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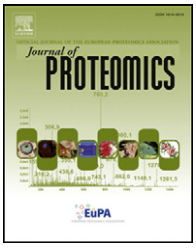

An investigation of boron-toxicity in leaves of two citrus species differing in boron-tolerance using comparative proteomics

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ABSTRACT

Limited data are available on boron (B)-toxicity-responsive proteins in plants. We first applied 2-dimensional electrophoresis (2-DE) to compare the effects of B-toxicity on leaf protein profiles in B-tolerant *Citrus sinensis* and B-intolerant *Citrus grandis* seedlings, and identified 27 (20) protein species with increased abundances and 23 (25) protein species with decreased abundances from the former (latter). Generally speaking, B-toxicity increased the abundances of protein species involved in antioxidation and detoxification, proteolysis, cell transport, and decreased the abundances of protein species involved in protein biosynthesis in the two citrus species. The higher B-tolerance of *C. sinensis* might include following several aspects: (a) protein species related to photosynthesis and energy metabolism in *C. sinensis* leaves were more adaptive to B-toxicity than in *C. grandis* ones, which was responsible for the higher photosynthesis and for the better maintenance of energy homeostasis in the former; and (b) the increased requirement for detoxification of reactive oxygen species and cytotoxic compounds due to decreased photosynthesis was less in B-toxic *C. sinensis* leaves than in B-toxic *C. grandis* ones. B-toxicity-responsive protein species involved in coenzyme biosynthesis differed between the two species, which might also contribute to the higher B-tolerance of *C. sinensis*.

Biological significance
B-toxicity occurs in many regions all over the world, especially in arid and semiarid regions due to the raising of B-rich water tables with high B accumulated in topsoil. In China, B-toxicity often occurs in some citrus orchards. However, the mechanisms of citrus B-tolerance are still not fully understood. Here, we first used 2-DE to identify some new B-toxicity-responsive-proteins involved in carbohydrate and energy metabolism, antioxidation and detoxification, signal transduction and nucleotide metabolism. Our results

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showed that proteins involved in photosynthesis and energy metabolism displayed more adaptive to B-toxicity in B-tolerant *C. sinensis* than in B-intolerant *C. grandis*, which might play a key role in citrus B-tolerance. Therefore, our results reveal some new mechanisms on plant B-response and tolerance.

1. Introduction

Boron (B) is an essential element for higher plants. While of lesser prevalence than B-deficiency, B-toxicity occurs in many regions all over the world, especially in arid and semiarid regions due to the raising of B-rich water tables with high B accumulated in topsoil [1].

B-toxicity affects plant growth. The typical symptom of B-toxicity in mature leaves is chlorosis and/or necrosis at the margins or tips or both [2]. Reid et al. [3] showed that the toxic effects of B on mature barley (*Hordeum vulgare*) leaves were caused by the accumulated retardation of many cellular processes, intensified in light by photo-oxidative stress, rather than by the disruption of a single process. Landi et al. [4] observed that B-toxic zucchini (*Cucurbita pepo*) and cucumber (*Cucumis sativus*) had increased activities of antioxidant enzymes, non-photochemical quenching (NPQ), concentration of malondialdehyde (MDA) and ratios of oxidized glutathione (GSSG)/reduced glutathione (GSH) and dehydroascorbate (DHA)/ascorbate (ASA), concluding that the up-regulation of both antioxidant enzymes and NPQ is not sufficient to cope with the negative effect caused by B-toxicity.

The mechanisms for B-tolerance in higher plants are not well elucidated yet. Different strategies exist to deal with B-toxicity depending on plant species/cultivars. Huang et al. [5] found that B-tolerant *Citrus sinensis* seedlings had lower leaf free B and higher bound B than B-sensitive *Citrus grandis* ones in response to B-toxicity, which might contribute to the higher B-tolerance of the former. Hamurcu et al. [6] reported that high B concentration in soils increased the activities of antioxidant enzymes due to a combination of activation of existing isoenzymes and newly induced isoenzymes in soybean (*Glycine max*) leaves, which might contribute to B-tolerance by reducing the level of lipid peroxidation. However, Karabal et al. [7] reported that reactive oxygen species (ROS) was not involved in B-toxicity-induced membrane damage in barley leaves. Schnurbusch et al. [8] showed that B-toxicity-induced decrease in the expression of the multifunctional aquaporin *HvNIP2;1* gene was responsible for barley B-tolerance. By contrast, transgenic *Arabidopsis* over-expressing a tonoplast aquaporin *AtTIP5;1* gene displayed enhanced B-tolerance [9]. Also, various efflux B transporters have been shown to be involved in plant B-tolerance [10–12].

Proteomics is a very powerful tool to investigate the complex responses of higher plants to environmental stresses. The aspect of mineral nutrient (i.e., Fe and B) deficiencies is an emergent application of proteomics in citrus plants [13,14]. To our best knowledge, limited data are available on B-toxicity-responsive proteins in plants. Using 2-dimensional electrophoresis (2-DE), Atik et al. [15] identified seven protein species with increased abundances including a vacuolar proton-translocating ATPase (V-ATPase) subunit E, from B-toxic barley leaves, concluding that V-ATPase subunit E might play a role in B-tolerance. Demiray et al. [16] used 2-DE to isolate six differentially abundant protein species from B-toxic callus of carrot (*Daucus carota*) roots. Patterson et al. [17] used isobaric tags for relative and absolute quantitation (iTRAQ) to compare the abundances of protein species from B-tolerant and -intolerant barley, and isolated 341 and 138 differentially abundant protein species from roots and leaves, respectively. Unfortunately, they did not investigate the effects of B-toxicity on proteomics. In summary, very little is known about B-toxicity-responsive proteins in woody plants.

In China, B-toxicity often occurs in some citrus orchards. During 1998–1999, Huang et al. [18] reported that up to 17.0% and 61.5% of 'Guanximiyou' pummelo (*C. grandis*) orchards in Pinghe, Zhangzhou, China were excess in soil water-soluble B and leaf B, respectively. Citrus B-deficiency rather than B-toxicity was extensively investigated by previous workers [19–23]. Limited studies investigated the effects of B-toxicity on citrus. For instance, it was found that B-toxicity affected nutrient efficiency and distribution in citrus [24]. Han et al. [25] demonstrated that B-toxicity reduced chlorophyll (Chl) concentration, CO_2 assimilation, photosynthetic enzyme activities and electron transport capacity, which might be caused by B-toxicity-induced oxidative damage in *C. grandis* leaves. Also, B-toxicity affected citrus root and leaf anatomy, particularly in B-intolerant species [5,26]. Keles et al. [27] demonstrated the vital role of ASA in protecting citrus leaves from B-toxicity. Cañon et al. [28] showed that *CmBOR1*, a B transporter isolated from *Citrus macrophylla*, conferred B-tolerance in a yeast complementation assay, while functional analysis in *Arabidopsis* indicated that it was a boric acid/borate transporter involved in plant tolerance to B-deficiency. Recently, we used cDNA-AFLP to assay the effects of B-toxicity on gene expression profiles in citrus leaves [29]. Here we investigated the effects of B-toxicity on leaf CO_2 assimilation, B concentration and protein profiles using 2-DE in B-intolerant *C. grandis* and B-tolerant *C. sinensis* seedlings [5] in order to understand the tolerant mechanisms of citrus plants to B-toxicity at translational level.

2. Materials and methods

2.1. Plant materials and culture

This study was conducted at Fujian Agriculture and Forestry University, Fuzhou, China. Plant culture and B treatments were performed according to Guo et al. [29]. Briefly, five week-old seedlings of 'Xuegan' (*C. sinensis*) and 'Sour pummelo' (*C. grandis*) were transplanted to 6 L pots (two per pot) containing river sand and grown in a greenhouse under natural photoperiod. Eight weeks after transplanting, each pot was supplied every other day until dripping with nutrient solution containing 10 μM (control) or 400 μM (B-toxic) H_3BO_3 for 15 weeks. At the end of