collected at different stages of growth after B deprivation.

2. Materials and methods

2.1. Plant materials and growth conditions

We used 8-month-old navel orange plants (*Citrus sinensis* cv. Newhall) grafted on Carrizo citrange (*Citrus sinensis* (L.) Osb. × *Poncirus trifoliate* (L.) Raf.) rootstock with uniform stem diameter (6–7 mm), height (18–22 cm) and total fresh weight (20–25 g). The selected plants all consisted of only one main shoot of the scion and had 12–15 leaves, which were not fully expanded at the beginning of the experiment.

All the plants were washed with distilled water to remove surface contaminants after soaking in tap water for 2 days, followed by transplantation to black pots (one plant per pot), each containing 8-L of nutrient solution. Prior to the experiment, the black pots were immersed in 1 mol L^{-1} HCl and washed with distilled water. Modified from Hoagland and Arnon (1950), the composition and salt contents of the basal culture solution were 1 mM KNO_3, 1.23 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.07 mM Na_2HPO_4, 0.16 mM NaH_2PO_4, 4.45 μM $MnCl_2$, 0.8 μM $ZnSO_4$, 0.16 μM $CuSO_4$, 0.18 μM Na_2MoO_4, and 28.7 μM Fe-EDTA. The nutrient solution was used at half strength for the first 7 days with 10 μM H_3BO_3. Subsequently, the nutrient solution was enhanced to the full strength with 20 μM H_3BO_3 (control plants) and 0 μM H_3BO_3 (B deficiency treatment). The solution was aerated for 10 min at 4 h intervals and replaced once a week. The pH of the nutrient solution was adjusted to 5.8–6.2 every day with 0.5 M H_2SO_4 or 1 M NaOH. Analytical-grade reagents were used to prepare nutrient solutions. Purified water was obtained by a system consisting of three units (active charcoal, ion exchanger, and reverse osmosis), and had an electric conductivity lower than 0.06 μS cm^{-1} and B concentration less than 0.5 μmol L^{-1}.

The experiment had a completely randomized design with the two treatments replicated nine times and each replication containing one plant. These plants were placed in April in a greenhouse under natural sunlight conditions at Huazhong Agricultural University, Wuhan, China.

2.2. Plant sampling and B analysis

Some new leaves became visible 10–15 days after the commencement of B treatments, and all of those were fully expanded at harvest when the experiment was terminated after 9 weeks. All leaves from each plant were further separated into lower leaves (existing before the experiment) and upper leaves (appearing after the experiment began). The leaves were oven-dried at 70 °C for 3 days, ground to fine powder, and stored in an air-tight glass container for subsequent analyses. To measure B content in leaves, finely powdered leaf samples were dry-ashed at 500 °C for 4 h, and then suspended in 0.1 mol L^{-1} HCl, followed by determination of B concentrations using the curcumin colorimetric method (Hitachi UV-3100 UV/VIS) (Dible et al., 1954).

2.3. Preparation of cell wall materials (CWM)

The cell wall was separated from the fresh leaves using the method described by Hu and Brown (1994). The leaf samples were ground into fine powder in liquid nitrogen with a mortar. The powder was then homogenized with 10 volumes of ice-cold water. The homogenate was centrifuged at 1000 *g* for 10 min. The precipitate was then washed with 10 volumes of ice-cold water and re-centrifuged. The residue was washed three times with 10 volumes of 80% ethanol and once with 10 volumes of a methanol:chloroform mixture (1:1, v/v). Finally, the precipitate was washed with 10 volumes of acetone. The final insoluble pellet was defined as CWM. The CWM samples were dried and weighed. Some of the dry CWM was ashed at 500 °C for cell wall B determination following the procedure above. Some was used as follows for fractionation of cell wall polysaccharides and determination of lignin. The rest of CWM was used for XPS and FTIR analysis.

2.4. Fractionation of cell wall polysaccharides

Sequential extraction of CWM was undertaken as described by Redgwell and Selvendran (1986) with slight modifications. Approximately 200 mg of CWM was suspended in 20 ml of 50 mM CDTA (trans-l, 2-diaminocyclohexane-N,N,N,N-tetraacetic acid, pH 6.5)