JOURNAL OF PROTEOMICS XX (2013) XXX–XXX

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot

iTRAQ protein profile analysis of *Citrus sinensis* roots in response to long-term boron-deficiency☆

Lin-Tong Yang[a,b], *Yi-Ping Qi*[c], *Yi-Bin Lu*[a,b], *Peng Guo*[b,d], *Wen Sang*[b,d], *Hui Feng*[a,b], *Hong-Xing Zhang*[b,d], *Li-Song Chen*[a,b,d,e,*]

[a]*College of Resources and Environmental Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China*
[b]*Institute of Horticultural Plant Physiology, Biochemistry and Molecular Biology, Fujian Agriculture and Forestry University, Fuzhou 350002, China*
[c]*Institute of Materia Medica, Fujian Academy of Medical Sciences, Fuzhou 350001, China*
[d]*College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou 350002, China*
[e]*Fujian Key Laboratory for Plant Molecular and Cell Biology, Fujian Agriculture and Forestry University, Fuzhou 350002, China*

ARTICLE INFO

Keywords:
Boron-deficiency
Citrus sinensis
iTRAQ
Proteomics
Respiration
Root

ABSTRACT

Seedlings of *Citrus sinensis* were fertilized with boron (B)-deficient (0 μM H_3BO_3) or -sufficient (10 μM H_3BO_3) nutrient solution for 15 weeks. Thereafter, iTRAQ analysis was employed to compare the abundances of proteins from B-deficient and -sufficient roots. In B-deficient roots, 164 up-regulated and 225 down-regulated proteins were identified. These proteins were grouped into the following functional categories: protein metabolism, nucleic acid metabolism, stress responses, carbohydrate and energy metabolism, cell transport, cell wall and cytoskeleton metabolism, biological regulation and signal transduction, and lipid metabolism. The adaptive responses of roots to B-deficiency might include following several aspects: (*a*) decreasing root respiration; (*b*) improving the total ability to scavenge reactive oxygen species (ROS); and (*c*) enhancing cell transport. The differentially expressed proteins identified by iTRAQ are much larger than those detected using 2D gel electrophoresis, and many novel B-deficiency-responsive proteins involved in cell transport, biological regulation and signal transduction, stress responses and other metabolic processes were identified in this work. Our results indicate remarkable metabolic flexibility of citrus roots, which may contribute to the survival of B-deficient plants. This represents the most comprehensive

Abbreviations: ABI3, abscisic acid insensitive 3; ACO, aconitase; AIP3, ABI3-interacting protein 3; ALDH, aldehyde dehydrogenase; APX, ascorbate peroxidase; B, boron; BTF3, basic transcription Factor 3; CBS, cystathionine-β-synthase; CS, citrate synthase; DHN, dehydrin; G6PDH, glucose-6-phosphate dehydrogenase; GPx, glutathione peroxidase; GR, glutathione reductase; GRF, general regulatory factor; GS, glutamine synthetase; KH, K-homology; LEA, late embryogenesis abundant; MBF1, multiprotein bridging factor 1; MDAR, monodehydroascorbate reductase; MSR, methionine sulfoxide reductase; NAC, nascent polypeptide-associated complex; NIP, nodulin 26-like intrinsic protein; PDI, protein disulfide isomerase; 6PGDH, 6-phosphogluconate dehydrogenase; PIP, plasma membrane intrinsic protein; PK, pyruvate kinase; PM, plasma membrane; POD, peroxidase; PPR, pentatricopeptide repeat; PR, pathogen-related; ROS, reactive oxygen species; SBP2, selenium-binding protein 2; SFGH, S-formylglutathione hydrolase; SIP, small basic intrinsic protein; SOD, superoxide dismutase; TF, transcription factor; TIP, tonoplast intrinsic protein; TPR, tetratricopeptide repeat; TRX, thioredoxin; UGPase, UDP-glucose pyrophosphorylase; VDAC, voltage-dependent anion channel

☆ This article is part of a Special Issue entitled: Translational Plant Proteomics.

* *Corresponding author at:* College of Resources and Environmental Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China. Tel.: +86 591 83854966; fax: +86 591 83727618.
E-mail address: lisongchen2002@hotmail.com (L.-S. Chen).

1874-3919/$ – see front matter
http://dx.doi.org/10.1016/j.jprot.2013.04.025

Please cite this article as: Yang L-T, et al, iTRAQ protein profile analysis of *Citrus sinensis* roots in response to long-term boron-deficiency, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.04.025

analysis of protein profiles in response to B-deficiency. This article is part of a Special Issue entitled: Translational Plant Proteomics.

Biological significance
In this study, we identified many new proteins involved in cell transport, biological regulation and signal transduction, stress responses and other metabolic processes that were not previously known to be associated with root B-deficiency responses. Therefore, our manuscript represents the most comprehensive analysis of protein profiles in response to B-deficiency and provides new information about the plant response to B-deficiency.

1. Introduction

Boron (B) has been known as an essential element required for the normal growth and development of higher plants as early as 1923 [1]. B-deficiency is a widespread problem in many agricultural crops, including citrus. Over 132 crops are susceptible to B-deficiency, and low B availability in soils inhibits vegetative and reproductive growth in a large number of crops [2]. In citrus, Haas first found that B is an essential element required for its normal growth and development by the use of sand cultures [3]. In China, B-deficiency is frequently observed in citrus orchards, and is responsible for loss of productivity and poor fruit quality [4,5].

B-deficiency in higher plants causes adverse effects on cellular functions and physiological processes [5–10]. B-deficiency symptoms mostly start in the actively growing parts of plants [7,9] as B is phloem immobile in many plant species, including citrus [11]. Root growth is more sensitive to B-deficiency than shoot growth. In higher plants, the most rapid response to B-deficiency is the inhibition or cessation of root elongation in both the main and lateral roots [7]. To cope with B-deficiency, plants have evolved a considerable degree of developmental plasticity, including adaptation *via* cascades of molecular networks. Increasing evidence has shown that B-deficiency affects the expression levels of genes related to B uptake and translocation [12–14], cell wall and membranes [16–18], N metabolism [17,19,20], stress responses [21,22], nucleic acid metabolism, signal transduction, and cell cycle [23]. While these data are very useful, the levels of mRNAs do not necessarily correspond directly to the levels of their corresponding proteins [24,25]. The level of a protein depends not only on transcription rates of the gene, but also on nuclear export and mRNA localization, transcript stability, translational regulation and protein degradation. Moreover, many differential effects on proteins themselves mainly come from PTMs such as glycosylation and phosphorylation or proteolytic cleavage [24,27]. Therefore, there is considerable variability on protein level *versus* mRNA level. Since biological processes are ultimately controlled by proteins, interrogation of B-deficiency-induced changes of the proteome is necessary to understand the adaptive mechanisms of plants to long-term B-deficiency.

While the transcriptional response of plants to B-deficiency has been examined in some detail [8,17,18,23], limited information is available with regard to the changes of protein profile upon B-deficiency. Analysis of *Lupinus albus* leaf apoplastic proteins and pea (*Pisum sativum*) root nodule proteins showed that B-deficiency induced the production of pathogen-related (PR)-1 like protein, β-1,3-glucanases, class III chitinases, thaumatin like protein, expansin-like protein [28], abscisic acid-responsive 17 (ABR17) and PR10.1 proteins [29]. In B-deficient *Brassica napus* roots, over 45 differentially expressed proteins related to carbohydrate and energy metabolism, stress responses, signaling and regulation, amino acid and fatty acid metabolism, nucleic acid metabolism, protein translation and degradation, cell wall structure, and transporter were identified [25,26]. Alves et al. identified 128 B-deficiency-responsive polypeptides related to cell wall metabolism, cell structure, defense, energy pathways and protein metabolism in *L. albus* roots [30]. Previously reported proteomic studies on plants in response to B-deficiency were limited to 2D gel electrophoresis analysis. Too large/small, too acidic/basic and too hydrophobic proteins, however, are difficultly observed on the 2D gel electrophoresis [27,31]. In addition, the method often falls to identify the low-abundance proteins such as membrane proteins, since the dynamic range is somewhat limited [27,32]. To overcome some of the shortcomings of the above technique, the non-gel-based quantitative proteomic methods have been established in recent years [27]. The iTRAQ-based quantitative proteomic approach was first reported by Ross et al. [33]. The advantages of the approach are that it can simultaneously identify and quantify proteins from multiple samples, while retaining important PTM information and it is unbiased toward these proteins not amenable to 2D gel technique.

In this study, we compared quantitative and qualitative changes in proteomes that occurred in B-deficient and -sufficient citrus roots using iTRAQ-based proteomics in order to determine the molecular mechanism of plants to cope with B-deficiency.

2. Materials and methods

2.1. Plant culture and B treatments

Seeds of 'Xuegan' [*Citrus sinensis* (L.) Osbeck] were germinated in plastic trays containing sand, and fertilized twice weekly with ¼ strength nutrient solution until dripping. Full strength nutrient solution contained 6 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2 mM $NH_4H_2PO_4$, 1 mM $MgSO_4$, 50 μM KCl, 10 μM H_3BO_3, 2 μM $MnSO_4$, 2 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.065 μM $(NH_4)_6Mo_7O_{24}$, and 40 μM Fe-EDTA [9]. In late-May (five weeks after germination), uniform seedlings with a single stem were selected, transplanted into 6 L pots containing sand and grown in a greenhouse under natural photoperiod at Fujian Agriculture and Forestry University, Fuzhou, Fujian Province, China. Each pot contained two seedlings, and was supplied with 500 mL of ½ strength nutrient solution every

Please cite this article as: Yang L-T, et al, iTRAQ protein profile analysis of *Citrus sinensis* roots in response to long-term boron-deficiency, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.04.025