Journal of Plant Physiology 175 (2015) 48–58

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Molecular Biology

Comparative proteomic analysis of tobacco expressing cyanobacterial flavodoxin and its wild type under drought stress

Javad Gharechahi [a], Mohammad-Reza Hajirezaei [b], Ghasem Hosseini Salekdeh [a,*]

[a] Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran
[b] Leibniz Institute of Plant Genetics and Crop Plant Research (Leibniz-IPK), Corrensstraße 3, 06466 Gatersleben, Germany

ARTICLE INFO

Article history:
Received 22 July 2014
Received in revised form 5 November 2014
Accepted 13 November 2014
Available online 20 November 2014

Keywords:
Drought stress
Ferredoxin
Flavodoxin
Proteomics
Tobacco

ABSTRACT

Tobacco plants expressing cyanobacterial flavodoxin (Fld) show enhanced tolerance to a wide range of abiotic stresses including drought, temperature and UV. The mechanisms of adaptation to stress conditions under Fld expression are largely unknown. Here, we applied comparative proteomic analysis to uncover the changes in the proteome profile of Fld-expressing plants in response to drought stress. Using high-resolution two-dimensional gel electrophoresis, we were able to detect 930 protein spots and compare their abundance. We found changes up to 1.5 fold for 52 spots under drought in transgenic and/or wild type plants. Using combined MALDI-TOF/TOF and ESI-Q/TOF analysis 39 (24 in wild type, 11 in transgenic, and 4 in both) drought-responsive proteins (DRPs) could be identified. The majority of DRPs are known to be involved in photosynthesis, carbohydrate and energy metabolism, amino acid and protein synthesis and processing, and oxidative stress responses. Among candidate DRPs, the abundance of remurin, ferredoxin-NADP reductase, chloroplast manganese stabilizing protein-II, phosphoglycerate mutase, and glutathione S-transferase decreased in drought stressed Fld-tobacco while S-formylglutathione hydrolase and pyridoxine biosynthesis protein abundance increased. In wild type plants, drought caused a reduction of proteins related to carbohydrate metabolism. These results suggest that the stress tolerance conferred by Fld expression is strongly related to control mechanisms regarding carbohydrate and energy metabolism as well as oxidative stress responses.

Introduction

Drought stress is one of the major environmental constraints that limit growth, development and productivity of plants worldwide. Developing crop plants with improved tolerance to drought is of great economic importance. The prerequisite to meet this goal is to understand the physiological, biochemical and molecular basis of the plant response to water deficiency which, from the perception of the drought signal to the molecular and physiological reprogramming, needs to be inspected at a global systems biology level to understand the integrated biological pathways involved in this process. Plants usually respond to water deprivation by activating cascades of signal transduction pathways and gene regulatory networks leading to an increase in stomatal resistance and consequently a reduction in water loss, an increase in water uptake by developing deep root systems, and the accumulation of compatible solutes such as proline, glutamate, glycine-betaine and sugars (mannitol, sorbitol and trehalose) (Cruz de Carvalho, 2008; Mahajan and Tuteja, 2005; Rampino et al., 2006; Yoo et al., 2009). In addition, upon perception of drought stress, plants usually activate defense mechanisms to re-establish the cellular homeostasis, to fine-tune the energy metabolism and to protect the cellular machinery from oxidative stresses imposed by prolonged drought stress (Zurbriggen et al., 2008).

Ferredoxin (Fd) is an iron-sulphur protein present in all photosynthetic organisms ranging from cyanobacteria to plants. It is the final electron acceptor of the photosynthetic electron transport chain (PETC) and essential for the distribution of low-potential reducing equivalents to the most energy-demanding metabolic steps in CO_2 fixation, nitrogen and sulphur assimilation, amino

Abbreviations: Fld, flavodoxin; Fd, ferredoxin; pfld, plastid Fld; DRPs, drought-responsive proteins; MALDI-TOF/TOF, matrix assisted laser desorption ionization-time of flight/time of flight; ESI-Q/TOF, electrospray ionization quadruple/time-of-flight; 2-DE, two-dimensional gel electrophoresis; PETC, photosynthetic electron transport chain; MS, Murashige and Skoog; DTT, dithiothreitol; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; IPG, immobilized pH gradient; IEF, isoelectric focusing; CBB G-250, coomassie brilliant blue G-250; ROS, reactive oxygen species.

* Corresponding author. Tel.: +98 263 2703536; fax: +98 263 2704539.
E-mail addresses: h_salekdeh@abrii.ac.ir, hsalekdeh@yahoo.com (G.H. Salekdeh).

http://dx.doi.org/10.1016/j.jplph.2014.11.001
0176-1617/

acid synthesis, fatty acid desaturation, as well as many regulatory (e.g. thioredoxin (Trx) redox regulation system) and dissipatory pathways (Hanke and Mulo, 2013; Hase et al., 2006; Knaff, 2004). Indeed, Fd determines the efficiency of electron transfer between the thylakoid membrane and the soluble stromal enzymes which are dependent on these electrons for their activities (Hanke and Mulo, 2013; Tognetti et al., 2006). Reduced Fd transfers electrons to several enzymes belonging to various metabolic, dissipative and regulatory pathways (Hanke and Mulo, 2013; Hanke et al., 2005). It serves as an electron donor for the reaction catalyzed by Fd-$NADP^+$ reductase (FNR) which generates NADPH as a reducing power for CO_2 fixation and other biosynthetic pathways. It also provides electrons for nitrogen and sulfur assimilation, amino acid, chlorophyll, phytochrome, and fatty acid biosynthesis (Hanke and Mulo, 2013; Hase et al., 2006). In addition, it plays a key role in the regeneration of antioxidants such as ascorbate (Miyake and Asada, 1994) and peroxiredoxin (Dietz et al., 2006), and it is involved in the regulation of enzyme activity via Fd-thioredoxin reductase (Schurmann and Buchanan, 2008). Photo-reduced Fd can transfer its electron to the cytochrome b_6f complex and creates a cyclic electron flow which bypasses the NADPH production by FNR (Johnson, 2011). It has been thought that the cyclic electron flow is activated to fulfill the ATP requirement of chloroplasts or to function as a safety valve to relieve the electron pressure from PETC under adverse environmental conditions such as high illumination, low CO_2 concentrations or drought stress (Hanke and Mulo, 2013; Lehtimaki et al., 2010).

At least two different isoforms of Fd are found in chloroplast that participate in separate electron transfer routes (Ceccoli et al., 2011; Hanke and Mulo, 2013). The expression of Fd is induced by light through a regulatory mechanism which requires active photosynthesis and is thought to be under the control of the redox status of the cell. When plants are exposed to environmental stresses such as iron deficiency, high illumination, salinity and drought, the Fd expression (both mRNA and protein) is significantly down regulated (Ceccoli et al., 2011; Tognetti et al., 2006; Zurbriggen et al., 2008). Deficiency in Fd expression could compromise cell survival and also modulates the response of the whole plant to situations of stress. It has been shown that Fd overexpression could not compensate for its loss during environmental adversities because Fd expression is thought to be regulated at the post-transcriptional level, which depends on cis-acting sequences integrated within the protein coding region of the gene (Zurbriggen et al., 2008). Repression of Fd expression is also observed in cyanobacteria, the closest living ancestors of modern day chloroplasts, when they encounter a similar set of environmental stresses (Singh et al., 2004). However, cyanobacteria and some algae can compensate for the loss of Fd expression by the induction of the expression of flavodoxin (Fld), an isofunctional electron carrier protein that contains flavin mononucleotide as the prosthetic group (Blanco et al., 2011; Singh et al., 2004). It has been shown that Fld could successfully substitute Fd in most electron transfer reactions in which Fd is involved (Blanco et al., 2011). During evolution, plants have lost the genes encoding for Flds and consequently the adaptive advantages arising from their induction during stress conditions. However, recent studies have shown that heterologous expression of cyanobacterial Fld in the chloroplast of tobacco plants could compensate for decreased Fd levels and enhance tolerance to a wide range of abiotic stresses including drought, extreme temperature, UV and high irrigation (Ceccoli et al., 2011; Tognetti et al., 2006). The tolerance toward stress is usually regulated by a coordinated action of metabolites and gene expression. However, the regulatory mechanisms are largely unknown for tobacco plants expressing the cyanobacterial Fld.

Comparative proteomics approaches have been successfully applied to study the response of plants to environmental stresses (Ghosh and Xu, 2014). Here, we applied a comparative 2-DE based proteomic approach in combination with protein identification using tandem mass spectrometry to decipher the change in the proteome profile of transgenic plants expressing cyanobacteral Fld in their chloroplasts in response to drought stress.

Materials and methods

Plant growth and water deprivation

The design and preparation of the homozygous plastid *fld* (pfld) line of tobacco (*Nicotiana tabacum* cv. Petit Havana) has been described elsewhere (Tognetti et al., 2006). Seeds of transgenic plants were provided by Prof. Nestor Carrillo. Seeds were germinated on Murashige and Skoog (MS) agar plates supplemented with 2% (w/v) sucrose and, in the case of transformants, with 100 $\mu g\,mL^{-1}$ kanamycin. After 4 weeks, seedlings were transferred to soil, watered daily with nutrient medium and grown at 200 $\mu mol\,m^{-2}\,s^{-1}$, 25 °C, a 16/8 h photoperiod and a relative humidity of 80%. Unless otherwise stated, experiments were performed using 6–8-week-old plants grown in soil. To improve reproducibility and to facilitate comparisons between lines, we performed side-by-side assays of the fifth and sixth fully expanded leaves belonging to the same node (counting from the apex). The presence of Fld in leaf extracts of the various lines was determined by SDS–PAGE and immunoblot analysis.

To perform water deprivation, plants were withdrawn from water for 12 d. Field capacity was measured every day and adjusted to 20% if needed to ensure that drought conditions were uniform between control and transgenic plants. Leaf material was harvested from the middle part of the fifth fully expanded leaf by removing the major vein of the leaf and frozen immediately in liquid nitrogen and stored at −80 °C for further analysis.

Protein extraction

Total leaf proteins were extracted from stressed pfld-expressing and wild type plants using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions with some modifications. Briefly, harvested leaves were finely ground in liquid nitrogen using a mortar and pestle. Subsequently, 0.2 mg of the resulting powder was resuspended in 1 mL TRIzol reagent. The suspension was vortexed and incubated for 5–10 min at room temperature, after which 200 μL of chloroform was added per 1 mL initial TRIzol, then mixed by inversion. The sample was centrifuged at 12,000 × *g* for 15 min at 4 °C. The upper layer was carefully removed and stored for RNA isolation. DNA was precipitated by the addition of 300 μL ethanol per 1 mL TRIzol reagent and the mixture was centrifuged for 5 min at 2000 × *g* and 4 °C. The supernatant was removed and proteins pelleted by the addition of three volumes of cold acetone followed by centrifugation for 10 min at 12,000 × *g* and 4 °C. The protein pellet was washed three times for 20 min in a solution containing 0.3 M guanidine chloride, 2.5% (v/v) glycerol and 95% ethanol. The final wash was performed in a solution containing 2.5% glycerol in 100% ethanol. After a brief air drying (10–15 min), the pellet was resuspended in lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2% IPG buffer pH 3–10 (Bio-Rad), 40 mM DTT and 35 mM tris). Protein concentration was measured by the Bradford assay using BSA as a standard (Bradford, 1976).

Two-dimensional gel electrophoresis (2-DE)

The IPG strips (pH 4–7, 24 cm length, Bio-Rad) were rehydrated at room temperature overnight in 450 μL rehydration solution (8 M urea, 2% CHAPS, 20 mM DTT, 2% IPG buffers (pH 3–10) and 0.002%