Plant Science xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

The beta subunit of glyceraldehyde 3-phosphate dehydrogenase is an important factor for maintaining photosynthesis and plant development under salt stress—Based on an integrative analysis of the structural, physiological and proteomic changes in chloroplasts in *Thellungiella halophila*

Q1 Lili Chang[a,b], Anping Guo[a,b,*], Xiang Jin[b], Qian Yang[b], Dan Wang[b], Yong Sun[b], Qixing Huang[b], Limin Wang[b], Cunzhi Peng[b], Xuchu Wang[a,b,*]

[a] *College of Agriculture, Hainan University, Haikou 570228, Hainan, China*
[b] *Key Laboratory of Biology and Genetic Resources for Tropical Crops, Institute of Tropical Biosciences and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, Hainan, China*

ARTICLE INFO

Article history:
Received 22 February 2015
Received in revised form 13 April 2015
Accepted 16 April 2015
Available online xxx

Keywords:
Chloroplast
Comparative proteomics
Glyceraldehyde 3-phosphate dehydrogenase
Halophyte
Thellungiella halophila

ABSTRACT

Thellungiella halophila, a new model halophyte, can survive under highly saline conditions. We performed comparative proteomics of chloroplasts from plants grown under different saline conditions. Seventy-five salt-responsive proteins were positively identified by mass spectrometry, which represented 43 unique ones. These proteins were categorized into 7 main pathways: light reaction, carbon fixation, energy metabolism, antenna proteins, cell structure, and protein degradation and folding. Saline conditions increased the abundance of proteins involved in photosynthesis, energy metabolism and cell structure. The results indicated that Thellungiella could withstand high salinity by maintaining normal or high photosynthetic capacity, reducing ROS production, as well as enhancing energy usage. Meanwhile, the ultrastructural and physiological data also agree with chloroplast proteomics results. Subsequently, the glyceraldehydes 3-phosphate dehydrogenase beta subunit (GAPB) involved in carbon fixation was selected and its role in salt tolerance was clarified by over-expressing it in Arabidopsis. ThGAPB-overexpressing plants had higher total chlorophyll contents, dry weights, water contents and survival rates than that of wild type plants. These results indicated that ThGAPB might improve plant salt tolerance by maintaining higher recycling rates of ADP and $NADP^+$ to decrease ROS production, helping to maintain photosynthetic efficiency and plant development under saline conditions.
© 2015 Published by Elsevier Ireland Ltd.

Abbreviations: 3-PGA, glycerate-3-phosphate; ATPB, ATP binding; ATPM, ATP metabolic process; CA, carbonic anhydrase; CBP, chlorophyll *a–b* binding protein; CU, carbon utilization; CP, chloroplast protein; Cytb559, cytochrome b559; FBA, fructose-bisphosphate aldolase; FNR, ferredoxin-NADP reductase; G3P, glyceraldehyde 3-phosphate; GAPB, glyceraldehyde 3-phosphate dehydrogenase beta subunit; GMP, glucose metabolic process; GPD, glyceraldehyde 3-phosphate dehydrogenase; IS, internal standard; OEC, oxygen-evolving complex; OEE, oxygen-evolving enhancer protein; ORP, oxidation-reduction process; PCL, protein-chromophore linkage; PET, photosynthetic electron transport; PLAP, plastid-lipid-associated protein; PRK, phosphoribulokinase; RCA, RuBisCO activase; SOTA, self-organizing tree algorithm; SMA, structural molecule activity; WT, wild type.

Q2 * Corresponding authors at: Chinese Academy of Tropical Agricultural Sciences, Key Laboratory of Biology and Genetic Resources for Tropical Crops, Institute of Tropical Biosciences and Biotechnology, Haikou, Hainan 571101, China. Tel.: +86 898 66987460; fax: +86 898 66987460.
E-mail addresses: xchwanghainan@163.com, xchwanghainanlab@163.com (X. Wang).

http://dx.doi.org/10.1016/j.plantsci.2015.04.010
0168-9452/© 2015 Published by Elsevier Ireland Ltd.

UNCORRECTED PROOF

1. Introduction

Q3 Soil salinity is one of the major abiotic stresses in nature [1]. Soil containing a high salt concentration often causes ion imbalance, hyperosmotic stress, and secondary stresses in plants, which will eventually inhibit plant growth and development [1,2]. Chloroplasts are the unique photosynthetic organelle in plants. Under salt stress, the photosynthetic activity in chloroplasts is decreased because of the peroxidation reaction of the membrane envelope, as well as the degradation of photosynthetic pigments [3–5]. The morphology of chloroplasts also changes with extended exposure time to increased salinity [1].

Thellungiella halophila, a close relative of Arabidopsis, is a new model halophyte owing to its small genome, short life cycle, and large seed yield [1,6–9]. There is strong evidence in support of its tolerance of extreme salt conditions being attributable to global changes in both gene and protein regulation levels [10,11]. In-depth analysis of altered protein expression profiles and functional analysis of certain differentially expressed proteins in the leaves and roots can help us unravel the salt tolerance mechanisms in this halophyte [11–14]. With a combination of traditional two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS) technology, Gao and colleagues identified 13 salt-responsive proteins from *T. halophila* leaves [12]. Comparative proteomics analyses of Arabidopsis and Thellungiella showed that more proteins changed in abundance in Arabidopsis than in Thellungiella, but that the proteins with altered expression were involved in more functional categories in the latter [14]. Additionally, phosphoproteomics of Thellungiella roots under high salinity conditions resulted in the identification of 20 phosphorylated proteins that are involved in signal transduction, ROS detoxification, protein synthesis and refolding [13]. Recently, our comparative proteomic analyses of Thellungiella leaves demonstrated that starch and sucrose metabolism in chloroplasts may play pivotal roles in salt tolerance [11]. Our morphological and physiological data also demonstrated that salt can stimulate starch accumulation in the chloroplasts of Thellungiella leaves. We identified 209 salt-responsive proteins and found that most of them are involved in carbohydrate metabolism, starch and sucrose metabolism, and photosynthesis. It was noteworthy that, among those identified proteins, 64 proteins were located in chloroplasts, and their expression changed significantly at both the mRNA and protein levels under high salinity conditions [11]. Several comparative proteomic studies of chloroplasts under saline stress were also performed in other plants to assess differentially expressed proteins: e.g., in maize [15], *Salicornia europaea* [16], wheat [17], and mangroves [18,19]. These proteomics data revealed that chloroplast proteins are important for the response to salt. Based on our observations and the published data, chloroplasts might be important organs for the response to salt stress, and some differentially expressed proteins related to photosynthesis, energy metabolism, detoxification and antioxidation in chloroplasts may play important roles in the tolerance of halophytes to extreme salt conditions [11].

Thus, in the present study, we further investigated the altered protein profiles of Thellungiella chloroplasts by combining 2D-DIGE and MALDI TOF/TOF MS technologies. The identification of the differentially expressed proteins, as well as investigation of the impact of salinity on chlorophyll fluorescence and chloroplast ultrastructure, revealed a potential link between specific proteins with altered abundance and the processes of light reaction and Calvin cycle in photosynthesis under different salinity conditions. Over-expression of the glyceraldehyde 3-phosphate dehydrogenase beta subunit gene (*ThGAPB*) in Arabidopsis demonstrated that *ThGAPB* may play important roles in plant salt tolerance.

2. Materials and methods

2.1. Plant materials and growth conditions

Seeds of Shandong ecotype *T. halophila* were sown on a 1:1 mixture (V/V) of cover soil and vermiculite irrigated with distilled water. After germination, seedlings were grown in a greenhouse with a day/night temperature regime of 22 °C/20 °C, a photoperiod of 16 h/8 h, and relative humidity of 65 ± 5%. Seedlings were irrigated from below to field capacity with half-strength Hoagland nutrient solution at 4-day intervals. Sixty days after sowing, the plants were divided into four groups and were watered with nutrient solutions supplemented with 0, 200, 400 or 600 mM NaCl for 7 days and were irrigated each day in the morning, before harvesting for morphological, physiological and proteomic analyses.

2.2. Determination of chlorophyll content and photosynthesis parameters

Chlorophyll was extracted from leaf powder (approximately 0.2 g) with 25 ml of 80% acetone. Samples were mixed in closed, darkened tubes for 1 h. The absorbance of extracted chlorophyll *a* was detected at 645 nm, and that of extracted chlorophyll *b* at 663 nm. Total chlorophyll levels were calculated as described previously [20,21]. Chlorophyll fluorescence emission was analyzed with a MINI-PAM chlorophyll fluorimeter according to the user's manual (Walz, Germany). To measure the minimal fluorescence (F_o) and the maximal fluorescence (F_m), the plants were dark-adapted for at least 30 min. Measurements were made for more than 20 plants under each of the four saline treatments.

2.3. Transmission electron microscopy

To characterize the ultrastructure of chloroplasts from *T. halophila* subjected to different salt stresses, a 0.3 cm × 0.2 cm slice from the middle part of each type of experimental leaf was fixed in 2.5% (v/v) glutaraldehyde in 0.1 M phosphate (pH 7.2) for 4 h at room temperature, followed by 2 h of fixation in 1% OsO_4 and rinsing with 0.1 M phosphate (pH 7.2). Then, the samples were dehydrated in graded ethanol solutions and embedded in Epon812. The blocks were sectioned with a glass knife using an ultramicrotome and were collected onto copper grids, which were then sequentially stained with uranyl acetate followed by lead citrate and examined under a JEOL JEM-1230 transmission electron microscopy (TEM, JEM 1230, Hitachi, Tokyo, Japan).

2.4. Chloroplast isolation, proteins extraction and DIGE analysis

The chloroplasts treated with 0, 200, 400, and 600 mM NaCl for 7 days were isolated from *T. halophila* leaves as described [22]. Then, the integrity of these purified chloroplasts was examined on an Axio scope microscopy (Carl Zeiss, Jena, Germany). Subsequently, the purity of chloroplast proteins was assessed by measuring the enzyme activity of catalase, which is an indirect cytosolic marker in plants [22]. In brief, soluble proteins in the crude extracts and the chloroplasts were extracted with potassium phosphate buffer, and the reaction mixture was prepared by adding additional amounts of protein to the potassium phosphate buffer. The reaction was started by adding H_2O_2. The absorbance values of the purified chloroplast fraction indicated no significant changes during the detection period. The assay was performed in triplicate, and the absorbance values were plotted as a function of time.

To analyze salt-response mechanisms at the protein level, these purified chloroplast proteins of *T. halophila* under 0, 200, 400 or 600 mM NaCl treatments were extracted via the BPP protocol [23]. The washed protein pellets were air-dried and dissolved with Lysis

Please cite this article in press as: L. Chang, et al., The beta subunit of glyceraldehyde 3-phosphate dehydrogenase is an important factor for maintaining photosynthesis and plant development under salt stress—Based on an integrative analysis of the structural, physiological and proteomic changes in chloroplasts in *Thellungiella halophila*, Plant Sci. (2015), http://dx.doi.org/10.1016/j.plantsci.2015.04.010

Download English Version:

https://daneshyari.com/en/article/2017033

Download Persian Version:

https://daneshyari.com/article/2017033

Daneshyari.com